10th TMD

Tooth Morphogenesis and Differentiation

Sept. 1 – 4, 2010
Berlin, Germany

Table of Content

Welcome Address

Dear colleagues,

with pride and thankfulness I am happy to welcome you to come to Berlin to participate in the 10th TMD-meeting. I am grateful that despite economic crisis and a severe shortage of money clearly more than one hundred scientists from all over the world found their way to participate and exchange their latest research results dealing with tooth morphogenesis and differentiation. This makes me also proud to show the public how much we, a small, but very much specialized group of scientists, can show the public how far we have arrived in understanding tissue morphogenesis in relation with dental development.

With our meeting we contribute to a long history of scientific meetings held in Berlin. This is a city where science and medicine underwent a transition from mystic conviction but tragic helplessness to a predictable medicine, based on science and experimental research. This transition started 300 years ago with the foundation of a hospital in 1710, which was named the „Charité“ by King Friedrich Wilhelm I in 1727. Here in Berlin, a vast quantity of innovative discoveries in the field of physiology, cellular pathology, hygiene, surgery, microbiology, and computer-assisted medicine took place over the last centuries and latest decades.

Our 10th TMD-meeting is one in a longer row of meetings to celebrate 300 years of medical research in Berlin this year, and we ourselves celebrate the 10th anniversary of the TMD-meetings.

So I wish you all a very fruitful meeting with many new insights in our latest research results, a productive exchange of ideas, and meeting old and finding new friends.

Ralf J. Radlanski

Contact Information

Local Organizing Committee

Congress President

Prof. Dr. Dr. Ralf J. Radlanski

Charité - Campus Benjamin Franklin at Freie Universität Berlin
Center for Dental and Craniofacial Sciences
Dept. of Craniofacial Developmental Biology
Assmannshauser Str. 4-6
14197 Berlin, Germany

Phone: +49 (0) 30-450-562-201
Fax: +49 (0) 30-450-562-902
E-Mail: ralfj.radlanski@charite.de
http://oralstrukturbiol.charite.de

Organizer

PCO

MCI – Berlin Office
MCI Deutschland GmbH
Markgrafenstr. 56
10117 Berlin, Germany

Phone: +49 (0) 30-20 45 90
Fax: +49 (0) 30-20 45 950
E-Mail: tmd2010@mci-berlin.de
www.mci-berlin.de/tmd2010

Scientific Information

The congress program is structured thematically, and the presentations appear in chronological order.

The abstracts of the oral presentations are listed according to their appearance in the scientific program.

The abstracts of the posters are listed by numbers.

Imprint

Editorial:
Local Organizing Committee and MCI – Berlin Office

Layout & Graphics:
X-Press Grafik & Druck GmbH

Photos:
Logo (c) Ralf J. Radlanski

Scientific Program

Thursday, September 2nd

9:30	**Opening**
	General Tooth Development
9:50	O1 – Early development of the lower incisor in mice Hovorakova, Maria
10:10	O2 – On the maintenance of the complex cusp pattern in continuously growing molars Tummers, Mark
10:30	O3 – Evaluation of apoptosis and proliferation in the area of rudimentary premolar tooth primordia in the mouse embryonic mandible Churava, Svatava
10:50	O4 – Primary cilia in tooth development Ghafoor, Sarah
11:10	**COFFEE BREAK**
11:25	O5 – Differential expression and functional significance of glucose transporters during murine tooth development Ida-Yonemochi, Hiroko
11:45	O6 – The cadherin-catenin complex during zebrafish tooth development Verstraeten, Barbara
12:05	O7 – Advances in understanding abnormalities of tooth number Nieminen P.
12:25	O8 – Eat it all: Tooth resorption in teleost fish Witten, P. Eckhard
12:45	**LUNCH**
	Stem Cells And Signalling
14:00	O9 – Purification and characterization of human dental epithelial stem cells Papagerakis, Petros
14:20	O10 – MicroRNA regulated tooth epithelial stem cell differentiation: Specific MicroRNAs target BMP, Wnt and chromatin remodeling complexes Amendt, Brad
14:40	O11 – Development of tooth cusps in the gecko Paroedura picta: Cusp generation without an enamel knot Zahradnicek, Oldrich
15:00	O12 – Ring1a/b polycomb proteins regulate the mesenchymal stem cell niche in continuously growing incisors Lapthanasupkul, Puangwan
15:20	**COFFEE BREAK**
15:35	O13 – Essential role of mesenchymal Notch-RBP-Jkappa signaling in controlling dental epithelial cell fate during mouse tooth development Hu, Bing
15:55	O14 – The interaction between Wnt, Shh and Sostdc1 governs the spatial patterning of teeth Cho, Sung-Won
16:15	O15 – Hedgehog signaling directs generation of progeny from adult stem cells in the continuously growing mouse incisor Seidel, Kerstin
16:35	O16 – Evc regulates symmetric and asymmetric responses to Shh signalling in tooth development Peters, Heiko
16:55	**COFFEE BREAK**
17:10	O17 – Downstream targets of Ectodysplasin in developing teeth Thesleff, Irma
17:30	O18 – Diverse roles of Lrp4 in craniofacial ectodermal organ development Porntaveetus, Thantrira
17:50	END
20:00	**Dinner at TV-Tower**

Scientific Program

Friday, September 3rd

Stem Cells And Signalling

9:30	O19 – Amelogenin and RANK pathway in root and bone phenotype of the Msx2 null mutant mouse Descroix, Vianney
9:50	O20 – The role of Pkd2 in tooth development Khonsari, Roman
10:10	O21 – A genetic pathway involving Msx1, Bmp4 and Pax9 connects growth and morphogenesis during upper lip development Nakatomi, Mitsushiro
10:30	O22 – Molecular regulations of supernumerary tooth formation Wang, Xiu-Ping

Ameloblasts and Amelogenesis

10:50	O23 – Structural and cytochemical analyses of enamel and enamel forming cells in the teeth of amelogenin-deficient mice Takano, Yoshiro
11:10	**COFFEE BREAK**
11:25	O24 – Induction of human keratinocytes into enamel-secreted ameloblasts Zhang, Yanding
11:45	O25 – Amelotin: Introducing a new player in the team of enamel matrix proteins Ganss, Bernhard
12:05	O26 – The role of Amelotin on differentiation of ameloblasts DenBesten, Pamela
12:25	O27 – A high calcium supplement partially prevents fluoride toxicity on secretory amelogenesis in developing hamster tooth germs in vitro: Ultrastuctural studies Lyaruu, Donacian
12:45	**LUNCH**
14:00	O28 – Ameloblastin in enamel formation: Rescue of amelogenesis imperfecta and processing by enamel proteinases Chun, Yong-Hee Patricia
14:20	O29 – Immunolocalisation of NBCe1 in the mouse enamel organ Bronckers, Ton
14:40	O30 – The recombinant human amelogenin protein and regeneration of mouse non-union calvarial defect Deutsch, Dan
15:00	O31 – Ameloblastoma characterization and in vitro stimuli response Catón, Javier
15:20	**COFFEE BREAK**
15:35	O32 – New hypothesis of cross-striation formation mechanism Sasaki, Ai

Scientific Program

Friday, September 3rd

Odontoblasts and Dentinogenesis

15:55	O33 – How odontoblasts can sense enamel erosion Bleicher, Françoise
16:15	O34 – Pathogen sensing by human odontoblasts Farges, Jean-Christophe
16:35	O35 – Overexpression of the Trps1 transcription factor in odontoblasts results in a dentinogenesis imperfecta-like phenotype Napierala, Dobrawa
16:55	**COFFEE BREAK**
17:10	O36 – The expression of GM-CSF and osteopontin in immunocompetent cells precedes the odontoblast differentiation following allogenic tooth transplantation in mice Saito, Kotaro
17:30	O37 – Analysis of Col1a1-2.3GFP transgene during odontoblast differentiation Mina, Mina **– CANCELLED** We make use of this time slot and gather for a group photo.

Dental Pulp

17:50	O38 – Neuronal differentiation of human dental pulp stem cells in vitro and in vivo - potential for tissue engineering Varga, Gabor
18:10	O39 – Establishment of in vitro culture system for evaluation of the dentin-pulp complex regeneration with special reference to differentiation capacity of the BrdU-label-retaining dental pulp cells Ohshima, Hayato
18:30	**COFFEE BREAK**
18:45	O40 – Responses of BrdU-label-retaining dental pulp cells to allogenic tooth transplantation into mouse maxilla Mutoh, Noriko

Tooth Bone Interface

19:05	O41 – The role of Wnt signaling for patterning of molar tooth roots in mammals Ota, Masato
19:25	O42 – Label-retaining epithelial cells in mouse epithelial cell rests of Malassez Honda, Masaki
19:45	**POSTERS with BarBQ**

Scientific Program

Saturday, September 4th

Tooth Bone Interface

9:30	O43 – The biology of periodontally accelerated tooth movement Gadban, Naser
9:50	O44 – Fibrillin-1 is indispensable for normal collagen fiber architecture and gene expression in periodontal ligament Suda, Naoto
10:10	O45 – Regulation of osteoblast differentiation and ECM remodeling by BMP2/4 in vitro Chen, Shuo
10:30	O46 – Non redundant role of Msx1 homeogene on neurectodermal osteogenesis Berdal, Ariane

Craniofacial Biology And Tissue Engineering

10:50	O47 – The planar cell polarity effector gene, Fuz is essential for craniofacial and tooth development Amendt, Brad
11:10	**COFFEE BREAK**
11:25	O48 – Interactions between Gas1, Cdo and Boc during early development of the craniofacial midline and dentition Cobourne, Martyn
11:45	O49 – Variation of tooth root lengths in human X chromosome aneuploids Lähdesmäki, Raija
12:05	O50 – Characterization of two novel genes expressed in tooth and associated epithelia Moffatt, Pierre
12:25	O51 – Genomic copy number alteration analysis of sporadic and Gorlin-syndrome associated keratocystic odontogenic tumour (odontogenic keratocyst) Heikinheimo, Kristiina
12:45	**LUNCH**
14:00	O52 – Tooth engineering: Organization of dental matrices in implanted dental cell-cell reassociations Lesot, Hervé

Scientific Program

Saturday, September 4th

Evolution And Development

14:20	O53 – Of teeth, pouches and pores: the ectoderm/endoderm connection in development and evolution Huysseune, Ann
14:40	O54a – Molecular regulatory mechanism of dental root development. Chai, Yang
	O54 – Rudimentary structures and dental anomalies Peterkova R. **– CANCELLED**
15:00	O55 - Signaling centers in mouse embryonic mandible reflect an ancestral tooth pattern Prochazka, Reata
15:20	**COFFEE BREAK**
15:35	O56 – Evolutionary analysis of enamelin in mammals, sauropsids and amphibians provides new insights on its function Delgado, Sidney
15:55	O57 – The evolution and development of the mammalian dentition: Insights from the marsupial Monodelphis domestica Moustakas, Jacqueline
16:15	O58 – Developmental mechanisms in the evolution of morphological key innovation in small mammals Renvoisé, Elodie
16:35	O59a – A new regulator of amelogenesis Klein, Ophir
16:55	**COFFEE BREAK**
17:10	O60 – How bats reach perfect occlusion? Late odontogenesis of tribosphenic molar Spoutil, Frantisek
17:30	O61 – Tooth replacement in Xenopus tropicalis: A WNT-WNT situation Willems, Maxime

WORKSHOP

17:50	W62 - In vitro investigations of the Pax9/Msx1/Bmp4 interrelationship in tooth bud mesenchyme Mues, Gabriele
18:35	Discussion about the 11th TMD-Meeting
19:00	END

Poster Session

P1 Timing of cranial neural crest cell migration is critical for tooth type formation in mice
Chen, Zhi

P2 Neural crest like cells from induced pluripotent stem cells for tooth regeneration
Kishigami, Ryota

P3 Patterning of the heterodont dentition in the house shrew (Suncus murinus)
Yamanaka, Atsushi

P4 Development of the oral cavity: From gene to clinical phenotype in human
Laugel, Virginie

P5 Molecular regulation of tooth replacement
Jussila, Maria

P6 Developmental analysis of limb development after temporal blocking of Hedgehog signaling
Kim, Eun-Jung

P7 Methylation status of the Runx2 P2 promoter in a family with ectopic maxillary canines
Camilleri, Simon

P8 Alteration of tooth development in two-phase organotypic cultures by transient Glycogen Synthase Kinase-3 (GSK-3) inhibition
Unda, Fernando

P9 Reduced expression of tight junction proteins ZO-1 and claudin-1 in ameloblasts and odontoblasts of Epiprofin/Sp6 deficient mice
Ibarretxe, Gaskon

P10 Caspase knock-outs and dental apoptosis
Matalova, Eva

P11 1,25 (OH)2 vitaminD3 regulate Msx1 antisense RNA expression in mouse incisor
Pinheiro Junior, Roberto Flavio

P12 Expression and function of microRNAs in tooth development
Otsuka-Tanaka, Yoko

P13 Ultrastructural localization of osteoadherin during early tooth development
Nikdin, Hero

P14 c-Myb in prenatal odontogenesis: a pilot study in mouse and minipig models
Lungova, Vlasta

P15 Roles of Wnt/ß-Catenin signaling in the formation of dentin and periodontium
Lim, Ka-Hae

P16 Smad4 mediated signaling is essential for dentin formation
Cho, Eui-Sic

P17 Expression patterns of the Fam83h gene during murine tooth development
Lee, Min-Jung

P18 Expression of prion gene and presence of prion protein during development of mouse molar tooth germ
Khan, Qalb-E-Saleem

P19 Early odontogenesis of Chamaeleo calyptratus
Balková, Simona

P20 Mechanism of dental lamina degradation
Buchtová, Marcela

P21 Dynamics movement of dental tissue during tooth development
Rothova, Michaela

P22 Visualizing cellular dynamics of tooth development
Otsu, Keishi

P23 Morphogenesis of the mouse third molar (M3)
Chlastakova, Ivana

P24 The developmental stage-dependent increase in expression of soluble guanylate cyclase during mouse tooth development
Beikler, Thomas

P25 Functional isolation of dental stem cells
Pang, Yvonne

P26 Characterization of the putative epithelial stem cells involved in continuous tooth replacement in Atlantic salmon (Salmo salar)
Vandenplas, Sam

P27 Cell culture of human dental epithelial cells and its approach for regenerative dentistry
Mayama, Hisayo

P28 Complexity of chiropteran dentition: the GIS approach
Spoutil, Frantisek

P29 Atavisms induced by Fgf3 loss of function point to a central role for FGF signaling in evolution of tooth shape in mammals
Charles, Cyril **– CANCELLED**

P30 Exploring the roles of core-binding factor (CBFB) in tooth development and ameloblast physiology
Yamashiro, Takashi

P31 Involvement of micro-RNAs in tooth morphogenesis and ameloblast differentiation
Juuri, Emma

Poster Session

P32 Distribution and structure of the initial dental enamel formed in incisors of young wild-type and Tabby mice
Sehic, Amer

P33 Fine structural and immunohistochemical observations of the collar enamel in Lepisosteus and Polypterus, actinopterygian fish
Sasagawa, Ichiro

P34 Identification of suppressor element in the amelogenin promoter.
Taniguchi, Akiyoshi

P35 Loss of transglutaminase 2 in the mouse results in an accelerated enamel mineralization
Felszeghy, Szabolcs

P36 Pfeiffer syndrome and amelogenesis imperfecta
Yamaguti, Paulo **– CANCELLED**

P37 A frameshift mutation in DSPP associated with dentinogenesis Imperfecta type II
Tallón-Walton, Victoria

P38 Fragile fracture of dental pulp chamber: a new method to obtain dental pulp stem cells
Manzanares-Céspedes, Maria-Cristina

P39 The vascularization of embryonic mouse molar papilla evaluated by CD34, CD31 antigen expression and morphological arrangement
Yang, Guobin

P40 Non-invasive imaging of human dental pulp stem cells
Lambrichts, Ivo

P41 Dental pulp progenitor / stem cells - dentin interactions in vivo
Kodonas, Kostantinos

P42 Influence of TGF-ß1 and culturing media on ALP expression in human pulp fibroblasts with different root development in vitro
Korneli, Nicole

P43 Implantation of odontoblast progenitors in the rat molar pulp leads to the formation of reparative osteodentin
Harichane, Yassine

P44 Expression pattern of Apin and Amelotin during formation and regeneration of the junctional epithelium
Wazen, Rima

P45 Cellular events in tooth root morphogenesis
Kim, Jae-Young

P46 Genome-wide screening of key molecules for tooth root development
Date, Yuki

P47 Establishment of immortalized Hertwig's epithelial root sheath cells and character of cell line
Fujiwara, Naoki

P48 Biological effects of cyclic diarylheptanoids on tooth root formation
Lee, Ji-Won

P49 Essential roles of Osx/Sp7 in the root dentin formation
Cho, Eui-Sic

P50 Formation of the maxillary alveolar bone in humans
Radlanski, Ralf

P51 Formation of the mandibular alveolar bone in mice
Radlanski, Ralf

P52 Formation of the maxillary alveolar bone in mice
Radlanski, Ralf

P53 Formation of the mandibular alveolar bone in humans
Radlanski, Ralf

P54 A new experimental model for monitoring bone neogenesis and osseointegration of implants in rats (OSSI model)
Blazsek, József

P55 Osteoblastic differentiation of transgenic mice overexpressing Apert syndrome-type mutant FGFR2 and its soluble form
Suzuki, Hiroyuki

P56 Characterizing the craniofacial and dental phenotype of Costello Syndrome patients
Goodwin, Alice

P57 miR-200b integrates Tgf-b signaling pathway in mouse palate development
Shin, Jeong-Oh

P58 Phenotypic characterization of seventeen families with nonsyndromic tooth agenesis.
Acevedo, Ana **– CANCELLED**

General Information

Congress Location
Charité Campus Virchow-Klinikum (CVK)
FORUM 3
Augustenburger Platz 1
13353 Berlin

Registration Counter
(opening hours)
The registration desk in the entrance hall of the Forum 3 will be open as follows:

Wednesday, Sep 1st	17.00 – 21.00 h
Thursday, Sep 2nd	08.30 – 18.00 h
Friday, Sep 3rd	09.00 – 19.00 h
Saturday, Sep 4th	09.00 – 18.30 h

Registration Fees
Registration fee for participants includes:
- Admission to Scientific Sessions
- Numerous Congress Documents
- Use of Public Transportation (BVG) within Berlin
- Lunch & Coffee Breaks
- Get Together on Wednesday, September 1st, 2010 (registration required)
- Gala Dinner at TV Tower on Tuesday, September 2nd, 2010 (registration required)
- Poster BBQ on Friday, September 3rd, 2010 (registration required)

Media Check
The Auditorium is equipped with computer projection. Speakers are asked to hand in their presentations at least two hours before their speech in the media check next to the registration desk. Speakers having a presentation during the first time slot in the morning are asked to hand in their slides the day before.

Congress Language
The congress language is English.

Poster Exhibition
Scientific Posters will be displayed in the entrance foyer of the Auditorium.

The size of your poster should not exceed 90 cm width and 120 cm height.

Posters should be hung up on Wednesday, Sept. 2, 2010 between 17.00 h and 19.00 h.

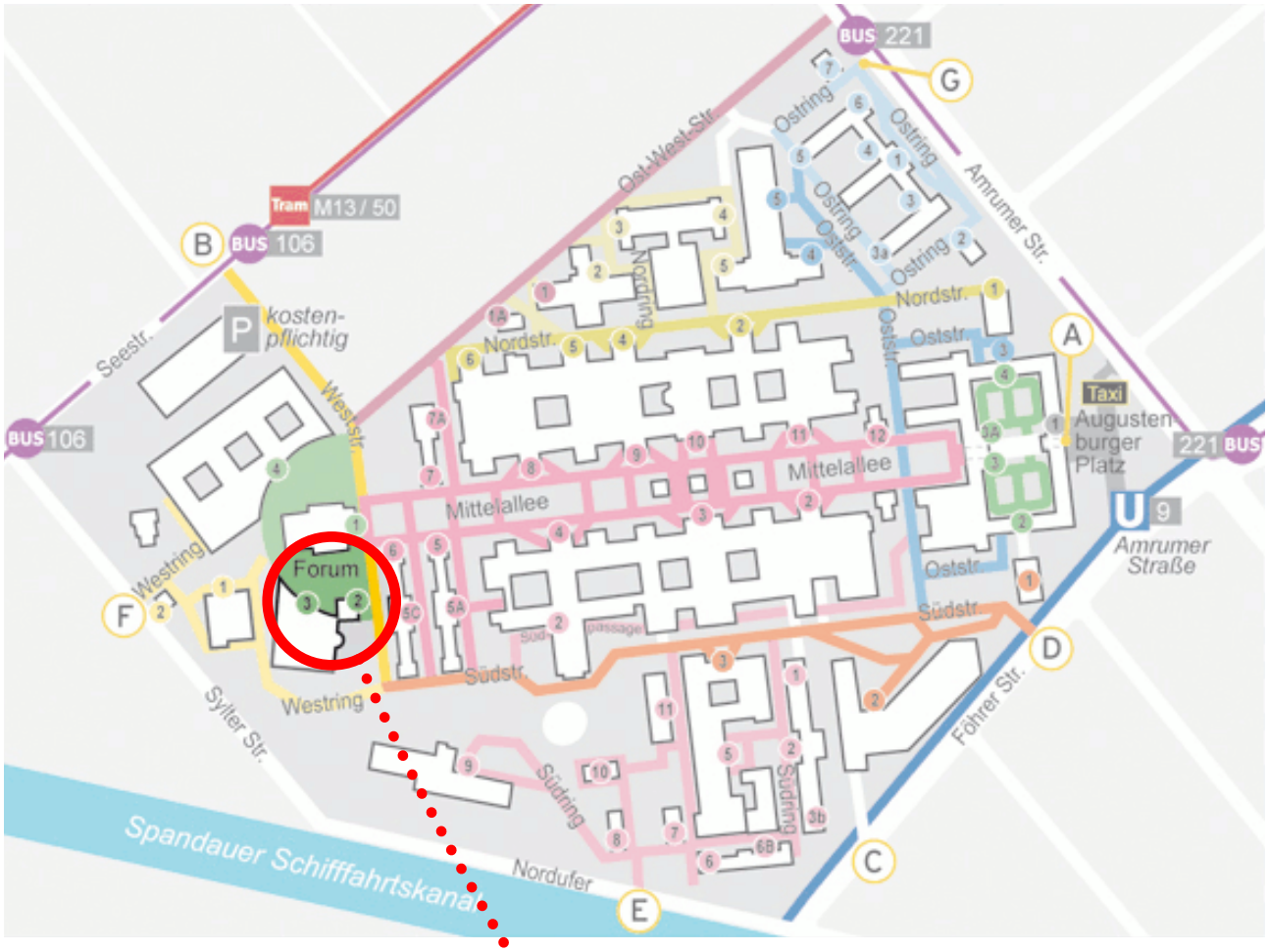
A poster information desk will be located next to the registration desk. Lists indicating title, author and poster number will be displayed at the desk. Pins for fixing the poster on the board will be available. The poster desk staff will be on your disposal to answer your questions and provide help locating the poster on site.

Please do not use your own adherence materials! In case of negligence, claims for compensation may be raised.

Coffee Breaks / Lunch
The coffee breaks and lunches are included in the registration fee. They will be served in the foyer of the Auditorium.

Certificate of Attendance
All participants receive a certificate of attendance with their conference material.

Congress Location



Congress Location

Charité Campus Virchow-Klinikum (CVK)
FORUM 3
Augustenburger Platz 1
13353 Berlin

Berlin Information

Climate
Berlin has a temperate/mesothermal climate (Cfb) according to the Köppen climate classification system.
Summers are warm with average high temperatures of 22–25°C (mid 70s F) and lows of 12–14°C (mid 50s F). Winters are cold with average high temperatures of 4°C (upper 30s F) and lows of –2 to 0°C (upper 20s and low 30s F). Spring and autumn are generally chilly to mild. Berlin‘s built-up area creates a microclimate, with heat stored by the city‘s buildings. Temperatures can be 4°C (7°F) higher in the city than in the surrounding areas.

Currency
The official currency in Germany is euros (EUR).

Electricity
In Germany electricity is supplied at 220V, 50Hz. For some devices from countries abroad converters will be needed.

Insurance
Neither the congress itself nor the PCO accept any liability for damages and/or losses of any kind which may be incurred by the congress participants or by any persons accompanying them, both during the official activities and excursions. Delegates participate in all tours/events at his/ her own risk. Participants are advised to take out insurances against loss, accidents or damage that could be incurred during the congress. Verbal agreements will not be binding unless they are confirmed in writing. Sole place of jurisdiction is Berlin. German law is applicable.

Time
Germany belongs to the Central European Time Zone. During the Conference the Central European Summer Time applies (GMT+2).

Tipping
In many areas such as gastronomy, in taxis, at hairdresser, at service stations, tipping is traditionally expected. Usually, the tip amounts to about 5 to 10 percent, but the size of the tip should be according to your satisfaction with the services rendered.

Taxi
Taxi’s are numerous and available at all times (almost). Taxi stands can be found at all main stations and airports as well as outside KADEWE and hotels. Most Berlin taxi drivers speak English, but don‘t take it for granted.

General Tooth Development

O1

Early development of the lower incisor in mice

Hovorakova M.[1], Peterkova R.[1], Prochazka J.[1,2], Lesot H.[3], Boran T.[4], Churava S.[1,4], Klein O.[5], Peterka M.[1]
[1]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, v.v.i., Department of Teratology, Prague, Czech Republic, [2]Faculty of Natural Sciences, Charles University, Department of Cell Biology, Prague, Czech Republic, [3]University of Strasbourg, UMR 977 and Dental School, Strasbourg, France, [4]Faculty of Natural Sciences, Charles University, Department of Anthropology and Human Genetics, Prague, Czech Republic, [5]School of Medicine, University of California at San Francisco, Department of Anatomy and Program in Developmental Biology, San Francisco, United States

Background: The multiple developmental origin of the upper incisor has been documented on serial histological sections and three-dimensional (3D) reconstructions during the period preceding a well-formed incisor bud (Peterkova et al., 1993). Using similar methods, the morphogenesis of the lower incisor from the bud stage to early bell stage has been systematically documented (Kieffer et al., 1999). However, there is still a lack of detailed information about the initial steps of lower incisor development, before this tooth enters the bud stage at embryonic day (ED) 13.5.
Aim of the study: Using serial histological sections and 3D reconstructions, we performed a detailed investigation of the initial development of the mouse lower incisor from the early epithelial thickening to the formation of the dental bud, and searched for its developmental origin in wild type mice.
Methods: Contours of the dental and adjacent oral epithelium were drawn from the serial histological sections and superimposed with respect to the middle line and to the horizontal level for correct spatial positioning of the reconstructed structures. The drawings were digitalized and 3D reconstructions were performed to visualize the dental and adjacent vestibular epithelia. 3D analysis was undertaken in the lower jaw quadrants at ED11.5-13.5.
Results: At ED 11.75, an epithelial thickening was formed in the prospective incisor area. At ED 12.0, it was possible to observe formation of several epithelial buddings in the lower incisor region. At ED 12.5, the antero-laterally located budding corresponded to the anlage of the lower oral vestibule. Compared to the upper oral vestibule area, where only indistinct thickenings were apparent at ED 13.5, the epithelium of the oral vestibule in the mandible formed a well-marked ridge. More posteriorly, the incisor bud formed at ED 12.5 and became clearly distinct at ED 13.5. In contrast to the upper jaw, where the initial epithelial budding could be still detected on the mesenchymal surface of the incisor bud, the remains of the initial epithelial budding in the incisor region of mouse mandible were not detectable on the mesenchymal surface of the incisor bud. An epithelial connection between the incisor bud and the externally located ridge of the vestibular epithelium was apparent.
Conclusion: The detailed temporo-spatial description of normal mouse incisor development and the differences between incisors in the upper and lower jaws can inform our understanding of abnormal incisor development in various genetic disorders and provides a basis for the investigation of molecular signalling pathways.
References:
Kieffer S, Peterkova R, Vonesch JL, Ruch JV, Peterka M, Lesot H. (1999) Morphogenesis of the lower incisor in the mouse from the bud to early bell stage. *Int J Dev Biol* 43: 531-539.
Peterkova R, Peterka M, Vonesch JL, Ruch JV. (1993) Multiple developmental origin of the upper incisor in mouse: histological and computer assisted 3-D-reconstruction studies. *Int J Dev Biol* 37: 581-588.
Acknowledgement: This work was supported by the Grant Agency of the Czech Republic (grants 304/09/1579 and 304/07/0223).

General Tooth Development

O2

On the maintenance of the complex cusp pattern in continuously growing molars

Tummers M.[1], Thesleff I.[1]
[1]*Helsinki University, Institute of Biotechnology, Helsinki, Finland*

Background: All rodents have continuously growing incisors and the developmental aspects of this regenerating system have been reasonably well studied. Some rodent species such as the guinea pig and the sibling vole also have continuously growing molars. From one developmental perspective these open-rooted molars are vastly more interesting than the incisors: they have a complex cusp pattern. This pattern needs to be maintained over time because it is worn down constantly. We examined in this study how this pattern is maintained.
Methods: We used basic immunohistochemistry and radioactive in situ hybridization techniques to analyze the patterns of proliferation and the distribution of regulatory molecules, and we added an extra level of information by generating 3D reconstructions of whole teeth showing the entire pattern. For this analysis tissues were used of the mouse, guinea pig and sibling vole.
Results: We show that the most apical part of the tooth, where normally root formation occurs, remains proliferation free in continuously growing molars. This is unlike the situation in non-regenerating molar of the mouse, which does grow roots. Here the most apical zone is originally also proliferation free, but the proliferation zone shifts towards the apical mesenchyme at the moment when root formation is initiated. In the continuously growing molars the mesenchymal proliferation never shifts, keeping the roots dormant. We also show that different zones are associated with different regulatory molecules.
Conclusion: We speculate that both processes, the maintenance of the complex cusp pattern and the postponement of root formation, are regulatory connected, and are possibly regulated by WNT signaling, through the spatial regulation of the proliferation in the mesenchymal component of the tooth.

General Tooth Development

O3

Evaluation of apoptosis and proliferation in the area of rudimentary premolar tooth primordia in the mouse embryonic mandible

Churava S.[1,2], Smrckova L.[1,3], Prochazka J.[1,3], Lesot H.[4], Klein O.D.[5], Peterka M.[1,2], Peterkova R.[1]
[1]Institute of Experimental Medicine AS CR, v.v.i., Department of Teratology, Prague, Czech Republic, [2]Faculty of Science, Charles University, Department of Anthropology and Human Genetics, Prague, Czech Republic, [3]Faculty of Science, Charles University, Department of Cell Biology, Prague, Czech Republic, [4]INSERM UMR 977 and Dental School, University of Strasbourg, Strasbourg, France, [5]School of Medicine, University of California at San Francisco, Department of Anatomy and Program in Developmental Biology, San Francisco, United States

Background: Mouse dentition consists of one incisor and three molars separated by a toothless gap - diastema, at the place of missing canine and premolars. In mouse embryos, two rudimentary tooth primordia sequentially develop in the premolar region of the diastema at embryonic day 12.5 and 13.5, respectively. They later regress or become incorporated into the first molar. However, just in the presumed premolar position in the diastema, some genetically modified mice show a supernumerary tooth (ST), which is variable in its size and shape. The ST has been proposed to result from a revitalization of a rudimentary premolar anlage in mutant mice. However, it is not known whether only one or both rudimentary tooth primordia can play a role in the ST origin.
Aim: We aimed to characterize the proliferation and apoptosis during development of the two rudimentary tooth primordia (called MS and R2) in the embryonic mandible of WT mice and of the mutant mice that will go on to form a ST.
Methods: We studied the dental epithelium in the cheek region of the mandible in wild type (WT) and mutant (Spry2-/-) mice at embryonic day (ED) 12.5 and 13.5. On series of frontal 7um thick histological sections, we measured the area of the dental epithelium, and counted the number of apoptotic elements and proliferating cells in the region of the rudimentary premolar primordia. The data were compared between WT and mutant embryos, and with the published data on the size, proliferation and apoptosis of the dental epithelium in the R2 bud and molar region at ED13.5 (Peterkova et al, 2009).
Results: First, we demarcated the region of the anterior rudimentary tooth primordium (MS) at ED 12.5. We performed shape and size analysis of the dental epithelium on histological sections. WT and mutant mice showed differences in the shape of dental epithelium on serial histological sections at ED 12.5. But it always remained possible to recognize the morphology characterizing the posterior end of the MS (peMS). We also analyzed the antero-posterior course of the size of the dental epithelium on graphical representations. The curves showed an ascent, peak and descent in the posterior direction. Although the antero-posterior position of the peMS was similar in all mice, the peMS was located at the peak of the Spry2-/-curves, but on the ascendant part of WT curves. The evaluation of the number of mitotic cells and apoptotic elements was made in the MS - on 5 sections located anteriorly to the peMS. The second evaluated region (corresponding to the R2) also comprised 5 sections, and was separated by a gap of 5 sections from the peMS. The number of apoptotic elements in MS and R2 was lower in Spry2-/- than in WT embryos. Furthermore, the mitotic index was higher in both rudimentary tooth primordia in the Spry2-/- mice than in WT at ED12.5. At ED13.5, the mitotic index in the mutant MS decreased and was similar to the MS and R2 of WT mandibles. However, the mitotic index in the mutant R2 stayed increased, being at a level similar to the molar region.
Conclusion: The increase of mitotic index and decrease of apoptosis in the mutant rudimentary tooth primordia suggested a sequential revitalization of the MS at ED12.5 and R2 at ED 13.5.
References: Peterkova et al. (2009). J Exp Zool B Mol Dev Evol 12B: 292-308.
Acknowledgement: The Grant Agency of the CR (grant 304/07/0223) and by the MSMT of the CR (MSM 0021620843).

General Tooth Development

O4

Primary cilia in tooth development

Ghafoor S.[1], Blackburn J.[1], Brugmann S.[2], Helms J.[2], Franco B.[3], Haycraft C.J.[4], Ohazama A.[1], Sharpe P.T.[1]
[1]Dental Institute at Guy's, King's College and St Thomas Hospitals London, Department of Craniofacial Development, London, United Kingdom, [2]Stanford University, Department of Plastic and Reconstructive Surgery, Stanford, United States, [3]Federico II University, Naples, Italy, [4]University of Alabama at Birmingham, Department of Cell Biology, Birmingham, United States

Primary cilia are surface organelles found on most cells in vertebrates that play critical roles in many aspects of biology, including development, and have recently been shown to be implicated in Shh signalling pathway that is also involved in tooth development. The aim of this study is to investigate the role of primary ciliary proteins in tooth development. Mice with mesenchymal conditional mutation of *Polaris* (*Polaris*$^{flox/flox}$*/Wnt1-Cre*) and *Kif3a* (*Kif3a*$^{flox/flox}$*/ Wnt1-Cre*) and *Ofd1* null mutant mice were used in this study. *Polaris*$^{flox/flox}$*/Wnt1-Cre* and *Kif3a*$^{flox/flox}$*/ Wnt1-Cre* mice lacked incisors as a result of down regulation of Shh signalling. *Ofd1* null mutants showed extra incisors. In the diastema and lingual region to the first molar, extra teeth were found in all three mutants, resulting both from ectopic Shh signalling. The action of primary cilia on Shh signalling is thus negative or positive, depending on the tissue context. In addition, different cilia proteins exert different functions on the mechanisms of cilia-directed regulation of Shh activity.

General Tooth Development

O5

Differential expression and functional significance of glucose transporters during murine tooth development

Ida-Yonemochi H.[1], Nakatomi M.[1], Harada H.[2], Ohshima H.[1]
[1]Niigata University Graduate School of Medical and Dental Sciences, Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata, Japan, [2]Iwate Medical University, School of Dentistry, Division of Oral Histology and Developmental Biology, Department of Oral Structure and Function Biology, Morioka, Japan

Background: Glucose is an essential source of energy for mammalian cells and plays an important role for body metabolism, in which the transport of glucose across the cell membrane is accelerated by glucose transporters (GLUTs). To date, thirteen GLUTs have been identified and their tissue-specific expression patterns are suggestive to reflect the various glucose requirements of different tissues. Class I transporter comprises the well-characterized GLUT1-4, and GLUT1 is known to be the major glucose transporter expressed in many tissues and be responsible for fundamental glucose uptakes. GLUT2 is a low affinity isoform with glucose, and GLUT3 and GLUT4 are high affinity isoforms, which are mainly expressed in brain. However, there has been no available data on the expression and the role of GLUTs during tooth development. This study aims to clarify the functional significance of GLUTs during murine tooth development using immunohistochemistry and organ culture experiment.
Methods: ICR mice from embryonic day 13 (E13) to postnatal day 10 (P10) were used in this study. All mice were administered with an intraperitoneal injection of BrdU 2 hrs before the fixation and perfused with 4% paraformaldehyde. Following decalcification in 10% EDTA when necessary, the heads were embedded in paraffin and the frontal or sagittal sections of teeth were cut at 4 μm. Immunohistochemistry was performed using antibodies against GLUT1-4, Ki67 and BrdU. For organ culture experiments, the mandibular molar germs were isolated from E13, E14 and E16 mice, and were cultured for 3, 7, 10 days with the inhibition of GLUTs by phloretin, a glucose transport inhibitor.
Results: An intense GLUT1-immunoreaction was localized in the enamel organ of bud-stage tooth germ (E13) of mandibular first molars as well as the oral epithelium, where the active cell proliferation occurred. By the cap stage (E14), the expression of GLUT1 in the dental epithelial cells was dramatically decreased in intensity, and subsequently began to appear in the stratum intermedium at the bell stage (E19). On the contrary, GLUT2-immunoreactivity was observed in the stellate reticulum and dental papilla throughout all stages. Secretary ameloblasts and odontoblasts also showed immunopositive reaction for GLUT2. The expression of GLUT1 and GLUT2 was not overlapped in the dental epithelial and mesencymal cells during tooth development. With regard to GLUT3 and GLUT4, their immunoreactions were not observed in the tooth germs.
In vitro organ culture experiments, the inhibition of GLUT1/2 by phloretin in the bud-stage tooth germs (E13) induced the developmental arrest of explants at the bud stage and the squamous metaplasia of dental epithelial cells lacking the enamel organ. On the other hand, the development of tooth germs of E14 and E16 was not inhibited by phloretin treatment. Three days after phloretin treatment of E13 tooth germs, cell proliferation was inhibited in the dental epithelial cells and the apoptotic features were observed in the primary enamel knot areas. These results suggest that the disturbance of primary enamel knot formation by lack of glucose uptake into the enamel organ cells is attributed to arrested tooth formation.
Conclusion: The expression of GLUT1 and GLUT2 in the dental epithelial and mesencymal cells seems to be precisely controlled spatiotemporally, and the glucose uptake mediated by GLUT1/2 plays a crucial role in the early tooth morphogenesis.

General Tooth Development

O6

The cadherin-catenin complex during zebrafish tooth development

Verstraeten B.[1], Sanders E.[2], D'heuvaert T.[1], van Hengel J.[2], Huysseune A.[1]
[1]Ghent University, Biology Department, Ghent, Belgium, [2]VIB / Ghent University, Department of Biomedical Molecular Biology, Ghent, Belgium

The development of teeth is the result of interactions between competent mesenchyme and epithelium, both of which undergo extensive morphogenesis. The importance of cell adhesion molecules in morphogenesis has long been acknowledged but remarkably few studies have focused on the distribution and function of these molecules during tooth development.
We analyzed the expression pattern of an important epithelial cadherin, E-cadherin, during the formation of first-generation teeth as well as replacement teeth in the zebrafish, using *in situ* hybridization and whole mount immunostaining to reveal mRNA expression and protein distribution. The uninterrupted expression of E-cadherin in the epithelial-derived part of the tooth indicates that downregulation of E-cadherin is not required for formation of an epithelial tooth bud.
In order to identify the intracellular partners in the adhesion complex, we are now embarking on a study of the expression pattern and distribution of several cadherin-associated molecules like p120catenin, β-catenin and plakoglobin and their role during the renewal of teeth.

General Tooth Development

O7

Advances in understanding abnormalities of tooth number

Nieminen P.[1], Arte S.[1,2]
[1]*University of Helsinki, Institute of Dentistry, Helsinki, Finland,* [2]*Helsinki University Central Hospital, Dept of Oral and Maxillofacial Diseases and Surgery, Helsinki, Finland*

The list of identified genes behind abnormal tooth number - either tooth agenesis or supernumerary teeth - is growing steadily. These genes involve all major signaling pathways or their downstream intracellular effectors. In humans, mutations have been identified mostly for syndromic tooth agenesis or supernumerary teeth and dominantly inherited isolated severe tooth agenesis. Recently apparently hypomorphic mutations in *EDA* and *WNT10A* have been associated with isolated tooth agenesis which is inherited in non-dominant manner. The identified mutations are often associated with strikingly characteristic phenotypes. Heterozygous mutations in *MSX1* and *PAX9* affect especially the posterior teeth, whereas anterior teeth are susceptible for impaired EDA signaling as shown in female EDA carriers and patients with hypomorphic *EDA* mutations. It is expected that hypomorphic mutations will also be found in other genes already associated with syndromic tooth agenesis. These may also shed light on the common types of tooth agenesis, incisor and premolar hypodontia and third molar agenesis. However, these candidate gene-based approaches must be complemented with whole genome searches which are able to reveal so far unknown connections to tooth development. We are currently performing both genome-wide and candidate gene-based approaches on tooth agenesis and supernumerary teeth. The results from this work including novel mutations will be presented and discussed.

General Tooth Development

O8

Eat it all: Tooth resorption in teleost fish

Witten P.E.[1], Huysseune A.[1]
[1]*Ghent University, Biology Department, Ghent, Belgium*

Teleost fish, like other non-mammalian vertebrates, replace lost teeth in a particular position by new teeth throughout life. Repeated tooth development requires bone and tooth resorption:
(a) to remove all or part of the old tooth,
(b) to move the successor tooth into the position of its predecessor, and
(c) to prepare the bone surface to allow the new tooth to attach. Advanced teleosts must also resorb bone
(d) for the downgrowth of the successional dental lamina into the medullary cavity of the jaw bone, and
(e) to create space for the growing tooth as it works its way through the jaw bone.
Remarkably, some teleosts, such as Atlantic salmon (*Salmo salar*), do not shed their teeth. Teeth are resorbed inside the oral mucosa likely to retain minerals (phosphorus), similar to mineral metabolism triggered scale resorption. Teleosts may also retain dentin and incorporate it into the jaw bone. Neither resorption nor preservation of dentin occurs in sharks (Chondrichthyans). Very different from teleosts, sharks shed entire tooth units (tooth + bony basal plate = odontode). Odontoclasts, osteoclasts or macrophages are not involved in this process. In teleosts the morphology of odontoclasts follows the osteoclast morphology. Basal teleosts with osteocyte-containing bone display mainly multinucleated odontoclasts, advanced teleosts with acellular bone display many mono- and oligonucleated odontoclasts. In Atlantic salmon, teeth are resorbed from inside the pulp cavity and from outside. Resorption from outside is clearly connected to - and likely mechanically triggered by - the growing replacement tooth. Indeed, studies on rodents have shown that the dental follicle, the dental epithelium, and the stellate reticulum release factors that regulate osteoclast activities such as CSF-1, RANK-L, OPG, TGF-b, and interleukin-1a (Il-1a) (reviewed by Witten & Huysseune 2009). Whether resorption from inside the pulp cavity is correlated to the outside-resorption, and how much the process depends on vascularization and innervation of the pulp cavity are questions that we address in our current studies.
Witten, PE & Huysseune, A, 2009. *Biol Rev* 84: 315-346

Stem Cells And Signalling

O9

Purification and characterization of human dental epithelial stem cells

Athanassiou-Papaefthymiou M.[1], Papagerakis S.[2], Mitsiadis T.[3], Ma P.[4], Honda M.[5], Papagerakis P.[1]
[1]University of Michigan, Orthodontics and Pediatric Dentistry, Ann Arbor, United States, [2]University of Michigan, Otolaryngology, Ann Arbor, United States, [3]University of Zurich, Zurich, Switzerland, [4]University of Michigan, Biological and Materials Science, Ann Arbor, United States, [5]Nihon University School of Dentistry, Tokyo, Japan

Background: Mesenchymal stem cells, which give rise to dentin, bone, and cementum, have been isolated and are being studied extensively in the context of tissue regeneration. However, no studies exist on the regenerative potential of epithelial stem cells that could lead to enamel reconstruction. This missing gap is critical because dental enamel, in contrast to bone and dentin, cannot regenerate. Furthermore, without cells that can give rise to ameloblasts complete biological restoration of human dental crown can not be achieved.
Our objective is to identify and characterize epithelial stem cells that can be differentiated to enamel producing cells. Our hypothesis is that human epithelial cell rests of Malassez (hERM), the only remaining dental-origin epithelial cells after tooth eruption, found in the periodontal ligament (PDL) of all teeth, have stem cell properties and can be isolated, expanded and induced to form enamel in vivo.
Methods: hERM cells were clonally isolated from PDL using cell surface markers and flow cytometry and were expanded through inhibition of anoikis and manipulation of cell proliferation pathways. hERM clones were tested for self-renewal and karyotype stability, for the expression of stem-cell markers using immunofluorescence microscopy and PCR arrays and for their ability to express enamel specific proteins upon differentiation induction using ameloblast lineage molecular determinants and/or co-culture and in vivo co-seeding systems with dental pulp stem cells (DPSC).
Results: We report a detailed protocol for the sorting and propagation of single live hERM expressing stem cell markers integrin-alpha6, Notch-1 and CD34. We also show evidence that hERM clones express the epithelial stem cell super-marker LGR5 and share genetic programs with other stem and progenitor epithelial cells suggesting that hERM have stem cell like properties. We are also able to expand hERM in vitro and to induce differentiation of hERM into cells that express ameloblast phenotypic markers and produce enamel-like matrix.
Conclusion: The isolation and characterization of hERM stem cells should help elucidate the molecular pathways that govern ameloblast lineage commitment and enamel tissue development. We are currently exploring combinations of epithelial and mesenchymal stem cells, biodegradable scaffolds and gene delivery systems to regenerate human enamel and dentin. These studies lay the foundation for innovative regenerative treatments in patients with defective or missing dental tissues.

Stem Cells And Signalling

O10

MicroRNA regulated tooth epithelial stem cell differentiation: Specific MicroRNAs target BMP, Wnt and chromatin remodeling complexes

Cao H.[1], Wang J.[1], Li X.[1], Florez S.[1], Venugopalan S.[1], Amendt B.A.[1]
[1]*Texas A&M Health Science Center, CEGM, Houston, United States*

Background: MicroRNAs are known to regulate gene function in many tissues and organs and in this study we investigate the functions of specific microRNAs in tooth development.
Methods: *Pitx2, K-14* and *Wnt-1 Cre's* were used to delete mature microRNAs in *Dicer1 floxed* mouse models. Transgenic mice are used to understand the effect of specific microRNAs on tooth and craniofacial development. Molecular and biochemical assays reveal molecular mechanisms of specific microRNAs.
Results: We have identified discrete sets of mircoRNAs expressed in molars compared to incisors as well as epithelium compared to mesenchyme at various stages of tooth development. Conditional knockout (cKO) of *Dicer1* (mature microRNAs) in the dental epithelium using the *Pitx2 Cre* mice results in multiple and branched enamel-free incisors and cusp-less molars. Analyses of differentiating dental epithelial markers reveal a defect in ameloblast differentiation. Conversely, the cervical loop (stem cell niche) is expanded in *Dicer1* cKO. Epithelial microRNAs control dental stem cell differentiation demonstrating a unique role for microRNAs in regulating in vivo dental stem cell biology. Noggin, a potent BMP inhibitor, Lef-1 an essential transcription factor and HMG-17 a chromatin-associated remodeling factor are up-regulated in the *Pitx2 Cre-Dicer1* knockout mouse. We have identified specific microRNAs that target these genes in vitro and in vivo and are associated with the tooth/craniofacial anomalies and cell differentiation defects.
Conclusions: These results demonstrate a critical role for microRNAs in regulating tooth and craniofacial development. Furthermore, we demonstrate microRNA regulation of Noggin/BMP signaling, Lef-1/Wnt signaling and chromatin remodeling during tooth/craniofacial development.
Support for this research was provided from grant DE13941 from the National Institute of Dental and Craniofacial Research.

Stem Cells And Signalling

O11

Development of tooth cusps in the gecko *Paroedura picta*: Cusp generation without an enamel knot

Zahradnicek O.[1], Horacek I.[1], Tucker A.[2]
[1]*Charles University, Department of Zoology, Prague, Czech Republic,* [2]*King's College, Guy's Hospital, Department of Craniofacial Development, London, United Kingdom*

Background: The evolution of tooth cusps has played a significant role in the radiation of the vertebrates. The molecular basis of cusp development is relatively well known in mammals, where cusp development is directed by the enamel knots, transient signalling centre which direct the growth and shape of inner enamel epithelium (IEE). In contrast to mammals, reptiles do not show any evidence of enamel knots. This poses the question, what mechanism directs cusp formation in this amniotic group and does this mechanism represent a pre-adaptation for mammalian cusp formation? To address this question we have investigated cusp development in a gecko, *Paroedura picta,* which has bi-cuspid teeth. The expression pattern of growth factors that play a role in directing cusp development in the mouse was followed in this reptile, with particular emphasis on Sonic Hedgehog (Shh). The expression and role of Shh has previously been described in unicuspid snake teeth, but its role in formation of bicuspid teeth has not been analysed.
Methods: Embryos of *P. picta* at different stages were analysed. Shh was detected by *DIG in situ* hybridization. Cell proliferation and programmed cell death were detected by PCNA and TUNEL respectively on serial sections. Fibronectin, a component of the extracellular matrix of the stellate reticulum, was also assessed. Trichrome staining was used for the basic histological observations.
Results: At the bell stage*, Shh* expression was localized in the apical zone of the IEE, prior to the cells differentiating into ameloblasts. At this point in development the tooth has a unicuspid morphology. As the apical cells mature, they enlarge. Cell proliferation was not observed in this cluster of cells. At this stage the stellate reticulum (SR) is almost free of fibronectin in the apical area, where it has a relatively solid cellular character. In contrast, the lateral sides of the SR contain a high density of fibronectin. The mesenchyme of the dental papilla is also full of fibronectin. Enlargement of the IEE cells of the apical complex results in the cells physically pushing into the dental papilla. The basic bicuspid shape is formed through this process. The shape is later fixed by the deposition of dentin. *Shh* expression in the apical ameloblasts disappears after the first signs of mineralization and the signal spreads along the differentiating cells of the IEE in the direction of the base of the tooth. The final shape of the cusps is re-enforced by the deposition of enamel. Proliferating cells, which direct the outgrowth of the tooth germ, were present in the non-differentiating area of the IEE and in the cervical loops from the bell stage until ankylosis. Apoptosis was detected in the apical zone of the SR during the mineralization stage, which may be connected with SR disintegration before tooth eruption. It would therefore appear that, unlike in the mouse, cell proliferation and apoptosis do not play significant roles in cusps formation.
Conclusions: Our studies indicate that tooth cusps develop in reptiles by a completely different from that reported in mammals. We saw no signs of any pre-adaptations that could have lead to the mechanism of mammalian cusp development. We plan to examine the expression patterns and functions of a number of other signalling factors (Fgfs, Bmps, Wnts) in the gecko in order to confirm this hypothesis.
This research was supported by GA AV CR (grant KJB601110910).

Stem Cells And Signalling

O12

Ring1a/b polycomb proteins regulate the mesenchymal stem cell niche in continuously growing incisors

Lapthanasupkul P.[1], Feng J.[1], Mantesso A.[1], Miletich I.[1], Takada-Horisawa Y.[2], Koseki H.[2], Vidal M.[3]
[1]Kings College London, Craniofacial Development, London, United Kingdom, [2]RIKEN, Centre for Allergy and Immunology, Yokohama, Japan, [3]Centro de Investigatciones Biologicas, Madrid, Spain

Background: Rodent incisors are capable of growing continuously. The renewal of dental epithelium giving rise to enamel-forming ameloblasts and dental mesenchyme giving rise to dentin-forming odontoblasts and pulp cells is achieved by stem cells residing at their proximal ends. Although the dental epithelial stem cell niche (cervical loop) is well characterized, nothing is known about the dental mesenchymal stem cell niche. Ring1a/b are the core Polycomb repressive complex1 (PRC1) components that have recently also been found in the protein complex with BCoR (Bcl-6 interacting corepressor) and FbxI10.
Methods: Expression of PRC1 components was mapped using in situ hybridization and conditional postnatal gene activation using tamoxifen-inducible Cre was used to effects of loss of gene function on incisor growth.
Results: During mouse incisor development, we found that genes encoding members of the BCoR/Ring1a/b complex are strongly expressed in the incisor apical mesenchyme. Analysis of *Ring1a$^{-/-}$;Ring1b$^{fl/fl}$* cre+ mice showed that loss of Ring1a/b postnatally results in defective cervical loop and disturbances of enamel and dentin formation in continuously growing incisors. To further characterize the defect found in *Ring1a$^{-/-}$;Ring1b$^{fl/fl}$* cre+ mice, we demonstrated that cell proliferation is dramatically reduced in mesenchyme and cervical loop epithelium of *Ring1a$^{-/-}$;Ring1b$^{fl/fl}$* cre+ incisors in comparison to *Ring1a$^{-/-}$;Ring1b$^{fl/fl}$* cre- incisors. Fgf signaling and downstream targets which have been previously shown to be important in the maintenance of the dental epithelial stem cell compartment in the cervical loop are downregulated in *Ring1a$^{-/-}$;Ring1b$^{fl/fl}$* cre+ incisors. In addition, expression of other genes of the BCoR/Ring1a/b complex is also altered.
Conclusions: These results suggest that the BCoR/Ring1a/b complex regulates the dental mesenchymal stem cell compartment and cell differentiation in developing mouse incisors. This is further supported by experiments showing that cells from this compartment are capable of directional migration in response to tooth damage and differentiation into odontoblast-like cells.

Stem Cells And Signalling

O13

Essential role of mesenchymal Notch-RBP-Jkappa signaling in controlling dental epithelial cell fate during mouse tooth development

Hu B.[1], Qiu W.[1], Dotto G.P.[1,2]
[1]*University of Lausanne, Department of Biochemistry, Epalinges, Switzerland,* [2]*Cutaneous Biology Research Center Massachusetts General Hospital and Harvard Medical School, Charlestown, United States*

Background: The tooth is a well-established model of epithelial-mesenchymal interactions, which controls histogenesis, morphogenesis, and cell differentiation during tooth development. Notch signaling is important in cell-cell communication and plays a key role in the control of cell fate, stem cell potential and differentiation. The best characterized “canonical” pathway of Notch activation involves translocation of NICD to the nucleus, where it associates with the DNA binding protein RBP-Jkappa (CBF-1 or CSL), converting it from a repressor into an activator of transcription. While attention have been already paid to the role of Notch signaling in the epithelial and mesenchymal compartments during tooth development, the role of this pathway in mediating mesenchymal-epithelial interactions has not been fully investigated.
Methods: In this study, we applied Cre-loxP system for targeting disruption of RBP-Jkappa, the key effector of “canonical” Notch pathway, in the mesenchymal compartments of the developing teeth. Transgenic mice with Cre transgene expression driven by the promoter of the collagen type I (a2 chain) gene were crossed with mice with the RBP-Jk gene flanked by loxP sites. The Cre activity was evaluated by further crossing with mice of ROSA26R background. Histogenesis, morphogenesis as well as the differentiation of the teeth in the mice with RBP-Jkappa deletion were evaluated by using different approaches. The target genes, were analyzed by real time RT-PCR, and confirmed by western blotting and/or immunohistomchemistry. To study whether the observed phenotype is intrinsic or secondary to systemic alterations resulting from the RBP-Jk deletion. To validate the *in vivo* gene regulation results, we applied adeno Cre virus in the cultured mouse dental mesenchymal cells to achieve acute deletion of RBP-Jkappa gene *in vitro*. To assess the functions of the relative signaling molecules, functional analysis was performed by applying recombinant proteins and/or chemical compound in the cultured tooth germs. Finally the direct down-stream targets of the RBP-Jkappa gene were confirmed by using chromatin IP.
Results and conclusion: The deletion of RBP-Jkappa, in the mesenchyme, validated by different techniques, resulted in smaller tooth size, with no apparent changes of crown shape and cusp numbers. The reduced tooth crown size was due to a decrease of epithelial cell proliferation of stratum intermedium cells. In the incisor teeth, an enlarged apical end could also be identified, which suggested a possible expended stem cell pool/niche. Similar observations in dental epithelial cells were also achieved in the cultured tooth germs, suggesting an intrinsic effect of losing RBP-Jkappa in the dental mesencymal cells. Molecular profiling indicated a common role of RBP-Jkappa, in regulating several key pathways involving in WNT, FGF and BMP signaling, in the mesenchymal cells including dental mesenchyme/pulp, dental sac, as well as surrounding bone tissues. Functional analysis suggests these signaling molecules could mediate the dental epithelial cell proliferation as well as differentiation. Thus our results suggest a novel role of Notch-RBP-Jkappa signaling is a key player in the mesenchyme compartment of a developing tooth in coordinating mesenchymal-epithelial interactions.

Stem Cells And Signalling

O14

The interaction between Wnt, Shh and Sostdc1 governs the spatial patterning of teeth

Cho S.-W.[1], Kwak S.[1], Lee M.-J.[1], Baker R.E.[2], Maini P.K.[2], Jung H.-S.[1]
[1]*Yonsei University College of Dentistry, Department of Oral Biology, Seoul, Korea, Republic of,* [2]*Mathematical Institute, University of Oxford, Centre for Mathematical Biology, Oxford, United Kingdom*

Each vertebrate species displays specific tooth patterns in each quadrant of the jaw: the mouse has one incisor and three molars, which develop at precise locations and at different times. Sonic hedgehog (Shh) is involved in pattern formation of tooth and other ectodermal organs such as limb, hair, mammary gland, and feather by controlling proliferation and differentiation of cells. It has been reported that Shh in tooth development induces cell proliferation and increases cell polarity and cell differentiation. Here, by utilizing maternal transfer of 5E1 (anti-Shh antibody) through the placenta, we found the fusion between first and second molar or the fusion between second and third molar depending on the embryonic stages when 5E1 was injected. In addition to Shh, Wnt and Sostdc1 are also key signaling molecules involved in the spatial patterning of teeth and other ectodermal organs. In this study, we also found the close interactions between Wnt, Shh and Sostdc1 governing the spatial patterning in teeth and proposed a new reaction-diffusion model confirming the interactions do lead to patterning consistent with tooth patterning of wild type mouse.

Stem Cells And Signalling

O15

Hedgehog signaling directs generation of progeny from adult stem cells in the continuously growing mouse incisor

Seidel K.[1], Ahn C.P.[2], Lyons D.[1], Nee A.[1], Ting K.[1], Brownell I.[3], Cao T.[4], Carano R.A.D.[4], Curran T.[5], Schober M.[6], Fuchs E.[6], Joyner A.[3], Martin G.R.[7], de Sauvage F.J.[2], Klein O.D.[1]
[1]University of California, San Francisco, Departments of Orofacial Sciences and Pediatrics and Program in Craniofacial and Mesenchymal Biology, San Francisco, United States, [2]Genentech Inc., Department of Molecular Biology, South San Francisco, United States, [3]Sloan-Kettering Institute, Developmental Biology Program, New York, United States, [4]Genentech Inc., Biomedical Imaging Group, Department of Tumor Biology and Angiogenesis, South San Francisco, United States, [5]The Children's Hospital of Philadelphia, Department of Pathology and Laboratory Medicine, The Joseph Stokes Jr. Research Institute, Philadelphia, United States, [6]Rockefeller University, Howard Hughes Medical Institute, Laboratory of Mammalian Cell Biology and Development, New York, United States, [7]University of California, San Francisco, Department of Anatomy and Program in Developmental Biology, San Francisco, United States

Background: The mouse incisor, unlike human teeth, grows continuously throughout the life of the animal. This constant growth requires the presence of both epithelial and mesenchymal stem cells that have the capacity to self-renew as well as to differentiate into enamel-producing ameloblasts, dentin-producing odontoblasts, and other cell types. Sonic Hedgehog (SHH) is expressed at multiple stages of tooth development, and Hedgehog (Hh) signaling has been proposed to regulate stem cells in several systems, including the adult brain. Here, we describe the role of Hh signaling in regulation of adult stem cells in the postnatal incisor. We show that SHH is a critical regulator of continuous incisor growth that directs progeny formation from stem cells.
Methods: K5tTA;H2B-GFP mice or BrdU-injected wildtype mice were used to visualize slowly cycling, label retaining cells (LRCs). Expression of Hh pathway members and cell type specific markers was detected using immunofluorescence, mRNA *in situ* hybridization, X-Gal staining or qRT-PCR. Genetic lineage tracing in Gli1CreER^{T2};R26R mice was used to identify Hh-responsive stem cells and to follow the lineage of their progeny *in vivo*. Effects of a Hh pathway inhibitor were studied using micro-CT, histological and marker analyses as well as cell proliferation assays and lineage tracing.
Results: LRCs were present exclusively in the posterior parts of both labial and lingual cervical loops and in the proximal incisor mesenchyme between the loops. *Shh* is expressed in pre-ameloblasts and ameloblasts, and expression analyses showed that it is the principal Hh ligand gene in the incisor. Using *Gli1^{lacZ}* and *Ptch1^{lacZ}* reporter mice, we found that high level Hh signaling is received specifically in those regions containing LRCs. The presence of double-labeled cells that were both BrdU label-retaining and *Gli1^{lacZ}* positive indicated that some of the putative incisor stem cells are Hh-responsive. Lineage tracing of *Gli1*-expressing cells confirmed that a subset of Hh-responsive cells are indeed stem cells in the mouse incisor that can give rise to progeny over extended periods of time. Furthermore, antagonizing Hh signaling using a small molecule inhibitor of Smoothened resulted in perturbed morphology and function of differentiated cell types, with ameloblasts being particularly affected. Interestingly, LRCs were not depleted after antagonizing Hh signaling for one month and the effects of the inhibitor were reversible. In addition, lineage tracing of Hh-responsive cells in inhibitor-treated mice indicated that antagonizing Hh signaling prevents generation of progeny that contribute to the ameloblast lineage. Significantly, after 14 days of antagonist treatment, proliferating cells in the transit-amplifying (T-A) region of the labial cervical loop were present only in one layer and stratum intermedium cells were still being generated from these T-A cells.
Conclusions: We showed that SHH signals to three stem cell niches in the incisor that contain Hh- responsive and Hh-non-responsive stem cells. Hh-responsive stem cells in the labial cervical loop are the progenitors of SHH secreting cells, thus, a positive feedback mechanism is in place between the stem cells and their progeny. Our data further suggest that Hh signaling is not required for stem cell survival but instead strongly support a role for Hh signaling in controlling generation of specific progeny- the ameloblasts- from epithelial stem cells.

Stem Cells And Signalling

O16

***Evc* regulates symmetric and asymmetric responses to Shh signalling in tooth development**

Nakatomi M.[1], Hovorakova M.[2], Gritli-Linde A.[3], Blair H.[1], Mc Arthur K.[1], Peterka M.[2], Lesot H.[4], Peterkova R.[2], Goodship J.[1], Peters H.[1]

[1]Newcastle University, Institute of Human Genetics, Newcastle upon Tyne, United Kingdom, [2]Institute of Experimental Medicine, Department of Teratology, Prague, Czech Republic, [3]University of Gothenburg, Department of Oral Biochemistry, Gothenburg, Sweden, [4]INSERM and University of Louis Pasteur, Strasbourg, France

Ellis-van Creveld (EvC) syndrome is an autosomal recessive disorder caused by mutations in *EVC* or *EVC2*. Both genes encode proteins that locate to the primary cilium, which is required for hedgehog signalling in mammals. Oral anomalies of EvC syndrome patients include multiple frenula, missing teeth and abnormal tooth morphogenesis. Consistent with a role for hedgehog signalling, we found an impaired response to Shh in the developing dentition of homozygous *Evc* mutant embryos. Remarkably, in the developing first molars of *Evc* mutants, the Shh pathway was transiently up-regulated at the lingual side but was subsequently inactive specifically at the buccal side, the latter associated with a severe, unilateral growth defect and a loss of the bucco-lingual symmetry. Unlike molars, the incisors of rodents deposit enamel only on the labial side and thus exhibit a highly asymmetric architecture along the oral-aboral axis. Paradoxically, in the lower incisors of *Evc* mutant mice this asymmetry is partly abrogated, a defect that is associated with an ectopic response to Shh signalling at the lingual cervical loop, while the response at the labial cervical loop is down-regulated. The results identified *Evc* as an important component of the spatio-temporal co-ordination of Shh pathway activities during tooth development. These modulations appear to contribute to the establishment of tooth-type specific symmetries and asymmetries along the oral-aboral axis of the mammalian dentition.

Stem Cells And Signalling

O17

Downstream targets of Ectodysplasin in developing teeth

Thesleff I.[1]
[1]*Institute of Biotechnology, University of Helsinki, Helsinki, Finland*

Ectodysplasin (Eda) is a signalling molecule which belongs to the Tumor Necrosis Factor family and is necessary for the normal development of teeth and other organs developing as appendages of the ectoderm. Mutations in the Eda pathway cause the hypohidrotic ectodermal dysplasia syndrome in humans and similar phenotypes in various animal models. Eda binds to its receptor Edar and the signalling is mediated via the NFκB transcription factor. In the teeth, the Eda pathway regulates the function of the epithelial signalling centers including the dental placode, as well as the primary and secondary enamel knots. We have searched for downstream target genes of the Eda pathway by microarray analysis of embryonic Eda mutant epithelium exposed to Eda protein. The results indicate that Ectodysplasin signalling regulates the expression of multiple genes which encode components of the FGF, BMP, Wnt as well as Hedgehog pathways. We conclude that Ectodysplasin functions as a modulator of all major developmental signalling pathways.

Stem Cells And Signalling

O18

Diverse roles of Lrp4 in craniofacial ectodermal organ development

Porntaveetus T.[1], Herz J.[2], Gritli-Linde A.[3], Basson M.A.[1], Sharpe P.T.[1], Ohazama A.[1]
[1]King's College London, Department of Craniofacial Development, London, United Kingdom, [2]UT Southwestern Medical Center, Department of Molecular Genetics, Dallas, United States, [3]Sahlgrenska Academy at Göteborg University, Department of Oral Biochemistry, Göteborg, Sweden

It has been known that ectodermal organs share common signaling pathways including Wnt, Shh, Bmp, and Fgf in their development and these pathways are functionally integrated in many biological processes. The precise mechanisms of the integration however are still unclear.
Lrp4 belongs to the low-density lipoprotein (LDL) receptor family which is a large evolutionarily conserved group of transmembrane proteins. Recent findings have shown that LDL receptor family members can also function as direct signal transducers or modulators for a broad range of cellular signaling pathways. *Lrp4* is expressed in the development of multiple craniofacial ectodermal organs including hair, tooth, palate, and tongue papillae.
Lrp4 mutant mice show fused molars and diastema teeth. Upregulation of Fgf, Bmp, and canonical Wnt signaling accompanied by changes in Shh activity are observed in the *Lrp4* mutant teeth. Molar fusion and/or diastema teeth are also found in mice with a mutation of *Wise* (a secreted BMP antagonist and Wnt modulator), *Sprouty2* (a negative feedback regulator of Fgf), and *Shh*[(1-3)].
Palatal rugae, corrugated structures on the hard palate, are also believed to develop through reciprocal epithelial-mesenchymal interactions similar to tooth development. *Lrp4* mutants present with disorganized palatal rugae. The palatal rugae of *Lrp4* mutants show upregulation of Fgf signaling and downregulation of Shh. Unlike tooth development, no changes in Bmp or canonical Wnt signaling are detected in developing palatal rugae. *Sprouty1/2* and *Shh* mutant mice also show similar palatal rugae deformities.
The data identify different molecular changes in tooth and palatal rugae development in *Lrp4* mutants and indicate that *Lrp4* regulates diverse signaling networks depending on the tissue context.

References:

1) Kassai et al. (2005) Regulation of mammalian tooth cusp patterning by ectodin. Science 309: 2067-2070.
2) Klein et al. (2006) Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. Dev Cell 11: 181-190.
3) Ohazama et al. (2008) Lrp4 modulates extracellular integration of cell signaling pathways in development. PLoS ONE 3: e4092.

Stem Cells And Signalling

O19

Amelogenin and RANK pathway in root and bone phenotype of the Msx2 null mutant mouse

Castaneda B.[1], Descroix V.[1], Molla M.[1], Hotton D.[1], Mueller C.[2], Lézot F.[1], Berdal A.[1]
[1]INSERM UMRS 872 Paris-Diderot University, Molecular Oral Physiopathology, Paris, France, [2]CNRS, UPR 9021, University of Strasbourg, Laboratory of Therapeutic Immunology and Chemistry, IBMC, Strasbourg, France

Background: The Msx2 null mutant mouse (Msx2-/-) presents a severe and complex phenotype. Msx2 -/- combines amelogenesis and dentinogenesis imperfecta and hypercementosis. In addition, regional RANKL down-regulation was suggested to be responsible for tooth eruption impairment associated to an alveolar osteopetrosis. Epithelial Malassez rests were hypothetised to be instrumental in this process. The aim of the present study was to analyse amelogenin and the impact of RANK pathway in this Msx2-/- radicular and alveolar phenotype.
Methods: Wild type, Msx2+/- and Msx2-/- mice were studied from birth until 3 months. Regarding RANK pathway implication, Msx2-/- mouse line was mated with a transgenic mouse line over-expressing RANK in the monocyte precursors (RTg) in order to compensate the RANKL expression decrease. Their root and bone phenotype was compared to single Msx2-/- mutant-ones. In situ hybridization, tartrate-resistant acid phosphatase TRAP-histoenzymology, immuno-histochemistry, histomorphometry and real-time PCR on RNA extracted from micro-dissected tissues were performed.
Results: Amelogenin expression was increased in pathological root epithelial Malassez rests of Msx2-/- mouse which continuously enlarged. RANK overexpression induces increased TRAP+ osteoclast number. In Msx2 RTg mouse, osteopetrosis was rescued as well as tooth eruption. Root elongation timing was normalized but the final root length remained similar to single Msx2-/- mouse. Interestingly, hypertrophic rests of Malassez seen in Msx2-/- mouse were transformed into massive epithelial cysts located within wide resorption lacunae in Msx2-/- RTg mouse.
Conclusions: Amelogenin expression level was shown to be Msx2-dependent in epithetial Malassez rests. Increased amelogenin levels in Msx2 -/- would inhibit osteoclast RANK pathway, as reversely documented in amelogenin -/- mouse line. The Msx2-/- RTg mouse analysis validates that RANKL expression decrease in Msx2-/- mouse is instrumental in the regional osteopetrosis. In addition, the data outlines the fact that such increased osteoresorption would stimulate growth of odontogenic epithelial cysts.

Stem Cells And Signalling

O20

The role of *Pkd2* in tooth development

Khonsari R.H.[1], Raouf R.[2], Wood J.N.[2], Ohazama A.[1], Sharpe P.T.[1]
[1]*Dental Institute, Department of Craniofacial Development, King's College London, London, United Kingdom,*
[2]*Molecular Nociception Group, Wolfson Institute for Biomedical Research, University College London, London, United Kingdom*

Pkd2 (encoding polycystin-2 or TRPP2) is a member of the transient receptor potential superfamily of cation channels. *Pkd2* has been implicated in various biological functions including cell proliferation, sperm fertilization, mechanoreception and asymmetric gene expression. The role of *Pkd2* in tooth development remains largely unknown.
We carried out expression analysis of *Pkd2* in tooth development as a starting point. *Pkd2* is expressed ubiquitously at early stages of tooth development and shows expression in developing tooth pulp and periodontal ligament at post-natal stages.
In order to investigate the role of *Pkd2* in tooth development, we examined mice with mesenchymal conditional *Pkd2* deletion (*Pkd2*$^{flox/flox}$*/Wnt1-Cre*). *Pkd2* mutant mice showed fractures of the middle portion of molar roots and thin roots at apical portion. Calcified structures were found in mutant molar pulp and blood vessels were abnormally expanded in mutant incisor pulp. *Pkd2* mutant mice also have multiple premature suture fusions, cleft palate and temporomandibular joint ankylosis.
The results suggest that *Pkd2* is involved in molar root and pulp development.

Stem Cells And Signalling

O21

A genetic pathway involving *Msx1*, *Bmp4* and *Pax9* connects growth and morphogenesis during upper lip development

Nakatomi M.[1], Peters H.[1]
[1]*Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom*

Background: Cleft lip (CL) is among the most frequent craniofacial birth defects and may result from a disrupted regulation of growth and morphogenesis of the MNP (medial nasal process) and MxP (maxillary process), which ultimately prevents their fusion. The multifactorial nature of CL formation suggests the importance of gene-gene interactions that co-ordinate MNP and MxP development; however, these interactions are poorly defined at present. We previously found that unilateral or bilateral CL manifest in a significant proportion of newborn *Pax9*$^{-/-}$;*Msx1*$^{-/-}$ mutant mice, but not in single homozygous mutants [1]. We therefore aim to understand how *Pax9*- and *Msx1*-regulated developmental pathways interact with each other and how these interactions affect upper lip formation.
Methods: The generation of *Pax9* [2] and *Msx1* [3] mutant mouse lines have been reported. Scanning electron microscopy (SEM) and *in situ* hybridisation were carried out as previously described [1]. Facial primordia at E10.5 were dissected for organ culture (Trowell's standard method) and incubated with implanted beads soaked in human BMP4 protein.
Results: During the early phase of upper lip development (E10.5 - E12.5) *Pax9* was mainly expressed in the mesenchyme of the MNP and LNP (lateral nasal process) surrounding the future nasal cavity, whereas expression of *Msx1*, *Msx2* and *Bmp4* was observed in overlapping patterns in the fusing regions of the MNP, LNP and MxP. SEM analyses at E11.5 demonstrated a hypoplastic MNP but also a clearly pronounced curvature of the MNP towards the MxP in *Msx1* mutants. In contrast, while MNP size was not affected in *Pax9* mutants, the curvature of the MNP was measurably impaired. These results indicate that the combination of a growth defect (caused by *Msx1*-deficiency) with a morphogenesis defect (caused by *Pax9*-deficiency) is the major reason for CL formation in double homozygous mutants. Moreover, the *Pax9*-dependent, pronounced curvature of the MNP in *Msx1* mutants suggests that this phenomenon might involve changes of *Pax9* expression. Indeed, we not only found spatially expanded *Pax9* expression in the MNP of *Msx1*$^{-/-}$ embryos but also down-regulated *Bmp4* expression in this region. Consistent with an important role of Bmp4 to regulate *Pax9* expression patterns, BMP4 soaked beads were sufficient to suppress *Pax9* expression in the MNP in organ culture experiments.
Conclusions: Our results identified *Msx1*, *Bmp4* and *Pax9* as components of a genetic pathway that connects MNP growth with MNP morphogenesis. The *Pax9*-dependent, enhanced curvature of the MNP in *Msx1*$^{-/-}$ embryos compensates for the MNP hypoplasia to allow sufficient contact between the MNP and MxP during a critical time-window of upper lip development. Impairment of this developmental robustness may underlie some sporadic CL cases that are occasionally seen in human oligodontia patients with *MSX1* mutations.
References:
[1] Nakatomi *et al*. (2010) Genetic interactions between *Pax9* and *Msx1* regulate lip development and several stages of tooth morphogenesis. *Dev Biol*. in press
[2] Peters *et al*. (1998) *Pax9*-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev*. 12: 2735-2747.
[3] Satokata and Maas (1994) *Msx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet*. 6: 348-356.

Stem Cells And Signalling

O22

Molecular regulations of supernumerary tooth formation

Wang X.-P.[1], Qiu Z.-F.[1], Ho J.[2], Kiezun A.[3], O'Connell D.J.[3], Lund J.J.[3], Saadi I.[3], Kuraguchi M.[3], Turbe-Doan A.[3], Kucherlapati R.[3], Park P.[2], Maas R.[3]

[1]Harvard School of Dental Medicine, Department of Developmental Biology, Boston, United States, [2]Boston Children's Hospital, Informatics Program, Boston, United States, [3]Brigham and Women's Hospital, Department of Medicine, Boston, United States

Teeth are excellent models for studying organogenesis and regenerations. Apc is an inhibitor of canonical Wnt signaling. We showed that Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood. Even adult oral tissues are still responsive to loss of Apc or activation of canonical Wnt signaling, and are able to form supernumerary teeth. These supernumerary teeth can form from multiple regions of the jaw. Some more mature developed supernumerary teeth are properly mineralized, vascularized, innervated, and even start to form roots, thus may function as nature teeth. Moreover, only a small number of *Apc*-deficient cells are sufficient to induce surrounding wild type epithelial and mesenchymal cells to participate in the formation of new teeth. Strikingly, *Msx1*, which is necessary for endogenous tooth development, is dispensable for supernumerary tooth formation, highlighting the similarities and differences between endogenous and supernumerary tooth development. By comparison of differentially expressed genes in Apc loss-of-function and b-catenin gain-of-function mouse tooth germs, we identified a number of Wnt/b-catenin target genes that may be responsible for the initiation of new tooth formation. Knowledge of exactly how these genes fit within the gene regulatory network will contribute to our understanding of tooth development, and will assist in efforts aiming at building teeth or tooth components using adult tissues.

Ameloblasts and Amelogenesis

O23

Structural and cytochemical analyses of enamel and enamel forming cells in the teeth of amelogenin-deficient mice

Takano Y.[1], Li Y.[2], Iseki H.[1], Uchida T.[3], Kulkarni A.B.[4], Gibson C.W.[2]
[1]Tokyo Medical and Dental University, Biostructural Science, Tokyo, Japan, [2]University of Pennsylvania School of Dental Medicine, Dept. of Anatomy and Cell Biology, Philadelphia, United States, [3]Hiroshima University, Oral Anatomy and Cell Biollogy, Hiroshima, Japan, [4]NIDCR, Bethesda, United States

The amelogenin proteins comprise approximately 90% of the organic phase of the enamel matrix during early development. It has been reported that the *amelx*-null mice show thin chalky white enamel with irregular surface and has disorganized crystals when viewed in the fractured planes, indicating a role of amelogenins for determination of enamel thickness and crystal arrangement. However, the previous phenotype analysis has been limited to macroscopic and SEM observations of the erupted teeth of the mutant mice and hence precise information of the intriguing process of amelogenesis without amelogenin gene and proteins has not been provided.
Methods: In this study we examined growing molars and incisors of the 14-day and 50-day-old *amelx*-null mice by light and electron microscopy, and focused on the structural and cytochemical aspects of the ameloblasts and the growing enamel matrix.
Results: In the incisors of *amelx*-null mice, the cells of the enamel organ showed normal arrangement and stage-related structural and functional features including the RA-SA modulation of maturation ameloblasts. The enamel matrix started to form on the mineralized dentin and the secretory ameloblasts developed Tomes' processes containing secretory granules in early development, although the thickness of the enamel was limited to 10 μm maximum. The immature enamel was immunopositive for both ameloblastin and enamelin although the enamelin reaction was limited to the “inter-rod” enamel in early development. Abnormal knob-like protrusions of enamel matrix up to 50 μm-long and 10 μm-thick were occasionally encountered on the growing enamel surface of the molars. Transmission electron microscopy of the undecalcified sections of the immature enamel revealed a halo of electron lucent layer along the individual ribbon-like crystals, highlighting the crystals from the surrounding amelogenin-free matrix that appeared relatively electron-dense. In some areas in immature enamel, the groups of enamel crystals were unusually thickened and some were apparently decorated with a bulk of irregular mineralized material. Such thickened crystals and deposits were lost by decalcification leaving a void space.
Conclusions: These data indicate that a total absence of amelogenin gene and proteins does not interfere with the development of the tooth germs and formation of dental hard tissues other than enamel. Unusually thickened crystals and irregular mineral deposition in the amelogenins-free immature enamel support the regulatory role of amelogenins in crystal growth and arrangement. The causative factors of knob-like protrusions of enamel in the molars of amelogenin-null mice need to be explored.

Ameloblasts and Amelogenesis

O24

Induction of human keratinocytes into enamel-secreted ameloblasts

Wnag B.[1], Li L.[1], Du S.[1], Liu C.[1,2], Hu X.[1], Chen Y.[1,2], Zhang Y.[1]
[1]Fujian Normal University, College of Life Sciences, Fuzhou, China, [2]Tulane University, Department of Cell and Molecular Biology, New Orleans, United States

Utilization of stem cells from a patient to develop bioengineered replacement teeth is the ultimate goal of regenerative dental medicine. Various postnatal mesenchymal stem cells have been shown capable of differentiating into odontoblasts and creating mineralized dentin. However, identification of stem cells that can be used as the epithelial component for tooth regeneration remains a challenge. We tested if keratinocytes could represent an applicable source for enamel production. Keratinocyte stem cells, isolated from circumcised human foreskins and confirmed by the expression of several molecular markers, were cultured to form confluent epithelial sheets. These epithelial sheets were recombined with E13.5 mouse molar mesenchyme that possesses an odontogenic potential. The recombinants developed into whole tooth crowns consisting of human and mouse tissues in 25% cases after 4-week culture under the mouse renal capsule. Immunohistochemical studies using specific antibodies against human or mouse MHC I antigens confirmed human origin of the epithelial component and mouse origin of the dental pulp, respectively. Histological examination revealed the presence of well-formed dentin, but a lack of enamel due to failed differentiation of the keratinocytederived epithelial cells into elongated ameloblasts. The human keratinocyte-derived epithelium thus supports mouse dental mesenchyme to form tooth structures but lacks ameloblastic differentiation capability under such condition. Next, we surveyed the potentials of several key growth factors to induce human keratinocytes into ameloblasts. Among them are SHH and BMP4 that are expressed in differentiating ameloblasts of mouse and/or human teeth, and FGF8 which is expressed in both odontoblasts and ameloblasts of developing human teeth. Growth factor-soaked beads were implanted in tissue recombinants of cultured human keratinocyte sheet and mouse molar mesenchyme, which were subsequently subjected to subrenal culture for 4-week. Neither SHH nor BMP4 induced ameloblast differentiation, while BMP4 rather caused bone formation instead of tooth in the recombinants. In contrast, inclusion of FGF8 beads, although did not enhance the success rate of tooth formation in the recombinants, indeed induced the elongation of keratinocyte-derived epithelial cells and the deposition of enamel in 6 cases of the 22 formed teeth. Immunostaining assays showed specific presence of ameloblastin and MMP-20, specific markers for differentiated ameloblasts, in the elongated epithelial cells and the enamel, confirming ameloblastic differentiation. It is interesting to note that in developing human embryo, the deciduous teeth begin to develop in the 6th week of gestation, but it is not until the 28th week the dental epithelium starts to differentiate into enamel-producing ameloblasts. However, in the human-mouse chimeric teeth, differentiated ameloblasts and deposited enamel are found within 4-week under the subrenal culture. This is likely due to a faster differentiation of the mouse odontoblasts, which in turn induces earlier ameloblastic differentiation of the human keratinocytes. These results demonstrate that human keratinocytes are capable of differentiating into ameloblasts and producing enamel, and identify the type of cell an appropriate source for generating bioengineered whole tooth crown for replacement therapy in the near future.

Ameloblasts and Amelogenesis

O25

Amelotin: Introducing a new player in the team of enamel matrix proteins

Ganss B.[1]
[1]*University of Toronto, Faculty of Dentistry, Group in Matrix Dynamics, Toronto, Canada*

Background: We have recently identified Amelotin (Amtn) as a unique gene that is highly and selectively transcribed in a transient fashion from birth to the time of tooth eruption in secretory stage ameloblasts of molars, and in late secretory to maturation stage ameloblasts of incisors. The AMTN protein is efficiently secreted *in vitro*. Studies in rat have located the protein to a basal lamina-like structure between ameloblasts and the enamel mineral surface, but the function of AMTN in amelogenesis remains unknown. The purpose of this project was to develop *in vivo* and *in vitro* models to provide insights into this functional role.
Methods: We have used immunohistochemical analyses to determine the developmental expression profile of AMTN in mouse molars and incisors, and compared it with the expression patterns of other known enamel matrix proteins (EMPs). We have also conducted a series of biochemical analyses on bacterially expressed recombinant AMTN to characterize the protein in more detail and to investigate its effects on activities such as cell adhesion. A yeast-two-hybrid system was used to identify any potential AMTN-interacting proteins. We have created several transgenic mouse lines that overexpress the AMTN protein under control of the amelogenin (Amel) promoter, and mice in which the *Amtn* gene has been disrupted, and have used histological and various imaging methods to characterize their phenotype with a particular emphasis on enamel formation. Lastly, we have used luciferase assays on several *Amtn* promoter constructs to delineate the regions controlling *Amtn* transcription.
Results: In close correlation with the mRNA expression profile, we found the murine AMTN protein expressed transiently in ameloblasts of molars from postnatal day 2 (P2) to the time of eruption, and in late secretory to maturation stage ameloblasts in incisors. Comparison with other EMPs revealed a distinct expression profile for AMTN at relatively late stages of enamel formation. This profile was found to be highly similar to that of APin (ODAM), another recently identified enamel protein. The largest concentration of AMTN was found at the interface between the distal end of ameloblasts and the enamel mineral surface. The transcription of *Amtn* was mediated by a short, ca. 200 bp region upstream of the translation start site. Recombinant AMTN protein did not have any effect on cell adhesion in a variety of cell types, but formed large, homomultimeric aggregates in solution. This aggregation was confirmed in yeast-two-hybrid experiments, which revealed not only AMTN-AMTN, but also AMTN-APin interactions. APin was found to interact with ameloblastin (AMBN) and to a lesser extent with amelogenin (AMEL). Transgenic mice overexpressing AMTN showed a dose-dependent effect on the structure and mechanical properties of enamel, and AMTN deficiency resulted in hypomineralized enamel and premature enamel attrition. Comparisons of expression levels for other enamel proteins in AMTN-overexpressing and -deficient mice revealed alterations for APin and kallikrein 4 (KLK4).
Conclusion: AMTN plays a critical role during amelogenesis, possibly forming co-polymeric aggregates with APin during the advanced stages of enamel mineralization. We hypothesize that AMTN and APin collectively provide the specialized matrix that is involved in the transition from highly organized and structured bulk enamel to the compact surface layer of “final” enamel.

Ameloblasts and Amelogenesis

O26

The role of Amelotin on differentiation of ameloblasts

DenBesten P.[1], He P.[1], Zheng L.[1], Zhu L.[1], Ganss B.[2], Li W.[1], Kim S.O.[3], Zhang Y.[1]
[1]University of California, San Francisco, Orofacial Sciences, San Francico, United States, [2]University of Toronto, Group in Matrix Dynamics, Toronto, Canada, [3]Yonsei University, Pediatric Dentistry, Seoul, Korea, Republic of

Background: Amelotin has been shown to be a marker for maturation ameloblasts in developing rodent incisors. However the function of amelotin in tooth formation remains unclear.
Methods: In this study we immunolocalized amelotin in developing human incisors by immunofluorescent staining of cryosectioned bell stage primary tooth organs. Laser capture microdissected ameloblasts were used to quantify and compare the relative amounts of amelotin mRNA at different stages of ameloblast differentiation. Primary ameloblast lineage cells were used to determine the effects of amelotin on ameloblast lineage cell proliferation and differentiation, and a SV40 transformed ameloblast lineage cell line with a relatively high expression of amelotin was used to determine effects of serum growth factors and basement membrane proteins on amelotin expression.
Results: Immunohistochemistry revealed prominent amelotin staining in human presecretory and secretory ameloblasts, as well as in the developing enamel matrix. Quantitative PCR analysis showed that mRNA expression of amelotin could be detected in preameloblasts, and was dramatically up-regulated in the presecretory stage of ameloblast differentiation. *In vitro*, amelotin mRNA expression by immortalized ameloblast-lineage cells was significantly down-regulated when grown in 3D Matrigel (basement membrane proteins), while the addition of fetal bovine serum up-regulated amelotin. The addition of recombinant amelotin (rhAMTN) to primary culture ameloblast lineage cells showed that rhAMTN significantly up-regulated the mRNA expression of apin and KLK4. Taken together, these data suggest that amelotin is upregulated by serum related growth factors, and upregulation of amelotin directs ameloblasts to a more mature phenotype, as indicated by increased expression of apin and KLK-4.
Conclusions: These studies show that while amelotin is highly upregulated at the maturation stage of ameloblast differentiation, it is present at all stages of ameloblast differentiation. Amelotin may partially regulate ameloblast maturation.

Ameloblasts and Amelogenesis

O27

A high calcium supplement partially prevents fluoride toxicity on secretory amelogenesis in developing hamster tooth germs *in vitro*: Ultrastuctural studies

Lyaruu D.M.[1], Bronckers A.L.J.J.[1]
[1]*ACTA, Oral Cell Biology, Amsterdam, Netherlands*

Background: Short-term exposure of fluoride (F^-) to the developing hamster tooth germ *in vitro* induces a double response in secretory stage enamel: the mineralizing enamel deposited *in vivo* before the onset of F^- exposure hypermineralizes and the enamel matrix secreted during F^- exposure fails to mineralize. Fluoride administration during the secretory phase of amelogenesis in vitro also results in enhanced uptake of radio-labeled calcium and phosphate mainly due to hypermineralization of the pre-exposure enamel layer while newly secreted enamel matrix remained unmineralized. To test the validity of the hypothesis that the failure of the fluorotic enamel matrix to mineralize was due to a local shortage of calcium ions, medium calcium levels were increased concomitant with F^- exposure. The effects of elevated calcium concentration on the toxicity of fluoride to developing enamel were investigated at the ultrastructural level in hamster second molar tooth germs grown during the secretory phase of amelogenesis *in vitro*.
Methods: Mineralizing 3-4 day-old hamster second maxillary tooth germs were cultured in BGJ_b medium using the grid method. Most of the ameloblasts are in the secretory stage and continue to deposit enamel in vitro. The BGJ_b culture medium was prepared in our laboratory in order to be able to adjust the medium calcium concentration. The culture medium was supplemented with 15% FCS. The tooth germs were cultured for 48 hours in the presence of high (4.2 mM Ca^{2+}) total calcium ions together with 1 mg/L F^-. The contralateral control tooth germs were cultured in standard medium (2.1 mM calcium) also containing 1 mg/L F^-. The medium containing standard calcium concentration used for the culture of the control germs support normal mineralization of enamel and dentine *in vitro*. Inorganic phosphate in the media was kept constant (1.6 mM). The culture media were changed every 24 hours. Some tooth germs were also cultured in standard medium but without fluoride but supplemented with an equimolar amount of Cl^- as control of the culture quality. At least six pairs of molars per group were examined.
Results: The most profound effect of culturing the explants in calcium-enriched media on the toxicity of fluoride was the absence of unmineralized enamel matrix on top of the pre-exposure enamel layer. Instead, a relatively normal *in vitro* enamel layer was deposited on top of the fluoride-induced hypermineralized pre-exposure enamel layer. Another effect was that high calcium levels inhibited both the fluoride-induced disorganization of the Tomes´ processes and improved the structural organization of secretory ameloblasts. However, the pre-exposure enamel deposited *in vivo* was still hypermineralized.
Conclusion: Based on the findings that hypermineralization of secretory enamel during exposure to F^- could not be altered by increasing calcium levels, we conclude that the primary target of fluoride action during secretory amelogenesis is the pre-existing mineral crystals rather than the organic matrix being secreted by the ameloblasts.

Ameloblasts and Amelogenesis

O28

Ameloblastin in enamel formation: Rescue of amelogenesis imperfecta and processing by enamel proteinases

Chun Y.-H.P.[1], Yamakoshi Y.[2], Lu Y.[2], Hu Y.[2], Yamakoshi F.[2], Yamada Y.[3], Hu J.C.-C.[2], Simmer J.P.[2]
[1]University of Texas Health Science Center at San Antonio, Periodontics, San Antonio, United States, [2]University of Michigan, Biologic and Materials Sciences, Ann Arbor, United States, [3]National Institute of Dental and Craniofacial Research, National Institutes of Health, Craniofacial Developmental Biology and Regeneration Branch, Bethesda, United States

Introduction: During enamel formation ameloblastin (Ambn) is expressed by secretory stage ameloblasts along with amelogenin and enamelin. Lack of Ambn or enamel proteinases results in amelogenesis imperfecta (AI). Mice with partial deletion of *Ambn* display hypoplastic AI. Disruption of proteolytic processing of enamel proteins causes hypomature enamel *in vivo*.
Objective: The first goal was to determine if Ambn is essential for enamel formation by rescuing the enamel defects in *Ambn* mutant mice using a transgenic mouse approach. The second goal was to identify the cleavage sites of Ambn recognized by enamel proteinases using recombinant Ambn and synthetic peptides.
Materials & methods: A transgenic mouse model was developed to express full-length mouse Ambn (396 amino acids) under the control of the amelogenin (Amel) promoter in the *Ambn* mutant mice. The *Amel* promoter (4.6 kb) and its *3′* UTR (1.1 kb) were cloned from mouse genomic DNA; the *Ambn* cDNA (1.7 kb) was cloned from mouse molar mRNA. pCR2.1-TOPO was used as cloning vector. The excised Ambn transgene (Tg) was injected into fertilized mouse oocytes. Ambn Tg expression levels of Ambn mutant mice were determined in the first molars of PN day 5. Proteins from molars were extracted with formic acid and analyzed by SDS-PAGE, CBB staining and Western blotting (WB). An anti-peptide antibody raised against a synthetic peptide from the deleted region was used in WB. The morphology of teeth was evaluated at 7 weeks. The thickness and organization of enamel were evaluated by scanning electron microscope (SEM) on cross-sectioned and fractured mandibular incisors. The enamel surface was analyzed on exposed labial surfaces. Recombinant pAmbn was expressed and secreted from a stable cell line (HEK293) and purified from medium. Ambn and synthetic peptides were incubated with rpMmp-20 and native Klk4. The digestion products were analyzed by SDS-PAGE, N-terminal sequencing and mass spectrometry.
Results: From 17 Ambn Tg mouse lines, 5 lines were established and analyzed. Among lines Ambn Tg expression levels ranged compared to wild-type mice from no Ambn expression Tg(0), below normal Tg(+), above normal Tg(++), to much higher Tg(+++). The morphology of incisors and molars of Tg(+) mice appeared to be similar to wild-type mice. Analysis by SEM revealed that the enamel thickness at the central labial position was restored to normal in Tg(+), (++), and (+++) mice; however the distal enamel thickness was restored to normal only in Tg(++) animals. In Ambn Tg(+) and Tg(+++) mice, the distal thickness of the enamel was reduced and had a rough surface. In fractured enamel the decussating pattern was rescued in all Tg expressing mice but Tg (+++) mice had defined interrods. The enamel surface of the incisors of Tg(++) animals was similar to wild-type mice during all stages of enamel formation. rpMmp-20 cleaved rpAmbn and the synthetic peptides at sites corresponding to those observed *in vivo*. Initial cleavages were made at the N-terminus, followed by cleavages at the C-terminus. In contrast, Klk4 cleaved rpAmbn and the synthetic peptides at sites not observed *in vivo*.
Conclusion: The enamel defects of the Ambn mutant mice were at least partially restored to normal by expression of the full-length Ambn Tg. Our data demonstrate that Ambn is essential for enamel formation. Mmp-20 is capable of catalyzing cleavages of Ambn during the secretory stage, while Klk4 likely degrades Ambn during maturation stage.

Ameloblasts and Amelogenesis

O29

Immunolocalisation of NBCe1 in the mouse enamel organ

Bronckers A.L.[1], Bervoets T.J.[1], Lyaruu D.M.[1], DenBesten P.K.[2]
[1]Universiteit van Amsterdam and Vrije Universiteit Amsterdam, Dept Oral Cell Biology ACTA, Amsterdam, Netherlands, [2]UCSF, Dept of Orofacial Sciences, San Francisco, United States

Background: Enamel crystal growth generates protons that need to be neutralized to sustain enamel mineralization. The presence of carbonic anhydrase II (Car-II), the anion exchanger-2 (Ae2) and Cystic fibrosis transmembrane regulator (Cftr) in maturation ameloblasts supports the view that these cells are involved in pH regulation. Recently it was reported that mice deficient in electrogenic sodium bicarbonate cotransporter-1 (Nbce1) have enamel defects (Gawenis et al; J Biol Chem.282:9042-52, 2007); in addition, transcripts for Nbce1 have been detected in ameloblast-like cells (Paine et al;. J Dent Res 87, 391-395; 2008). This suggests that Nbce1 is operating in cells of the enamel organ during amelogenesis.
The aim of this study was to examine a potential role of Nbce1 in pH regulation by ameloblasts by establishing its location during amelogenesis.
Methods: Mouse jaws were fixed in buffered paraformaldehyde, histologically processed in paraffin, serially sectioned and stained with rabbit anti-NBCe1 polyclonal antibodies.
Results: Staining for Nbce1 was located in the enamel organ during both secretion and maturation stage of amelogenesis. In early secretory ameloblasts, staining was located in membranes of stratum intermedium and cytoplasmically in secretory ameloblasts. In more advanced stages secretory ameloblast staining was very strong in the apical end of the ameloblast cell body near the proximal part of the Tomes' process. The distribution of staining for Nbce1 changed dramatically in maturation phase. The plasma membranes of the papillary layer adjacent to maturation ameloblasts became intensely stained whereas staining in maturation ameloblasts was reduced. The apical staining of the ameloblast membrane had disappeared with the onset of maturation stage but weak staining in the cytoplasm and in basolateral membranes was still apparent in these cells.
Conclusions: The presence of electrogenic Nbce1 in both secretory ameloblasts and maturation ameloblasts suggests that this co-transporter functions in both stages of amelogenesis, likely involved in pH regulation during mineralization of enamel. In secretory stage the *apical* NBCe1 near the proximal ends of the Tomes' processes may be involved in bicarbonate extrusion into the enamel space in close vicinity of the plasma membranes of the Tomes processes where enamel crystals are forming.
In maturation stage, as mineral accretion increases, proton production in enamel is intensified which requires more bicarbonate secretion. The joint activity of (cytosolic) Car II, basolateral Nbce1 and Ae2, and apical Cftr in maturation ameloblasts provides a basic mechanism to extrude bicarbonates into maturation stage enamel. The intense staining of the papillary layer cells for Nbce1 strongly suggests that the basal plasma membrane of the ameloblasts that is in contact with the papillary layer is a major route to import bicarbonates into the maturation ameloblast cell body.
The present data further add to the concept that ameloblasts contain the machinery for local extracellular pH regulation in the enamel space.
Acknowledgments: Part of this work was funded by NIH (grant DE13508-06; DL, THB, PdB and AB).

Ameloblasts and Amelogenesis

O30

The recombinant human amelogenin protein and regeneration of mouse non-union calvarial defect

Sharon S.[1], Haze A.[1], Shilo D.[1], Silverstein N.[1], Blumenfeld A.[1], Deutsch D.[1]
[1]*The Hebrew University of Jerusalem, Institute of Dental Sciences, Jerusalem, Israel*

Background: The amelogenin protein constitutes 90% of the forming extra-cellular enamel matrix proteins and its function is to regulate the size, shape and direction of the mineral crystal growth. We have previously reported that amelogenin is expressed in cells of alveolar bone, long bone, cartilage and bone marrow (Deutsch et al. 2006, Haze et al. 2007, 2009). Later we showed that the recombinant human amelogenin protein ($rHAM^+$) alone, produced in eukaryotic system in our laboratory (Taylor et al. 2006), induces the regeneration of the periodontal tissues; cementum, periodontal ligament and alveolar bone, after induction of experimental periodontitis in the dog model, through recruitment of mesenchymal stem cells (Haze et al. 2009). We have also observed high amelogenin expression in active bone regions (growth and remodeling). The dynamic spatio-temporal pattern of expression of amelogenin in the developing mouse embryonic craniofacial complex strongly suggests that amelogenin has an important role during normal bone development (Gruenbaum-Cohen et al. 2009).
Objectives: To investigate the ability of amelogenin to repair / regenerate critical size calvarial defect (a membranous bone not associated with PDL and cementum).
Methods:
(1) Creation of critical size defect (5 mm, Aalami et al. 2004) in the parietal bone of the mouse.
(2) Application of collagen sponge into the defect. Suture soaked with $rHAM^+$ (or its carrier PGA alone-control) was placed above the calvarial bone defect.
(3) Characterization of the regenerated bone tissue 2-5 months after amelogenin application, using micro-CT.
(4) Histological, immunological and molecular biology analyses of the regenerated bone (compared to control with PGA alone).
Results: Micro-CT analysis showed some calvarial bone regeneration 7 weeks after $rHAM^+$ application, and the regeneration progressively increased at 3 to 5 months. In the experimental mice the regeneration process generally initiated at the sagital line, just below the suture (soaked with $rHAM^+$) and progressed laterally towards the defect boarder. 3-5 month after application of $rHAM^+$, the regenerated bone was continuous at the sagital line. Generally, in contrast, in control mice, no regenerated bone to very little bone regeneration was observed. In some defects sporadic islands of bone were observed, mostly close to the edges of the defect but not at the sagital line. A direct correlation was apparent between $rHAM^+$ concentration and the degree of regeneration at fixed 5 months period. Sagital micro-CT section of the regenerated bone demonstrated full bone thickness as compared to non-operated bone. Histological and immunological analyses of the regenerated bone showed new bone formation, characterized by expression of specific bone markers.
Conclusions: Amelogenin induces calvarial bone regeneration, most probably through recruitment of mesenchymal stem cells.
Funded by the Israeli Ministry of Health, Grant number (D.D.): 3-4064

Ameloblasts and Amelogenesis

O31

Ameloblastoma characterization and *in vitro* stimuli response

Catón J.[1], Mitsiadis T.[2], Heikinheimo K.[3], Morgan P.[1]
[1]King's College London, Clinical & Diagnostic Sciences, London, United Kingdom, [2]University of Zurich, Institute of Oral Biology, Zurich, Switzerland, [3]University of Turku, Department of Oral and Maxillofacial Surgery, Turku, Finland

Background: Ameloblastomas are odontogenic neoplasms with a predilection for the posterior mandible although other jaw sites may be involved. They grow slowly, do not mineralize and are rarely metastatic. The usual treatment is surgical resection with the attendant disadvantages of significant morbidity and tendency for recurrence. Strategies for alternative therapies are therefore needed. Although still debated, it is generally accepted that these tumours originate from ameloblast precursors. Ameloblastomas have been reported to express genes important for enamel formation such as amelogenin and tuftelin and their histological appearance resembles pre-secretory ameloblasts. There is no established *in vitro* system developed to study these neoplasms. Attempts have been made to isolate stable ameloblastoma cell lines, but to date only one line transfected with a human papilloma-virus type-16 is available. The aim of this project was to establish an *in vitro* system to better understand how ameloblastomas interact with their local environment and maintain themselves in a tumourogenic state. The better understanding of tumour maintenance can lead to the development of new and effective therapies. Ameloblastomas grown in organ cultures can help us to understand them in 3-D context dissecting the behavior of the specific cell types within the tumour when challenged in a controlled environment. These tumours can be challenged with embryonic dental cells in co-cultures. These cells have been shown to produce factors capable of inducing adjacent tissues driving them towards their programmed fate.
Methods: We co-cultured ameloblastoma organs with murine embryonic dental epithelium or mesenchyme.
Results: We established that the murine dental cells change the rate of proliferation and apoptosis on the cultured ameloblastomas. We also determined by *in situ* hybridization that the dental cells have an effect on the expression of genes important for tooth development such as BMP2, BMP4 and TGFb1 in the cultured ameloblastomas. In parallel experiments we used beads soaked with SHH, BMPs and FGFs proteins to determine the effects of these factors on the cultured ameloblastomas. These factors also have an effect on the rate of proliferation and apoptosis on the ameloblastomas.
Conclusion: These results demonstrate the ability of these tumours to respond to stimuli derived from dental environment *in vitro*.

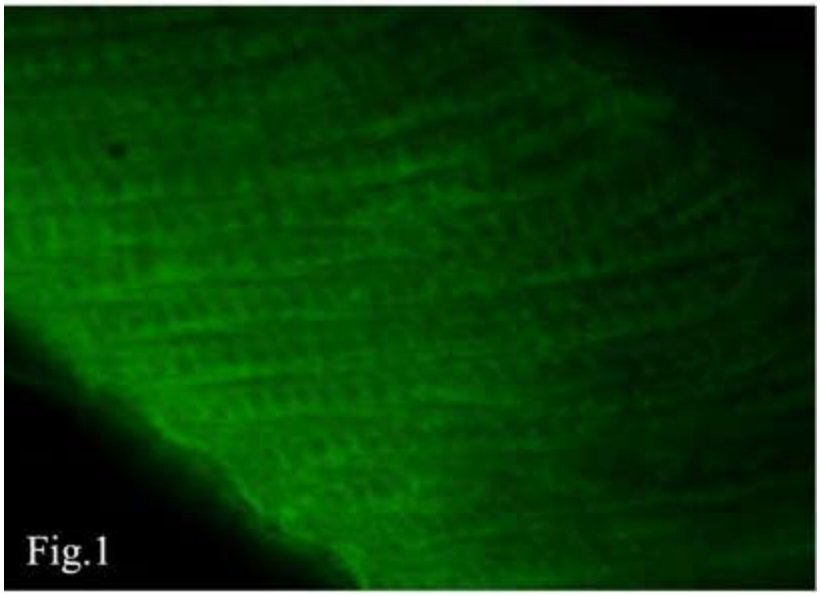
Ameloblasts and Amelogenesis

O32

New hypothesis of cross-striation formation mechanism

Sasaki A.[1], Otsu K.[1], Fujiwara N.[1], Xu L.[2], Taniguchi A.[2], Harada H.[1]
[1]Iwate Medical University, Division of Oral Histology and Developmental Biology, Department of Oral Biology, Morioka, Japan, [2]National Institute for Materials Science, Advanced Medical Materials Group, Biomaterials Center, Tsukuba, Japan

In the enamel rods, periodic bands referred to as “cross-striation” are observed as incremental lines of circadian rhythm, and in some textbooks it has been written that human enamel is formed at a rate of approximately 4 μmper day and cross-striations appear at 4-μmintervals across enamel rods. However, the formation mechanism has not been elucidated clearly. The enamel formation proceeds through two-step process. In the first step the enamel protein has been deposited within the predicted enamel thickness and the second step involves mineral influx coincident with the removal of protein and water. Accordingly, cross-striation may not directly reflect the periodicity of mineralization in the enamel. Hematoxylin-eosin staining of non-calcified enamel matrix in canine tooth germ showed dark and light coloring pattern like cross-striation. And the pattern of fluorescent dynamic by amelogenin immunostaining is also very similar to cross-striation (Fig.1).



[Fig.1]

Therefore, we consider that cross-striation reflects the contrasting density of amelogenin protein in the process of calcification.

Firstly, to examine the diurnal fluctuation of amelogenin expression in ameloblasts, we utilized the transcriptional assay of amelogenin promoter using luciferase. After transfecting the construct of amelogenin promoter into rat ameloblast cell line, the reporter activity was measured by luminometer and was observed by lumino-microscopy, time-dependently. The results showed that transcriptional activity of amelogenin promoter repeated the cycle of increase and decrease with a period of 20-26h.

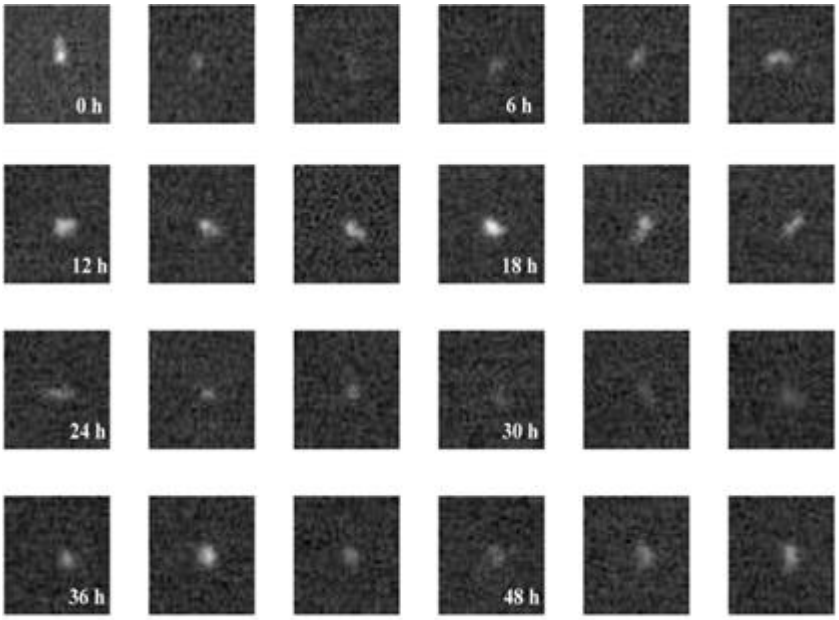


Fig.2

[Fig.2]

Next, to search the regulatory region controlling the transcriptional activity periodicity of amelogenin promoter, we designed the deletion mutant of amelogenin promoter and carried out the transcriptional assay in a same fashion. The rhythm was observed similarly in the deletion-mutant using -74bp promoter, the specific region controlling the periodicity could not be detected in the amelogenin promoter.

Thirdly, because it has been reported that there are several CBP/αbinding motifs in the promoter region and the activity is inhibited through Msx2 binding to CBP/α, we examined the effect of Msx2 on the periodicity of the amelogenin promoter activity. Overexpression of Msx2 led to the disappearance of the periodicity of amelogenin promoter activity. The results suggested that the binding motifs of CBP/α and Msx2 might be associated with the periodicity of transcriptional activity, however, further study is needed to clarify this.

Taken together, we would like to hypothesize that cross-striation is the low-mineralization line which reflects the higher-density line of amelogenin at the stage of enamel matrix deposition.

Odontoblasts and Dentinogenesis

O33

How odontoblasts can sense enamel erosion

Bleicher F.[1,2,3], Shibukawa Y.[4], Couble M.-L.[1,2,3], Maurin J.-C.[5,6], Thivichon-Prince B.[1,2,3], Magloire H.[1,2,3]
[1]Université Claude Bernard Lyon1, Institut de Génomique Fonctionnelle de Lyon, Lyon, France, [2]UMR 5242 CNRS, Lyon, France, [3]ENSL, Lyon, France, [4]Oral Health Science Center, hrc7, Department of Physiology, Tokyo Dental College, Chiba, Japan, [5]University of Reims Champagne-Ardennes, Faculty of Odontology, Reims, France, [6]INSERM UMR S926, Reims, France

Enamel erosion leads to dentine hypersensitivity, a painful disease that affects today a younger population. The exposure of a dentinal surface results in pain arising in response to thermal, tactile, osmotic or chemical stimuli. The widely accepted hydrodynamic theory states that the consequent opening of dentinal tubules to the oral environment allows, under these stimuli, the movement of the dentinal fluid inside the tubules, indirectly stimulating the extremities of the pulp nerves, causing the pain. Nevertheless, growing evidence suggests that odontoblast could be a key cell in the detection of enamel erosion and the tooth pain transmission. We have demonstrated the presence of mechanosensitive ion channels at the surface of odontoblasts :BKCa, TREK-1 which is involved in polymodal pain perception, and $Ca_{v2.2}$ clustering at the base of the primary cilium of odontoblasts. Very recently, mechanosensitive TRP channels (transient receptor potential channels, a family of non-selective cation permeable channels) such as TRPV1-4 and TRPM3 were identified in odontoblasts at the gene expression and functional level (1). Moreover, we demonstrated the presence of TRPP1 and TRPP2, forming a mechanosensor / Ca^{2+} permeable channel heterodimeric complex, at the base of the primary cilium of odontoblasts. Therefore, odontoblasts are at least as well equiped as sensory nerves to sense enamel erosion. Moreover, we have demonstrated that these cells are able to generate action potentials and therefore are excitable cells. Taken together, all these data allow to devise a new schema to explain dentinal sensitivity pain where odontoblasts could sense the fluid displacement consecutive to stimuli, integrate this mechanical stress into a biological response that could be transduced to the sensory nerve endings. However, we need to precisely identify the proteins that mediate signals between odontoblasts and nerves although some candidate mediators are emerging.
Therefore, the early step underlying dentin pain perception and transmission is a much more intricate mechanism than initially proposed by the hydrodynamic theory as odontoblasts are key cells involved in the pulp/dentin strain sensor complex. This explains the difficulty to develop more effective therapeutic strategies for the treatment of dentin sensitivity.

References:
(1) Son AR, Yang YM, Hong JH, Lee SI, Shibukawa Y, Shin DM. J Dent Res. 2009; 88:1014-1019.
(2) Thivichon-Prince B, Couble ML, Giamarchi A, Delmas P, Franco B, Romio L, Struys T, Lambrichts I, Resnikoff D, Magloire H, Bleicher F. J Dent Res. 2009 ; 88 :910-915.
(3) Magloire H, Maurin JC, Couble ML, Shibukawa Y, Kimura-Tsumura M, Thivichon-Prince B, Bleicher F, J Pain, accepted

Odontoblasts and Dentinogenesis

O34

Pathogen sensing by human odontoblasts

Farges J.-C.[1,2,3], Keller J.-F.[1,2,3], Carrouel F.[1,3], Kufer T.A.[4], Baudouin C.[5], Msika P.[5], Bleicher F.[1,3], Staquet M.-J.[3]
[1]Université Claude Bernard Lyon1, Faculté d'Odontologie, Lyon, France, [2]Hospices Civils de Lyon, Service d'Odontologie, Lyon, France, [3]Institut de Génomique Fonctionnelle de Lyon, Lyon, France, [4]University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Cologne, Germany, [5]Laboratoires Expanscience, Département Innovation, Recherche et Développement, Epernon, France

Human odontoblasts are neural crest-derived, dentin-producing mesenchymal cells aligned at the periphery of the dental pulp. They become exposed to cariogenic oral bacteria as these progressively demineralise enamel then dentin to gain access to the pulp. Due to their situation at the dentin-pulp interface, odontoblasts are the first cells encountered by invading pathogens and/or their released components, and represent, in the tooth, the first line of defence for the host. Previous studies have shown that odontoblasts are able to sense pathogens and elicit innate immunity. In particular, they express several pathogen recognition receptors of the Toll-like receptor (TLR) and nucleotide-binding oligomerisation domain (NOD) families, which allow them to recognize specific bacterial and viral components. So far, most studies aiming at elucidating the role of odontoblasts in the dental pulp innate response have focused on Gram-positive bacteria, as these largely dominate the carious microflora in initial and moderate dentin caries lesions. In vitro, odontoblasts were found to be sensitive to Gram-positive bacteria-derived components, mainly lipoteichoic acid which is recognized through cell membrane TLR2. Our studies have shown that engagement of odontoblast TLR2 by LTA triggers TLR2 and NOD2 up-regulation, NF-kB nuclear translocation, production of various chemokines including CCL2, CXCL1, CXCL2, CXCL8 and CXCL10, while promoting immature dendritic cell recruitment. Conversely, LTA down-regulates major dentin matrix components, including collagen type I and dentin sialophosphoprotein, as well as TGF-b1, a known inducer of dentin formation. We provide here additional data showing the fine localization of NOD2 in healthy dental pulps, as well as differential regulation of *TLR2, TLR4, NOD2, CCL2 and CXCL8* genes by LTA and the synthetic TLR2 agonists Pam2CSK4 and Pam3CSK4.
It appears from the aforementioned data that odontoblast-triggered immune events constitute potential targets for interrupting the signaling cascades which lead to excessive immune response and necrosis in the dental pulp tissue challenged with cariogenic bacteria. In particular, preventing Gram-positive bacteria recognition or signal transduction by pattern recognition receptors may represent a valuable strategy to achieve this goal. Future studies in the field will pave the way for designing novel therapeutic agents which modulate odontoblast behaviour to promote pulp healing and repair. This work was supported by University Lyon 1, CNRS, IFRO and Région Rhone-Alpes.
TAK acknowledges support from the Deutsche Forschungsgemeinschaft (DFG), SFB670.

Odontoblasts and Dentinogenesis

O35

Overexpression of the Trps1 transcription factor in odontoblasts results in a dentinogenesis imperfecta-like phenotype

Napierala D.[1], Sun Y.[2], Maciejewska I.[2], Munivez E.[1], Bertin T.[1], Dawson B.[3], D'Souza R.[2], Qin C.[2], Lee B.[3]
[1]Baylor College of Medicine, Human and Molecular Genetics, Houston, United States, [2]Baylor College of Dentistry, Texas A&M University System Health Science Center, Biomedical Sciences, Dallas, United States, [3]Howard Hughes Medical Institute, Baylor College of Medicine, Human and Molecular Genetics, Houston, United States

Dentinogenesis imperfecta (DI) is a disorder of dentin formation. It can be manifested as a part of the osteogenesis imperfecta (OI) phenotype or the phenotype can be restricted to dental findings only. In the latter case, DI is caused by mutations of the *DSPP* gene encoding for dentin sialophosphoprotein (DSPP), a matrix protein expressed by odontoblasts. In dentin DSPP is cleaved to dentin sialoprotein (DSP) and dentin phosphoprotein (DPP), which together constitute the majority of non-collagenous dentin matrix proteins. Here we demonstrate that mice overexpressing *Trps*1in odontoblasts (*col1a1-Trps1* transgenic mice) present with a phenotype resembling DI. Trps1 is a GATA-type transcription factor that has been shown to play role in endochondral bone formation. Our previous studies suggested that Trps1 may be a negative regulator of mineralization. To understand the function of the Trps1 transcription factor in mineralizing tissues we generated transgenic mice overexpressing *Trps1* under the control of a 2.3 kb fragment of collagen 1a1 promoter. *Col1a1-Trps1* mice develop severe post-weaning growth retardation and lethality which are secondary to malnutrition. MicroCT and histological analyses revealed tooth fragility due to diminished dentin layer in the teeth of *col1a1-Trps1* mice. Biochemical analyses of SIBLING proteins extracted from dentin of wild type and *col1a1-Trps1* transgenic mice demonstrated decreased levels of both Dsp and Dpp proteins in transgenic animals, while there was no change in the level of dentin matrix protein 1 (Dmp1). Additionally, analyses of gene expression by RNA in situ hybridization demonstrated that overexpression of *Trps1* in odontoblasts results in inhibition of the *Dspp* expression in odontoblasts, but not in amelobalsts. Furthermore , by using chromatin immunoprecipitation (ChIP) assay in odontoblastic cell lines, we have shown that Trps1 can bind GATA consensus sites in the *Dsp*p promoter. Interestingly, during tooth development *Trps1* is expressed in preodontoblasts, but not in mature odontoblasts secreting dentin matrix. These data collectively demonstrate that *Trps1* is a negative regulator of dentin formation and serves this function, at least in part, through repression of the *Dspp* gene.

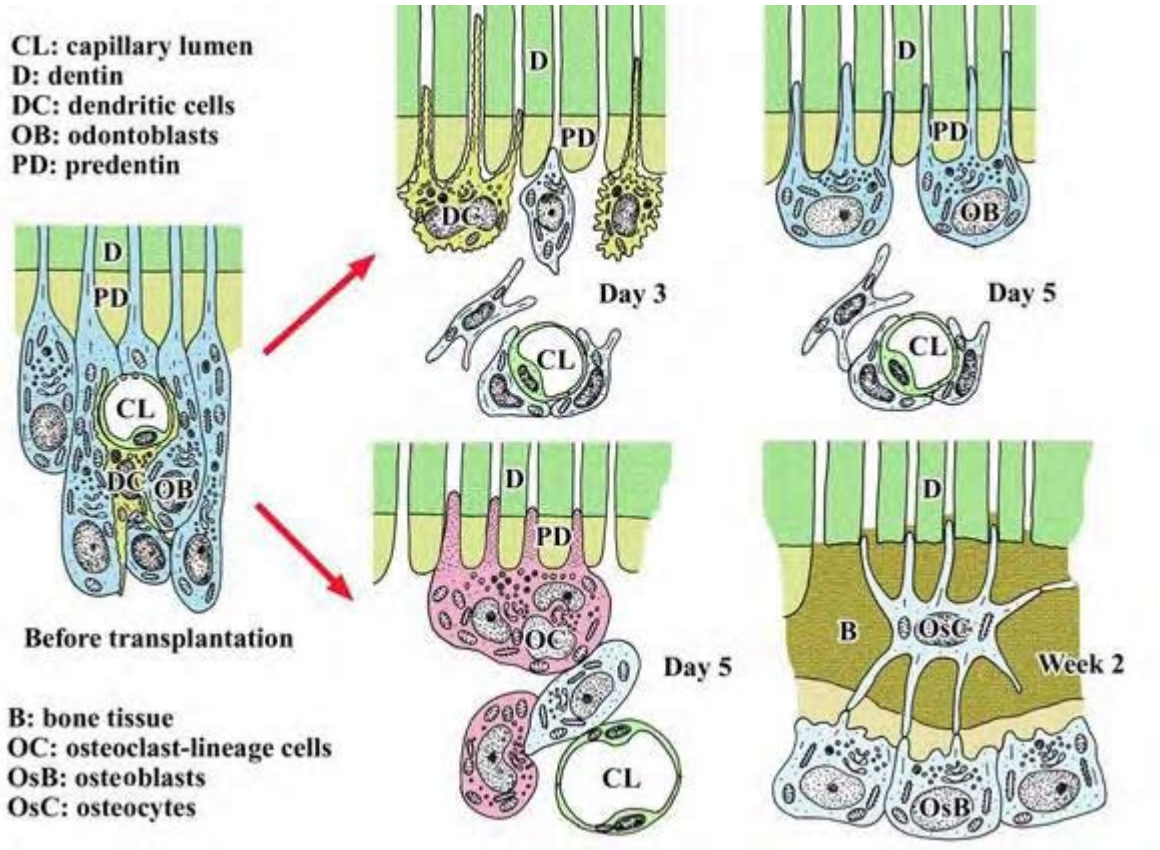
Odontoblasts and Dentinogenesis

O36

The expression of GM-CSF and osteopontin in immunocompetent cells precedes the odontoblast differentiation following allogenic tooth transplantation in mice

Saito K.[1], Nakatomi M.[1], Ida-Yonemochi H.[1], Kenmotsu S.[1], Ohshima H.[1]
[1]*Niigata University Graduate School of Medical and Dental Sciences, Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata, Japan*

Background: Dental pulp elaborates both bone and dentin under pathological conditions such as tooth replantation/transplantation. Once osteoclast-lineage cells appear at the pulp-dentin border, bone-like matrix deposition can be induced, even beneath the pre-existing dentin, whereas the temporal appearance of dendritic cells there induces the tubular dentin formation (Figure 1). This study aims to clarify the expression of granulocyte macrophage colony-stimulating factor (GM-CSF) and osteopontin (OPN) in the process of reparative dentin formation by allogenic tooth transplantation using *in situ* hybridization for OPN and immunocytochemistry for GM-CSF and OPN at the both level of light and electron microscope.
Methods: Crlj:CD1 (ICR) mice, 3 weeks old, were used in this study. The upper-right first molar was extracted under anesthesia, and the roots and pulp floor were resected. The coronal portion of the sample without the periodontal tissue was immediately transplanted into the sublingual region after cutting the ventral side of the tongue of the littermates. Materials were collected in groups of animals at intervals of 1, 3, 5, 7, and 14 days after allogenic tooth crown transplantation. The upper-left M1 of the same animal was used as control.
Results: In the control group, GM-CSF-positive reactions were not observed in either the dental pulp or the periodontal ligament, whereas OPN-positive reactions were observed in the dentinal tubules at the pulp horn in addition to the osteoblasts, cementoblasts, and the matrix of cementum and bone. On the other hand, nestin-immunoreactivity was exclusively expressed in the coronal and root odontoblasts, and the other types of cells lacked nestin-positive reactions in the dental pulp. On Days 1-3, immunocompetent cells such as macrophages and dendritic cells expressed both GM-CSF and OPN, and some of them were arranged along the pulp-dentin border and extended their cellular processes into the dentinal tubules. On Days 5-7, tubular dentin formation commenced next to the pre-existing dentin at the pulp horn where nestin-positive odontoblast-like cells were arranged. Although the expression of GM-CSF and OPN disappeared in the pulp tissue except for the prolonged expressions of OPN and rarely GM-CSF in the lesion lacking the regenerated odontoblasts, the OPN-immunopositive matrices were recognized between the pre-existing and postoperative dentin. Until Day 14, bone-like tissue formation occurred in the pulp chamber, where OPN-positive osteoblasts surrounded the bone matrix.
Conclusion: These results suggest that the secretion of GM-CSF and OPN by immunocompetent cells plays a role for the maturation of dendritic cells and the differentiation of odontoblasts, respectively, in the regenerated pulp tissue following tooth transplantation.



[Figure 1]

Figure 1. Cell differentiation and the induction of hard tissues following tooth replantation/transplantation (modified from *Clin Res Dent 4: 49-57, 2007)*.

Dental Pulp

O38

Neuronal differentiation of human dental pulp stem cells in vitro and in vivo - potential for tissue engineering

Király M.[1], Porcsalmy B.[1], Kádár K.[1], Gera I.[2], Lacza Z.[3], Zsembery Á.[3], Gerber G.[4], Varga G.[1]
[1]Semmelweis University, Department of Oral Biology, Budapest, Hungary, [2]Semmelweis University, Department of Periodontology, Budapest, Hungary, [3]Semmelweis University, Department of Human Physiology, Budapest, Hungary, [4]Semmelweis University, Department of Anatomy, Histology and Embriology, Budapest, Hungary

Background: The plasticity of human dental pulp stem cells (DPSCs) has been demonstrated by several studies. The aim was to differentiate these cells to mature neuronal cells showing functional evidence of voltage gated ion channel activities. In addition, the fate of predifferentiated, implanted DPSC cells were studied in young rats following mild brain cortical lesion.
Methods: Normal impacted human third molars were used following extraction. DPSCs were isolated and cultured as previously reported. For neuronal differentiation we developed a three-set developed protocol. First, cultures were pretreated with a medium containing the demethylating agent 5-azacytidine to dedifferentiate the cells. Then neural induction was performed by the simultaneous activation of protein kinase C and the cAMP pathways. Finally, maturation of the induced cells was achieved by a neurodifferentiation medium. Then Vybrant DiD labeled neuronally induced DPSCs were transplanted into the cerebrospinal fluid of 2-days-old male rats. Cortical lesion was induced by cold exposure applying metal stamp to the calvaria over the motor cortex. For visualization fluorescent and immunohistochemistry were applied. Voltage-dependent sodium and potassium channels were investigated by patch clamp recording.
Results: Noninduced DPSCs cells already expressed vimentin, nestin, N-tubulin, neurogenin-2 and neurofilament-M. The in vitro neuroinductive treatment resulted in decreased vimentin, nestin, N-tubulin and increased neurogenin-2, neuron-specific enolase, neurofilament-M and glial fibrillary acidic protein expression. By the end of the maturation period all investigated genes were expressed at higher levels than in undifferentiated controls except vimentin and nestin. Patch clamp analysis revealed the functional activity of both voltage-dependent sodium and potassium channels in the differentiated cells in vitro. In non-injured brain implanted cells migrated into the progenitor zones of the brain. The cells displayed modest TTX sensitive voltage dependent sodium and TEA sensitive delayed rectifier potassium currents. In injured brain, four weeks after damage the DPSC cells were found the lesion, and expressed multiple neuronal marker proteins. Their sodium currents and potassium currents were about three times larger than those in uninjured brain.
Conclusion: Our results demonstrate that DPSC cells can be efficiently differentiated into functional neurons in vitro. In addition, these differentiated cells are able to maintain their functional characteristics when implanted into injured brain. These data clearly indicate that the dental pulp contains a cell population that is capable of neural commitment, a valuable potential source for tissue engineering.
Supported by the Hungarian National Scientific Research Fund (NI 69008 and CK 80928).

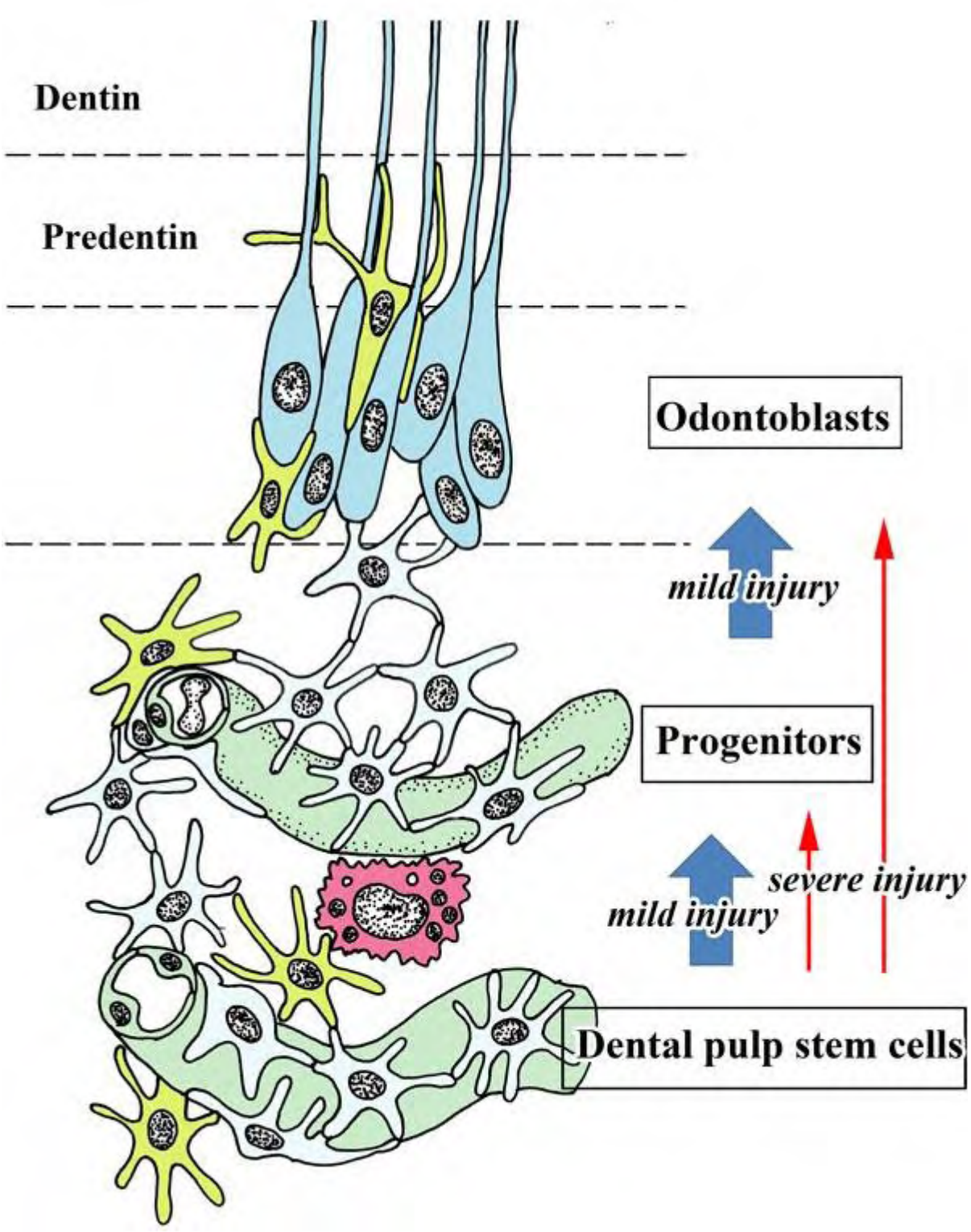
Dental Pulp

O39

Establishment of *in vitro* culture system for evaluation of the dentin-pulp complex regeneration with special reference to differentiation capacity of the BrdU-label-retaining dental pulp cells

Ohshima H.[1], Nakagawa E.[2], Ida-Yonemochi H.[1]
[1]Niigata University Graduate School of Medical and Dental Sciences, Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata, Japan, [2]Yonsei University College of Dentistry, Division of Anatomy and Developmental Biology, Seoul, Korea, Republic of

Background: Our recent study has demonstrated that a pulse of the thymidine analog BrdU given to the prenatal animals revealed the existence of slow-cycling long-term label-retaining cells (LRCs), putative adult stem cells, reside in the pulp tissue. Furthermore, we have proposed the new hypothesis that both progenitors and dental pulp stem cells (DPSCs) are equipped in the dental pulp and that the DPSCs with proliferative capacity play crucial roles in the pulpal healing process following the exogenous stimuli in cooperation with the progenitors (Figure: modified from Niigata Dent J 39: 171-176, 2009). This study aims to establish *in vitro* culture system for the evaluation of the dentin-pulp complex regeneration with special reference to differentiation capacity of the LRCs using immunocytochemistry for BrdU, Ki67, nestin, and α-smooth muscle actin (SMA), and to compare the results obtained from *in vitro* system with those obtained from *in vivo* animal models such as tooth replantation/transplantation for the verification of our hypothesis.
Methods: Three peritoneal injections of BrdU were given to pregnant Crlj:CD1(ICR) mice to map dense LRCs in the mature tissues of born animals. The labeled born animals or GFP-transgenic mice were used for *in vitro* and *in vivo* experiments. The upper-right first molars (M1) of BrdU-labeled or GFP-transgenic mice (3 weeks old) were extracted under anesthesia. For *in vitro* culture, the extracted teeth were divided into the two pieces and cultured for 0, 1, 3, and 7 days using the Trowel's method. For *in vivo* experiments, the extracted teeth were replanted in the original socket or the crown portion without roots were allogenic transplanted in the sublingual region of non-labeled animals.
Results: We succeeded to establish the *in vitro* culture system for the evaluation of the dentin-pulp complex regeneration, where most odontoblasts were occasionally degenerated and lost nestin-immunoreactivity because of the separation of cell bodies from cellular processes in the dentin matrix until the beginning of *in vitro* culture. Numerous dense LRCs were mainly resided in the center of the dental pulp associating with blood vessels throughout the experimental periods. On postoperative Days 1-3, the periphery of pulp tissue including the odontoblast layer showed the degenerative features, although some odontoblasts survived throughout the experimental periods. Until Day 7, nestin-positive odontoblast-like cells were arranged along the pulp-dentin border and dense LRCs were committed in the odontoblast-like cells. These chronological changes in the pulp-dentin border *in vitro* organ culture were similar to the changes in the *in vivo* experimental models.
Conclusions: These results suggest that dense LRCs in the center of the dental pulp associating with blood vessels were supposed to be dental pulp stem cells possessing regenerative capacity for forming newly differentiated odontoblast-like cells.


Dentin
Predentin
Odontoblasts
mild injury
Progenitors
severe injury
mild injury
Dental pulp stem cells

[Figure]

Dental Pulp

O40

Responses of BrdU-label-retaining dental pulp cells to allogenic tooth transplantation into mouse maxilla

Mutoh N.[1], Nakatomi M.[2], Ida-Yonemochi H.[2], Nakagawa E.[3], Tani-Ishii N.[1], Ohshima H.[2]
[1]Kanagawa Dental College, Division of Endodontics, Department of Oral Medicine, Yokosuka, Japan, [2]Niigata University Graduate School of Medical and Dental Sciences, Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata, Japan, [3]Yonsei University College of Dentistry, Division of Anatomy and Developmental Biology, Seoul, Korea, Republic of

Background: Allogenic tooth transplantation is now a common procedure in dentistry for replacing a missing tooth. However, there are many difficulties in clinical application of allogenic tooth transplantation because of immunological rejection. Recently, we have established the successful experimental animal model using mice for allogenic tooth transplantation into the maxilla (Anat Rec 292: 570-579, 2009). Furthermore, our recent study has demonstrated that BrdU given to the prenatal animals revealed the existence of slow-cycling long-term label-retaining cells (LRCs), putative adult stem cells, reside in the pulp tissue. This study aims to clarify responses of BrdU-label-retaining dental pulp cells to allogenic tooth transplantation into mouse maxilla using *in situ* hybridization for osteopontin (OPN) and periostin and immunocytochemistry for BrdU, nestin, OPN, and periostin. Furthermore, the relationship between donor and host cells in the healing process has been analyzed using GFP mice.
Methods: Two to 3 peritoneal injections of BrdU were given to pregnant Crlj:CD1(ICR) mice to map dense LRCs in the mature tissues of born animals. The labeled born animals at 2 weeks after birth were used for tooth transplantation. The upper-right first molars (M1) of BrdU-labeled and non-labeled mice (2 weeks old) were extracted under anesthesia, and the extracted teeth were allografted in the original socket in the non-labeled and BrdU-labeled mice, respectively, after the extraction of M1. Materials were collected in groups of animals at intervals of 1, 3, 5, 7 days, 2, 4, and 8 weeks after allogenic tooth transplantation. Furthermore, the allogenic tooth transplantation was performed between GFP and non-GFP mice. The upper-left M1 of the same animal was used as control.
Results: In the control group, nestin-immunoreactivity was exclusively expressed in the odontoblasts, and numerous dense LRCs were mainly resided in the center of the dental pulp of BrdU-labeled animals, associating with blood vessels. Tooth transplantation caused degeneration of the odontoblast layer, resulting in the disappearance of nestin-positive reactions in the dental pulp. On postoperative Days 1-3, the pulp chamber was mainly occupied by inflammatory lesions including numerous neutrophils, fibrin networks, and a hemorrhage. On postoperative Days 5-7, tertiary dentin formation commenced next to the preexisting dentin where nestin-positive odontoblast-like cells were arranged in the successful cases. Three types of healing patterns were recognized until Day 14: tertiary dentin, the mixed form of dentin and bone-like tissue formation, and immunological rejection. In the case of BrdU-labeled transplanted teeth, dense LRCs were maintained in the center of the dental pulp beneath the newly differentiated odontoblast-like cells, whereas LRCs disappeared in the area beneath the bone-like tissue. On the other hand, LRCs were not recognized in the pulp chamber of non-labeled transplants through the experimental period. Interestingly, the periodontal tissue recovered even in the case of immunological rejection in which the pulp chamber was replaced by sparse connective tissue. In such cases, the donor periodontal tissue was replaced by the host tissue.
Conclusions: These results suggest that the maintenance of BrdU-label-retaining dental pulp cells is the decisive factor for the regeneration of odontoblast-like cells in the process of pulpal healing following tooth transplantation.

Tooth Bone Interface

O41

The role of Wnt signaling for patterning of molar tooth roots in mammals

Ota M.S.[1,2], Nakahara T.[3], Ohazama A.[4], Schmidt-Ullrich R.[5], Kanri Y.[6], Kozawa Y.[7], Aoba T.[6], Sharpe P.T.[4], Kondo T.[2], Iseki S.[1]

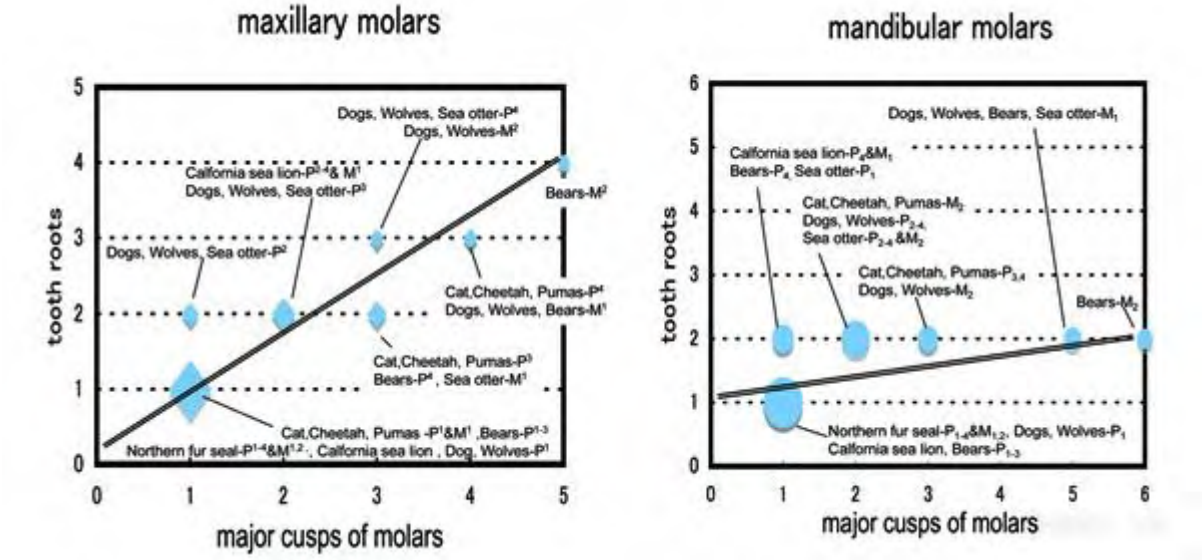
[1]Tokyo Medical and Dental University, Section of Molecular Craniofacial Embryology, Tokyo, Japan, [2]Brain Science Institute, RIKEN, Kondo Research Unit, Wako, Japan, [3]The Nippon Dental University, Section of Development and Regenerative Dentistry, Tokyo, Japan, [4]GKT Dental Institute, Guy's Hospital, Department of Cranifacial Development, London, United Kingdom, [5]Max-Delbrück-Center of Molecular Medicine, Berlin, Germany, [6]The Nippon Dental University, Department of Pathology, Tokyo, Japan, [7]Nohon University School of Dentistry at Matsudo, Matsudo, Japan

Background: The diet is one of the essential factors for survival. The tooth crown structure is adapted for diet, and thought to contribute to the diversification and adaptation of extant mammals. Importantly, the structure of tooth roots also varies to effectively support the applied force. Tooth morphogenesis is regulated by reciprocal interactions between the dental epithelium and odontogenic mesenchyme. Following tooth crown formation, the dental epithelium forms a double-layered Hertwig's epithelial root sheath (HERS) derived from inner and outer enamel epithelium. Previous studies have reported that HERS plays an important role in tooth root development. The morphology of tooth roots is considered to be determined by genetic and environmental factors, but basic information on the morphology of tooth root patterning and the molecular mechanism of root morphogenesis is largely unavailable in mammals.

Methods: The jaws of extant mammals were collected to examine correlation between the number of major tooth cusps and tooth roots in molar teeth. The jaws of Tabby, Downless, Crincle, and NFkBdN mutants indicating microdontia, and of Sostdc1 null, Lrp4 null mutants indicating macrodontia were collected and analysed by microCT. The tooth raw were dissected from Top-GAL and Axin2-LacZ mice at postnatal day 5, 6, 7, 8, 9, and 10 and performed whole-mount LacZ staining and histological analysis.

Results: Firstly, we studied about the correlation between the number of major cusps of the tooth crown and number of tooth roots of molars and last premolar teeth in several extant mammals. The number of molar tooth roots have strong coorelation in maxilla but weak in mandible. Next, similar analysis was performed with several mouse mutants indicating abnormal molar morphology, similar coorelation was identified. The dynamic change of LacZ-staining was observed on early development of molar root in Axin2-LacZ and TOP-GAL mice, and LacZ-positive tissues were identified as developing HERS.

Conclusion: The number of tooth roots was correlated to the major tooth cusps in extant mammals strongly in maxilla but weakly in mandible. The number of molar tooth roots are determined by the elongation pattertn of epithelial diagram of HERS in early development of tooth roots, and the staining pattern of HERS of Axin2-LacZ suggested that Wnt-signaling play important role for patterning of molar tooth roots.



[FIG.1 (OTA)]

Tooth Bone Interface

O42

Label-retaining epithelial cells in mouse epithelial cell rests of Malassez

Honda M.[1], Oka K.[2], Isokawa K.[1]
[1]Nihon University School of Dentistry, Department of Anatomy, Chiyoda-ku, Japan, [2]Fukuoka Dental College, Department of Anatomy, Fukuoka, Japan

Slow-cycling somatic stem cells have been identified in some tissues by their ability to retain bromodeoxyuridine (BrdU) label for long periods of time. Using these BrdU pulse-chase experiments, lineage-specific stem cells have been identified in epithelium such as skin, intestine, and cornea. However, epithelial stem cells have not yet been identified in ERM.
Epithelial cell rests of Malassez (ERM) are located in the periodontal ligament (PDL). ERM are clusters of epithelial cells that are derived from fragments of Hertwig's epithelial root sheath (HERS). Epithelial cell rests of Malassez in the PDL are thought to be completely quiescent. On the other hand, ERM actively proliferate when they are expanded *in vitro*, suggesting that some ERM are mitotically active.
This study was designed to identify putative epithelial stem cells in ERM, based on their retention of BrdU label *in vivo*. C57BL6 mice were injected subcutaneously with BrdU daily for three days. Tissues were obtained at postnatal day 5, 10, 32, and 90 (P5, P10, P32, and P90) to identify and characterize the label-retaining epithelial cells (LRECs) in the ERM. At P5, several BrdU-stained cells were detected in both inner and outer enamel epithelia in HERS. BrdU and cytokeratin double-stained cells were seen in the clusters of ovoid cells in the PDL at P10, P32, and P90. Furthermore, cytokeratin and Ki67 double-staining of cells with a proliferative activity was apparent in the clusters of ovoid cells in the PDL at P32. This study suggested that populations of LRECs that were quiescent, slow-cycling, or undergoing asymmetric division were identified in the ERM using BrdU pulse-chase experiments.

Tooth Bone Interface

O43

The biology of periodontally accelerated tooth movement

Gadban N.[1], Binderman I.[1]
[1]*Tel Aviv University, Oral Biology, Ramat Aviv, Tel Aviv, Israel*

It is apparent that tooth movement is enhanced by procedures that elevate the remodeling of alveolar bone, and of periodontal and gingival fibrous tissues. The periodontally accelerated osteogenic orthodontics (PAOO) involves full-thickness labial and lingual alveolar flaps accompanied with limited selective labial and lingual surgical scarring of cortical bone (corticotomy). Most of the authors suggest that the RAP is the major stimulus for alveolar bone remodeling, enabling the PAOO.We have shown that detachment of the bulk of dento-gingival and interdental fibers from coronal part of root surfaces by itself suffice to stimulate alveolar bone resorption mainly on its PDL surfaces which largely attributes to PAOO.Our results propose that unstrained gingival fibroblasts secrete ATP which activates P2X7 purinoreceptors in human gingival fibroblasts. Also, stanniocalcin-1 is upregulated which in turn stimulates Pi transport. It seems that extracellular ATP is modulating the pathway leading to alveolar bone remodeling. Moreover, marginal gingival fibroblast closely control the remodeling of alveolar bone. Therefore, by fiberotomy the physiological strain of marginal gingiva cells is reduced, thus activating alveolar bone resorption, this way increasing the efficiency of orthodontic tooth movement. Also, normal tension integrity of the marginal gingiva is important for normal function of the periodontium.

Tooth Bone Interface

O44

Fibrillin-1 is indispensable for normal collagen fiber architecture and gene expression in periodontal ligament

Suda N.[1,2], Ganburged G.[1,2], Saito M.[3], Yamazaki Y.[4,5], Isokawa K.[4,5], Moriyama K.[1,2]
[1]Tokyo Medical and Dental University, Maxillofacial Orthognathics, Tokyo, Japan, [2]Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan, [3]Tokyo University of Science, Research Institute for Science and Technology, Chiba, Japan, [4]Nihon University School of Dentistry, Department of Anatomy, Tokyo, Japan, [5]Nihon University School of Dentistry, Division of Functional Morphology, Dental Research Center, Tokyo, Japan

Background: Periodontal ligaments (PDLs) are a soft connective tissue between the cementum covering the tooth root surface and alveolar bone. PDLs are composed of collagen and elastic system fibers, blood vessels, nerves, and various types of cells. Elastic system fibers are distributed among various types of connective tissue where elasticity is required. Depending on the relative amount of elastin, they are classified into elastic, elaunin and oxytalan fibers. Oxytalan fibers, lacking elastin, are made up of bundles of 10-12-nm microfibrils which are predominantly composed of glycoproteins, fibrillin-1 and -2. In PDLs, the main elastic system fibers are oxytalan fibers oriented in an occluso-apical direction. Compared to collagen fibers, there is only limited information on microfibrils in PDLs. In order to clarify the unknown role of microfibrils in PDLs, mice underexpressing fibrillin-1 (MgR mice) were examined.
Materials and methods: Light and electron microscopic analysis was performed on PDLs of 2 to 8 week-old MgR and wild-type (WT) mice. *In situ* hybridization was carried out to examine the expression of PDL-related genes.
Results: In PDLs, homozygous mgR mice had only one quarter of the elastic system fibers compared to WT mice. Electron microscopic observation showed that microfibrils were seen adjacent to each PDL-cell, suggesting a close relationship between the cell and the microfibril in both WT and MgR mice. An association between the elastic system fibers and capillaries was noted in wild-type (WT), homozygous, and heterozygous mgR mice, and capillaries in PDLs of homozygous mice were dilated or enlarged compared to those of WT mice. Interestingly, there were degenerative PDL-cells exclusively around blood vessels in homozygous mice but not in other genotypes. Next, structure of collagen fibers in mgR mice was examined. *In situ* hybridization showed that a comparable level of type I collagen, which is the most major collagen in PDLs, was expressed in PDL-cells of mice with three genotypes. Sections were stained with Picrosirius red solution and observed three dimensionally using a polarizing light microscope to examine the collagen fiber architecture in PDLs. The observation showed that well-organized definite collagen fiber bundles were seen in WT mice. In contrast, multi-oriented collagen fiber bundles with a thinner appearance were noted in homozygous mice. Moreover, there was a marked decrease in periostin expression in homozygous mice.
Conclusion: PDLs are well vascularized tissue, which reflects the high levels of cellular and extracellular constituents. The present study indicates the fibrillin-1 is required to maintain the normal vascularization. The exact reason for the abnormal collagen architecture in homozygous mice is unknown; however, it is interesting to speculate that the architecture is related to the suppressed expression of periostin, which is known to regulate collagen crosslinking and fibrillogenesis. The present study opens a new insight that the microfibrillar protein, fibrillin-1, is indispensable for the normal tissue architecture and gene expression in PDLs.

Tooth Bone Interface

O45

Regulation of osteoblast differentiation and ECM remodeling by BMP2/4 in vitro

Wu L.-A.[1,2], Feng J.[1], Mu Y.-D.[1], Gluhak-Heinrich J.[3], Donly K.J.[1], Wang L.[1], Harris S.[4], MacDougall M.[5], Chen S.[1]
[1]University of Texas Health Science Center at San Antonio, Pediatric Dentistry, San Antonio, United States, [2]The Fourth Military Medical University, Pediatric Dentistry, Xi-an, China, [3]Universit y of Texas Health Science Center at San Antonio, Orthodontics, San Antonio, United States, [4]University of Texas Health Science Center at San Antonio, Periodontics, San Antonio, United States, [5]University of Alabama, Oral Surgery, Birmingham, United States

Both of bone morphogenetic proteins 2 and 4 (Bmp2 and Bmp4) are two closely related members of the transforming growth factor beta superfamily and play diverse roles in normal and pathological processes. However, detail understandings of mechanisms through which Bmp2 and Bmp4 exert their effects remain elusive due to their functional compensations each other.
Objective: To study roles of Bmp2/Bmp4 in osteoblast differentiation and extracellular matrix (ECM) remodeling.
Methods: Bmp2/4 conditional mice with Cre recombinase recognition site (loxP) were isolated from calvarial osteoblasts and transfected with simian virus 40 large antigen to generate immortalized $BMP2^{C/C}4^{C/C}$ (iBMP2 $^{C/C}/4^{C/C}$) osteoblast lines. The BMP2/4 genes in the $iBMP2^{C/C}/4^{C/C}$ cells were double knocked out by Cre recombinase. Expression of bone-relate genes in $iBMP2^{C/C}/4^{C/C}$ knock-out (KO) cells were detected by immunohistochemistry, western blot and real time quantitative PCR analyses. ECM remodeling was also observed in fluorescent microscope.
Results: Expression of Runx2, Osx, AFT4, DMP1, OC and other genes were dramatically decreased in the $iBMP2^{C/C}/4^{C/C}$ KO cells compared to that of the $iBMP2^{C/C}/4^{C/C}$ osteoblasts. Cell differentiation and mineralization were also reduced in these KO cells by alkaline phosphatase and alizarin red S staining. Furthermore, Bmp2/4 double knock-out cells have major defects in remodeling the ECM as reflected by changes in collagen type I, IV and gelatin processing.
Conclusions: We for the first time demonstrate the establishment of immortalized floxed $Bmp2^{C/C}/4^{C/C}$ cells and Bmp2/4 double null $iBmp2^{C/C}/4^{C/C}$ cells Expression of bone-relate genes and cell differentiation in the $iBmp2^{C/C}/4^{C/C}$ KO cells were decreased. Furthermore, ECM processing in these KO cells was impaired. This indicates the BMP2/4 play important roles in osteoblast differentiation and ECM remodeling.

Tooth Bone Interface

O46

Non redundant role of Msx1 homeogene on neurectodermal osteogenesis

Berdal A.[1,2], Meary F.[1], Babajko S.[1], van den Boogaard M.-J.H.[3]
[1]Centre de Recherche des Cordeliers, Inserm UMRS-872, Equipe 5, Universités Paris 5, 6 and 7, France, Paris, France, [2]Reference Center for Rare Buccal and Facial Malformations, Odontology Unit, Hôtel-Dieu Hospital, AP-HP, Paris, France, [3]University Medical Center Utrecht, Department of Medical Genetics, Utrecht, France

Background: Anodontia and cranio-facial dysmorphology characterise Msx1 -/- null mutant mice. Long bone defects require additional Msx2 gene invalidation. The proper Msx1 impact on osteogenic differentiation was studied here. The divergent cell origin (mesoderm/neurectoderm) or site (limb/maxilla) were taken into account.
Methods: Histology, Von Kossa staining and µCT were realised at 18 days of gestation and birth in Msx1 -/- (versus Msx1 +/- and Msx1 +/+ controls). Osteogenic and chondrogenic markers were screened in microdissected oral (maxilla and mandible without teeth) and tibial bones by RT-qPCR and in situ. In vitro, cell-cycle phases were studied under transient Msx1 overexpression.
Results: Msx1 was shown to alter cell differentiation program: in vitro, Msx1 overexpression induced G0/G1 phase increase. In the reverse in vivo situation (Msx1 -/- mice), late bone differentiation markers were up-regulated and mineralization, enhanced. These defects were significant in oral osteoblasts where Msx2 expression was interestingly found Msx1-dependent.
Discussion: Our results showed a non-redundant role of Msx1 on oral osteoformation. This indicates that a proper regulation of homeoprotein expression levels is independently critical for tooth and bone morphogenesis. Such a proposal was revisited by the analysis of a Utrecht/Paris cohort. Oral phenotype variability (oligodontia/clefts) were relied to specific Msx1 RNA expression levels and gene mutations.

Craniofacial Biology And Tissue Engineering

O47

The planar cell polarity effector gene, *Fuz* is essential for craniofacial and tooth development

Zhang Z.[1], Venugopalan S.[1], Wlodarczyk B.J.[1], Finnell R.H.[1], Amendt B.A.[1]
[1]*Texas A&M Health Science Center, CEGM, Houston, United States*

Background: *Fuz* has been identified as a planar cell polarity effector gene and shown to be involved in ciliogenesis. We asked if *Fuz* expression was critical for craniofacial and tooth development.
Methods: *Fuz*$^{-/-}$ mutant mice and mouse embryo fibroblasts were used to analyze the effect of *Fuz* expression on tooth development and gene expression pathways, respectively. Immunohistochemical, molecular and biochemical assays were used to understand the defects and molecular mechanisms underlying the phenotype of the *Fuz*$^{-/-}$ mice.
Results: The *Fuz*$^{-/-}$ mutant mice exhibit massive craniofacial deformities such as a hypoplastic mandible, a complete lack of upper and lower incisor development, malformed molars, hyperplastic Meckel's cartilage and missing eyes and tongue. *Fuz* has an essential role in ciliogenesis, and the primary cilium has been shown to repress canonical Wnt signaling. We demonstrate that canonical Wnt signaling is up regulated in *Fuz* mutant mice, shown by increased β-catenin expression. Given the critical function of Wnt signaling in craniofacial development, we believe that this up-regulation of Wnt signaling results in hyperplasic cartilage formation and disruption of incisor and tongue development as well. Over-expression of *Fuz* represses the Topflash reporter demonstrating a role for Fuz in canonical Wnt signaling. The *Fuz* 2.4kb promoter contains eleven Wnt response elements, and LiCL treatment can activate both Fuz 2.4kb and 1.1kb promoter constructs and endogenous *Fuz* in cell cultures. We have identified several transcription factors that are regulated indirectly by Fuz through Wnt and Shh signaling mechanisms, which are associated with specific craniofacial developmental processes.
Conclusions: These data suggest that *Fuz* is involved in a negative feedback loop of canonical Wnt signaling regulation, and this feedback loop is essential for precise regulation of craniofacial and tooth development.
Support for this research was provided from grant DE13941 from the National Institute of Dental and Craniofacial Research.

Craniofacial Biology And Tissue Engineering

O48

Interactions between Gas1, Cdo and Boc during early development of the craniofacial midline and dentition

Seppala M.[1], Sharpe P.T.[1], Cobourne M.T.[1]
[1]*Kings College London, London, United Kingdom*

Background: Sonic hedgehog (Shh) is a secreted signaling molecule essential for normal craniofacial development. Disruptions within the Shh signalling pathway can result in Holoprosencephaly (HPE), a developmental disorder characterized by failure of the forebrain to divide and varying degrees of midline facial anomalies. Gas1, Cdo and Boc are membrane proteins able to bind to Shh and regulate transduction of the signal. Both *Gas1*$^{-/-}$ and *Cdo*$^{-/-}$ mice exhibit disrupted craniofacial midline patterning and microform HPE as a result of reduced Shh signalling activity; however, Boc$^{-/-}$ mice have normal craniofacial structures. Interestingly, in the absence of Gas1 function, supernumerary premolar teeth also form in the diastema region of both jaws with complete penetrance.
Methods: We have investigated the relationship between *Gas1*, *Cdo* and *Boc* during early craniofacial development using phenotypic analysis of varying combinations of *Gas1/Cdo* and *Boc* mutant mice.
Results: We find combinatorial expression of these genes within overlapping domains in the embryonic head. Loss of a single allele of either *Gas1* or *Cdo* on a *Gas1*$^{-/-}$ or *Cdo*$^{-/-}$ background respectively, results in exacerbation or increased penetrance of the midline craniofacial phenotype. Moreover, *Gas1*$^{-/-}$/*Cdo*$^{-/-}$ mice display a severely dysmorphic craniofacial midline, with many structures either synostotic or absent. In contrast, the loss of *Boc* does not affect gross craniofacial development in combination with either *Gas1* or *Cdo*. However, whilst an important individual role and strong interaction was demonstrated between *Gas1* and *Cdo*, but not *Boc* during midline craniofacial patterning, different requirements amongst all these genes were clear during tooth development. Whereas *Gas1*$^{-/-}$ mice display both maxillary and mandibular supernumerary premolar teeth, tooth morphology and number in both *Cdo*$^{-/-}$ and Boc$^{-/-}$ mice was normal.
Conclusions: This phenotypic analysis has demonstrated a strong interaction between *Gas1* and *Cdo* that influences Shh signaling activity during early craniofacial and tooth development. However, these requirements appear to vary between different regions of the craniofacial complex during development.

Craniofacial Biology And Tissue Engineering

O49

Variation of tooth root lengths in human X chromosome aneuploids

Lähdesmäki R.E.[1,2], Alvesalo L.J.[1], Kvantti Research Project
[1]Institute of Dentistry, University of Oulu, Oral Development and Orthodontics, Oulu, Finland, [2]University Hospital of Oulu, Dental and Oral Diseases, Oulu, Finland

Background: Human tooth development is a continuous process from primary to permanent teeth, the permanent molars developing as clones from the second primary molars. Teeth are single organs and develop from the tip of the crown to the root, and their size is final once the root apex has closed. Murine studies have shown that separate homeobox-containing genes and signalling molecules are expressed in the anterior and posterior fields during dental development. Mutations in tooth-specific genes usually affect all teeth of the same type in mice, but in humans the teeth in the clone (ICM shape series) that develop later are the first to be absent. Tooth patterning in humans seems to be more complex, indicating that each clone is separately controlled. More than 300 genes are now known to be involved in tooth development. Human sex chromosomes also influence human tooth root lengths, men having longer roots than women. The aim of this study was to compare and describe the variation of the X chromosome influence on tooth root lengths in human X chromosome aneuploids.
Methods: Permanent tooth root lengths in 56 normal women were compared with those in seven 47,XXX, three 48,XXXX and fifteen 45,X/46,XX females studied in the Kvantti research project headed by Professor Alvesalo. Measurements were made on panoramic radiographs of the permanent teeth (except third molars) on both sides of the jaws available from three 48,XXXX females and three female relatives using a digital calliper and following established procedures. The other root lengths, measured in the same manner, have been published earlier.
Results: The influence of the X chromosome on tooth root lengths differed between the maxilla and mandible in all the groups studied. Its additive effect of the X chromosome was obvious in the mandible and was larger anteriorly. In the maxilla the root lengths with one extra X chromosome were similar to those in normal women but those with two extra X chromosomes were posteriorly longer than in any other group, and while root development was inhibited anteriorly. A lack of sex chromosome material had an overall inhibiting effect on root length development.
Conclusions: The results showed variations between the jaws and between the anterior and posterior regions of the permanent maxillary dentition in the tooth root length differences between X chromosome aneuploids and normal women. It is suggested that the genetic influence of the X chromosome on permanent tooth root development is mediated by a number of inductive and inhibiting agents.
Keywords: Aneuploidy, chromosomes human X, dental field, growth and development, tooth root

Craniofacial Biology And Tissue Engineering

O50

Characterization of two novel genes expressed in tooth and associated epithelia

Moffatt P.[1,2], Lopez Di Armas N.E.[2], Wazen R.[2], Smith C.E.[2], Nanci A.[2]
[1]Shriners Hospital for Children / McGill University, Human Genetics, Montreal, Canada, [2]Université de Montréal, Faculté de Médecine Dentaire / Laboratory for the Study of Calcified Tissues and Biomaterials, Montréal, Canada

Background: Our group previously conducted a functional genomic screen for cDNAs encoding secreted and membrane proteins in rat enamel organs (EOs). This allowed us to identify and characterize Amelotin and Apin (Odam) as novel secretory products of maturation stage ameloblasts and junctional epithelial (JE) cells. We present here two additional novel genes expressed in the tooth referred to as clones *EO063* and *EO463*.
Methods and results: Northern blotting on a panel of RNAs extracted from rat tissues reveals that the expression of *EO063* and *EO463* is restricted to tooth. In microdissected strips of rat EOs, transcripts of *EO063* are more abundant in the secretory stage, whereas those of *EO463* are highly enriched during the maturation stage. In silico data mining indicates that *EO063* matches a mouse Riken clone (5430401F13). The gene is present in 3 copies in the mouse (chr. 6F3) and in a single copy in the rat (chr. 4q42), but it is apparently absent in human. *EO063* encodes for a secreted protein having 145 residues and a C-terminal enriched in glycine, glutamine, and lysine. To understand the function of EO063 in vivo, we generated a transgenic mouse model overexpressing Flag-tagged-EO063 under the ameloblastin promoter. No obvious alterations on molars and incisors were observed. This was probably due to the fact that EO-063-Flag protein expression was below immunodetectable levels despite obvious transgenic expression at the mRNA level. Hence, this model did not allow us to determine the role of EO063 in tooth formation and mineralization. The *EO463* clone does not correlate to any known sequences available in various databases. The gene encoding *EO463* is also not annotated in existing genomes and is considered totally novel. Cloning of the cDNA for *EO463* allowed us to precisely map the gene and to determine its chromosomal architecture. The *EO463* gene resides within the so-called 'secretory calcium binding phosphoprotein' (SCPP) cluster. In several mammal genomes EO463 contains 10 exons transcribed in the same orientation as the adjacent *SPARCL1* gene. Very recently, Kawasaki (Dev. Genes Evol. (2009) 219:147-157) identified EO463 gene by in silico cloning and named it *SCPPPQ1*. EO463 is a highly conserved short 75 residue long secreted protein rich in proline, leucine, glutamine and phenylalanine. Using an antibody generated against the bacterial recombinant protein, EO463 was strongly immunodetected along the apical surfaces of maturation stage ameloblasts, in line with its gene expression profile. No or very little signal was found over the enamel layer suggesting it does not accumulate to any significant extent within the calcified matrix. The only other site immunoreactive for EO463 was the JE which attaches to erupted tooth surfaces. The immunolabeling pattern for EO463 in the EO and JE is reminiscent of Amelotin and Apin.
Conclusion: Together with their respective sequence features, clustering and phylogenic evolution within the SCPP locus, their expression at a developmental time when the enamel layer becomes almost fully mineralized is consistent with the intriguing possibility that Amelotin, Apin and EO463 may have evolved as “hypermineralizing” molecules. Their presence at the cell-matrix interface where an atypical basal lamina is present further suggests that these unique molecules may be part of the multimolecular complex involved mediating attachment of epithelia to mineralized tooth surfaces.

Craniofacial Biology And Tissue Engineering

O51

Genomic copy number alteration analysis of sporadic and Gorlin-syndrome associated keratocystic odontogenic tumour (odontogenic keratocyst)

Heikinheimo K.[1], Morgan P.R.[2], Jee K.J.[3], Leivo I.[3], Berdal A.[4], Bouattour A.[4], Ruhin-Poncet B.[4], Knuutila S.[3]
[1]Institute of Dentistry, University of Turku, Oral and Maxillofacial Surgery, Turku, Finland, [2]King's College London, Clinical and Diagnostic Sciences, London, United Kingdom, [3]University of Helsinki, Department of Pathology, Helsinki, Finland, [4]Centre de Recherche des Cordeliers, Laboratoire de Physiopathologie Orale, Paris, France

Background: Apart from reported aberrations in the PTCH1 gene (9q22-31), very little is known about the genomic structural rearrangements in sporadic and Gorlin syndrome (basal cell nevus syndrome, BCNS, OMIM #109400) associated keratocystic odontogenic tumour (KCOT). The aim of the present study was to find out deletions and duplications in the genome, collectively referred to as copy number alterations (CNA), in KCOT. Further aim was to find out potential differences in the CNA regions between the sporadic and syndrome associated forms of the tumour to aid their differential diagnosis.
Methods: DNA originating from 20 sporadic and seven Gorlin-syndrome associated KCOT samples were included in this study. All samples were hybridized on Genome-Wide Human SNP arrays 6.0 (Affymetrix®) and the chromosomal regions with copy number changes (gains and deletions) were analyzed using Genotyping Console 3.0 (Affymetrix®) software. Additional analyses included loss of heterozygosity (LOH). Principal component analysis (PCA), hierarchical clustering and Pearson correlation coefficient were adopted for bioinformatics analyses.
Results: Gorlin-associated KCOT samples exhibited more CNA and LOH regions than the sporadic tumours. PCA and hierachical clustering mapped most of the sporadic tumours and three Gorlin-associated tumours together indicating similarity in their molecular pathogenesis, whereas the remaining four Gorlin-associated tumours formed its own cluster.
Conclusions: Gorlin-associated KCOT shows more genomic aberrations than sporadic KCOT, which is in accordance with its more aggressive behaviour. Furthermore, neither sporadic nor Gorlin-associated KCOT appears to share common CNA regions, which could have offered an option for future discovery of a diagnostic biomarker.
Keywords: DNA segments, genome-wide studies, molecular biology, intermediate-scale, structural abnormalities, odontogenic tumours

Craniofacial Biology And Tissue Engineering

O52

Tooth engineering: Organization of dental matrices in implanted dental cell-cell reassociations

Nait Lechguer A.[1], Couble M.-L.[2], Labert N.[3], Kuchler-Bopp S.[1], Keller L.[1], Magloire H.[2], Bleicher F.[2], Lesot H.[1]
[1]University of Strasbourg, UMR INSERM 977 and Dental School, Strasbourg, France, [2]University of Lyon, IGFL CNRS UMR 5242, Ecole Normale Supérieure, Lyon, France, [3]University of Lyon, IGFL CNRS UMR 5242, Ecole Normale supérieure, Lyon, France

Background: Embryonic dental cells have been used to set up an experimental approach for tooth engineering (1) and check a set of criterions to be achieved for this purpose. We have successively answered a series of constraints to be respected in order to obtain crown morphogenesis, epithelial histogenesis, organ vascularization, as well as root and periodontium development (1,2,3). The purposes of the present work were to investigate the organization of the different dental matrices (predentin/dentin, enamel and cementum), which form and mineralize after implantation of cultured cell-cell reassociations, and compare these matrices to those forming after implantation of cultured tooth germs.
Methods: First lower molars from ED14 mouse embryos were dissected and dissociated in epithelium and mesenchyme using trypsin as previously published (2). For each tissue, cells were further dissociated as single cells. Pellets of epithelial cells were cultured in contact with mesenchymal cells for 8 days on a semi-solid medium and implanted for two weeks under the skin of adult ICR mice (1,3). These implants were processed for histology and TEM. For comparison, molar tooth germs were cultured for 6 days before implantation.
Results: After two weeks of implantation, the cultured cell reassociations were fully re-vascularized as cultured tooth germs (3). In the reassociations, gradients of odontoblasts differentiated. These cells were in close contact with capillaries. They were ciliated, polarized and extended cell process in predentin and dentin. This context allowed the induction of ameloblast functional differentiation. These cells accumulated enamel, which also mineralized. Enamel crystals organized in implanted cell-cell reassociations as in tooth germs. Finally in the developing root, odontoblast differentiation proceeded, cementogenesis occurred, and periodontal ligament fibers could form and interact with the root surface and newly formed bone. This was observed in both types of implants.
Conclusion: This work shows that the implantation of cultured dental cell-cell re-associations allows reproducing a correct functional differentiation of odontoblasts, ameloblasts and cementoblasts. The same will have to be searched for, in reassociations where one dental compartment is replaced by non-dental cells.
References:
1) Hu B, Nadiri A, Kuchler-Bopp S, Perrin-Schmitt F, Peters H, and Lesot H. Tissue engineering of tooth crown, root and periodontium. Tissue Engineering, 2006, 12, 2069-2075.
2) Hu B, Nadiri A, Bopp-Kuchler S, Gendrault JL and Lesot H. Dental epithelial histo-morphogenesis in vitro. J. Dent. Res., 2005, 84, 521-525.
3) Nait Lechguer A, Kuchler-Bopp S, Hu B, Haïkel Y, and Lesot H. Vascularization of engineered teeth. J Dent Res. 2008, 87, 1138-1143.

Evolution And Development

O53

Of teeth, pouches and pores: the ectoderm/endoderm connection in development and evolution

Huysseune A.[1], Witten P.E.[1]
[1]*Ghent University, Biology Department, Ghent, Belgium*

In basal osteichthyans, teeth are distributed throughout the oropharynx, including the roof of the oral cavity, and the surface of the hyoid and five more posterior pharyngeal arches. In actinopterygians and sarcopterygians, teeth became restricted to the mandibular and to the last pharyngeal arch (constituting the oral and pharyngeal dentition, respectively). We postulate that the presence of oral and pharyngeal teeth in the various osteichthyan lineages is linked to the potential of competent tooth-forming ectoderm to penetrate into the oropharyngeal cavity via regular openings, such as the mouth, the spiraculum, the opening of the nasopharyngeal duct or of the pharyngeal pouches (gill slits), and we propose that the ectoderm acts instructively or permissively in tooth formation. The fossil record presents many examples that support this view.
Zebrafish (*Danio rerio*), an increasingly popular model to study non-mammalian teeth, possesses pharyngeal teeth only. We use zebrafish to monitor the sequence of events and the cellular changes that characterise the formation of the endodermal pharyngeal pouches and their contact with the ectoderm (skin). Contacts are established already at 24 hrs post-fertilisation as an extremely flattened epithelial sheet connecting the two body sides, followed by substantial proliferation and shape changes within this epithelium. Pharyngeal tooth formation precedes cavitation within this solid epithelial strand. Zebrafish *van gogh* mutants, defective in the transcription factor *tbx1*, display impaired pouch formation (Piotrowski et al., 2003). Interestingly, these mutants show defects in their pharyngeal dentition, coinciding with the level of impairment of ectodermal-endodermal contacts. These data are in support of our hypothesis that the loss of ectodermal-endodermal contacts, and thus of (open) gill slits, could be responsible for the evolutionary loss of pharyngeal teeth in tetrapods (Huysseune et al. 2009).
Huysseune et al. 2009. *J. Anat.* 214: 465-476
Piotrowski et al. 2003. *Development* 130: 5043-5052

Evolution And Development

O54a

Molecular regulatory mechanism of dental root development.

Chai Y.[1], Huang X.[1], Mayo J.[1]
[1]*Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, USA*

During tooth development, dental epithelial cells, known as the Hertwig's epithelial root sheath (HERS), participate in root formation following crown development. However, the functional significance of HERS and the molecular regulatory mechanism of root development are still largely unknown. In our recent study, we investigated the mechanism of Smad4-mediated BMP signaling in HERS in regulating root development. Tissue-specific inactivation of *Smad4* in HERS results in abnormal enamel and dentin formation in *K14-Cre;Smad4*$^{fl/fl}$ mice. HERS enlarges but cannot elongate to guide root development without Smad4. At the molecular level, BMP signaling determines the number of roots in a developing tooth. Smad4-mediated TGF-β/BMP signaling is required for *Shh* expression in HERS and *Nfic* (nuclear factor I c) expression in the CNC-derived dental mesenchyme. *Nfic* is crucial for root development and loss of *Nfic* results in CNC-derived dentin defect similar to the one of *K14-Cre;Smad4*$^{fl/fl}$ mice. Significantly, we show that ectopic Shh induces *Nfic* expression in dental mesenchyme and partially rescues root development in *K14-Cre;Smad4*$^{fl/fl}$ mice. Furthermore, Smad4 also regulates other important growth factor signaling during the interaction between HERS and the CNC-derived dental mesenchyme to control root formation. The continuous epithlial-mesenchymal interaction controls the size, shape and number of tooth root. This study was supported by National Institute of Dental and Craniofacial Research (NIDCR), NIH.

Evolution And Development

O55

Signaling centers in mouse embryonic mandible reflect an ancestral tooth pattern

Prochazka J.[1,2], Pantalacci S.[3], Churava S.[1,4], Rothova M.[1,2], Lambert A.[3], Lesot H.[5], Klein O.[6], Peterka M.[1,4], Laudet V.[3], Peterkova R.[1]

[1]Institute of Experimental Medicine AS CR, v.v.i., Department of Teratology, Prague, Czech Republic, [2]Faculty of Science, Charles University, Department of Developmental Biology, Prague, Czech Republic, [3]Institut de Génomique Fonctionnelle de Lyon, Universite de Lyon, Universite Lyon 1, CNRS, INRA, Ecole Normale Supérieure de Lyon, Lyon, France, [4]Faculty of Science, Charles University, Department of Anthropology and Human Genetics, Prague, Czech Republic, [5]University of Strasbourg, INSERM UMR 977 and Dental School, Strasbourg, France, [6]University of California, San Francisco, Program in Craniofacial and Mesenchymal Biology, Departments of Orofacial Sciences and Pediatrics, San Francisco, United States

Background: Mouse molar development has long been an important experimental model. Although mice have only three molars and no premolar, two large rudimentary (vestigial, diastemal) buds transiently develop in both maxilla and mandible of mouse embryos in front of molars, and have been correlated with premolars lost during mouse evolution (Peterkova et al, 2000). Since the transient development of these rudimentary buds has been claimed for years only on the basis of morphological criteria, we used an experimental approach to further support their existence and their dental origin in the mouse embryonic mandible, where they are called MS and R2.
Methods: We used Shh reporter mice, whole mount in situ hybridization, histology and 3D reconstructions in mouse embryonic mandible. The mouse embryos were precisely staged on the basis of day of embryonic development (ED) combined with the wet body weight of embryos (Peterka et al., 2002).
Results: The Shh signal was co-localized with the rudimentary buds on frontal sections and 3D reconstructions made from the whole mount in situ hybridized mandibles. These data showed that each rudimentary diastemal bud has its own signaling centre with a transient Shh activity. These Shh-signaling centres appeared sequentially at embryonic day (ED) 12.7 and 13.3, and the duration of the Shh expression could be determined on the basis of a range of embryonic body weight: MS at ED12.7, range 65 - 100 mg, R2 at ED13.3, range 125 - 160mg. The Shh expression in the primary enamel knot of the first molar (M1 pEK) started at ED 14.3, in embryos heavier than 210mg. Both MS and R2 signaling centers also expressed other markers that have been reported in the signaling center M1 pEK (Jernvall et al, 1994).
Conclusion: These data document that the two rudimentary diastemal buds are tooth primordia which exhibit their own transient signaling centres, develop in the mouse embryonic mandible anterior to and before the forming M1, and thus reflect the tooth pattern in the ancestors of muroids.
Acknowledgement: Financial support by the Grant Agency of the CR (grant 304/07/0223) and by the MSMT CR (MSM 0021620843).
References:
Peterková R, Peterka M, Viriot L, Lesot H (2000). Dentition development and budding morphogenesis. J Craniofac Genet Dev Biol 20:158-72.
Peterka M, Lesot H, Peterkova R (2002). Body weight in mouse embryos specifies staging of tooth development. Connect Tissue Res 43:186-190.
Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I (1994). Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: non-dividing cells express growth stimulating Fgf-4 gene. Int J Dev Biol 38:463-469.

Evolution And Development

O56

Evolutionary analysis of enamelin in mammals, sauropsids and amphibians provides new insights on its function

Delgado S.[1], Al-Hashimi N.[1], Lafont A.-G.[2], Kawasaki K.[3], Sire J.-Y.[1]
[1]Université Pierre et Marie Curie, UMR 7138, Paris, France, [2]Université Pierre et Marie Curie, Paris, France, [3]Pennsylvania State University, University Park, United States

Enamelin (ENAM) plays an important role in the mineralization of the forming enamel matrix but the functions of its different regions and residues are still poorly understood. Here, we have compiled the results of an evolutionary analysis of mammalian ENAM and the characterization, for the first time, of *ENAM* in non mammalian vertebrates. The main objectives of these studies were to
(i) identify highly conserved residues or regions that could have important function (selective pressure),
(ii) predict mutations that could be associated with *amelogenesis imperfecta* in humans,
(iii) reveal possible adaptive evolution of ENAM during 200 millions years (Ma) of mammalian evolution, and
(iv) improve our understanding of ENAM origin and evolution. Indeed, although ENAM was known only in mammals, previous molecular analyses strongly suggested that this protein was present early in vertebrates. This finding was supported both by the ENAM relationships with the other enamel matrix proteins (amelogenin and ameloblastin) and the presence of enamel/enameloid tissues in early jawless vertebrates, 450 Mya.
We have obtained the sequences of
(i) *ENAM* mRNA in two reptiles, a lizard and a crocodile, and in a frog, and
(ii) the gDNA of frog and lizard *ENAM*.
Using these sequences we have been able to find the putative sequence of *ENAM* pseudogene in the chicken genome.
Taken together our results show a remarkable conservation pattern in the region of the 32 kDa fragment of ENAM, especially its phosphorylation, glycosylation and proteolytic sites. In primates and rodents, we have also identified several sites under positive selection, which could indicate recent evolutionary changes in ENAM function. Twenty five amino acids have been conserved unchanged during 350 Ma of tetrapod evolution. These data provide new directions for further investigation of ENAM functions and reveal several positions that are predicted to lead to *amelogenesis imperfecta* when changed.
Furthermore, our studies indicate
(i) the recruitment, in mammals, of an unusual signal peptide that provides new insights on the possible regulation of ENAM secretion,
(ii) the presence of an additional coding exon 8b in the common amniote ancestor, and
(iii) many sequence variations in the large exons compared to conserved regions in the small exons encoding the N-ter of the protein.
Furthermore, lizard *ENAM* was identified in a region that was not expected when considering gene synteny in mammals. This discovery allowed to find the *ENAM* pseudogene in an homologous region of the chicken genome. In contrast in the frog the enamel gene cluster is conserved in a similar synteny as in mammals. This result indicates that the enamel gene cluster was probably translocated to another chromosome in an ancestor of the sauropsid lineage.
Our studies indicate that *ENAM* origins have to be found earlier in vertebrate evolution and *ENAM* invalidation in chicken confirms that this gene encodes a tooth specific protein.

Evolution And Development

O57

The evolution and development of the mammalian dentition: Insights from the marsupial *Monodelphis domestica*

Moustakas J.E.[1,2], Smith K.K.[3], Hlusko L.J.[1]
[1]*University of California, Berkeley, Integrative Biology and Museum of Paleontology, Berkeley, United States,* [2]*Institute of Biotechnology, University of Helsinki, Helsinki, Finland,* [3]*Duke University, Biology, Durham, United States*

To understand developmental mechanisms of evolutionary change, we must first know how different morphologies are formed. The vast majority of our knowledge on the developmental genetics of tooth formation derives from studies in mice, which have only molars and incisors and only one tooth generation. In contrast, the marsupial *Monodelphis domestica* has a heterodont dentition with incisors, canines, premolars, and molars on both the upper and the lower jaws. The complexity of the *M. domestica* dentition ranges from simple, unicusped incisors to conical, sharp canines to multicusped molars.
Here, we present data on the development of the teeth in *M. domestica* that reveals the normal program of development of these teeth as compared to mouse wild-type and mutant dentitions. We show that the tooth germs of *M. domestica* express fibroblast growth factor (FGF) genes and Sprouty genes in a manner similar to wild-type mouse molar germs, but with a few key differences. We find differences in the expression of *Fgf10* and *Sproutys 2* and *4* along the tooth row of *M. domestica* as compared to mouse and hypothesize that these differences are attributable to differences in tooth shape.

Evolution And Development

O58

Developmental mechanisms in the evolution of morphological key innovation in small mammals

Renvoisé E.[1], Evans A.[2], Jebrane A.[3], Labruère C.[3], Laffont R.[4], Montuire S.[4,5], Jernvall J.[1]
[1]University of Helsinki, Institute of Biotechnology, Helsinki, Finland, [2]Monash University, School of Biological Sciences, Victoria, Australia, [3]Mathematical Institute of Burgundy, UMR 5584 CNRS, Dijon, France, [4]University of Burgundy, UMR 5561 CNRS, Dijon, France, [5]Ecole Pratique des Hautes Etudes, Paléobiodiversité et Préhistoire, Dijon, France

Since mammalian appearance in the Triassic period, different morphological key innovations in mammalian tooth evolution were involved in their adaptive radiation 65 My ago (for example, strengthen of the enamel structure, cusps appearance, hypsodonty…). Despite the more recent evolutionary origins of voles (5-6 My), they show all the features of an explosive radiation leading to the appearance of modern voles 2 My ago. In temperate climates, voles supplanted mice species by a rapid ecological and morphological diversification. We have already demonstrated that, during this radiation event, vole tooth evolution is characterized by a sudden and stable elongation of the first lower molar (m1). Moreover, this radiation event is contemporaneous with the onset of the Pliocene glacial/interglacial cycles. There is an ongoing debate about the factors affecting phenotypic evolution between developmental constrains and environmental pressure.
Our aim is to understand the developmental mechanisms involved in the m1 elongation of voles. Two first objectives are developed in this direction:
1) which specific genes can be expressed in the m1 anterior part of voles? and
2) which growth factors are involved in the elongation of the m1 anterior part of voles?
The first objective is studied through gene expression experiments with *in situ* hybridization of the molars at early embryonic developmental stages (E13 and E14). Results of this first objective are used to perform *in vitro* experiments to functionally target the main growth factors responsible in the m1 elongation of voles. These experiments will help to uncover developmental mechanisms that allow rapid evolutionary change in small mammals.

Evolution And Development

O60

How bats reach perfect occlusion? Late odontogenesis of tribosphenic molar

Spoutil F.[1], Horacek I.[2]
[1]*University of South Bohemia, Deparment of Zoology, Ceske Budejovice, Czech Republic,* [2]*Charles Uninversity, Department of Zoology, Prague, Czech Republic*

Background: The vast majority of odontogenetical studies are concerned with the early stages of molars and incisors of mouse. Little attention was paid to developmental dynamics of the tribosphenic molar, i.e. the tooth type ancestral to all diverse types of mammalian molar teeth. The complicated system of crests and cusps and perfect pattern of interlocking between the occluding upper and lower teeth are perhaps the most prominent characteristics of the tribosphenic dentition.
Methods: To understand the way the interlocking pattern is established, we studied postnatal dental development (including premature enamel coat) in a bat, *Myotis myotis*, from newborns where molars were already in bell stage, enamel maturation was not finished and tooth eruption was about to start, to the stage of completely established adult dentition pattern. The complete series covered 98 juvenile individuals (mostly of known age). We studied them with the aid of detailed craniometry and scanning electron microscopy.
Results & conclusion: We found that
(i) molars exhibit a considerable expansion in size during their eruption,
(ii) mutual position of particular molar elements (cusps, crests) is not entirely fixed until the moment of eruption what together with
(iii) delayed enamel maturation make teeth flexible and capable to refine the crest interlocking pattern during first occlusion efforts.
(iv) The eruption of lower molars (especially trigonids) is faster and their posteruptional dimensions are more constant, what suggests they can serve as the template for the upper molars.

Evolution And Development

O61

Tooth replacement in *Xenopus tropicalis*: A WNT-WNT situation

Willems M.[1], Vleminckx K.[2], Huysseune A.[1]
[1]*Ghent University, Biology, Ghent, Belgium,* [2]*Ghent University, VIB, Department for Molecular Biomedical Research, Ghent, Belgium*

The most popular model to study tooth development, the mouse, has an evolutionary very derived dentition in which teeth are never replaced. To understand the process of continuous tooth replacement which characterizes all non-mammalian vertebrates, an experimental model is required for which a large and diverse set of genetic and molecular tools is readily available. The frog *Xenopus tropicalis* has recently emerged as a popular model to address developmental questions. For instance, the WNT pathway, suggested to play a crucial role during continuous tooth replacement (Huysseune & Thesleff 2004), can be visualized using a transgenic approach and studied functionally by gain- and loss-of-function strategies. Although the dentition of *X. tropicalis* in many ways resembles that of its close relative *X. laevis*, aspects relevant to the tooth replacement process have not been well documented. Such knowledge is nevertheless required to correctly interpret data from functional studies. Here we describe tooth formation and replacement in *Xenopus tropicalis* using histological sections, focusing on the identification of the dental lamina, and mapping the epithelial connectivity. A multi-layered dental lamina, running parallel with the oral epithelium, links all teeth (whether developmental stages or functional teeth) to each other and to the oral epithelium. Also, another cell layer links the outer dental epithelia of a functional tooth and its successor within a tooth family. We discuss the role of these different cell layers during tooth replacement.
In addition, we have analyzed the activity of the WNT pathway using a new transgenic reporter line. Preliminary results suggest activation of the pathway in early cytodifferentation, but not in initiation stages of tooth germs. We discuss the potential role of the WNT pathway during continuous tooth replacement in this species and elaborate on the similarities and differences to what is known on tooth development in other model organisms.
Huysseune, A. and I. Thesleff. 2004. Continuous tooth replacment: Possible involvement of epithelial stem cells. *BioEssays* 26: 665-671.

W62

***In vitro* investigations of the Pax9/Msx1/Bmp4 interrelationship in tooth bud mesenchyme**

Wang Y.[1], Hui K.[1], Kapadia H.[2], Mues G.[1], D'Souza R.[1]
[1]Texas A&M Health Science Center Baylor College of Dentistry, Dallas, United States, [2]New York University Medical Center, Institute of Reconstructive Plastic Surgery, New York, United States

In vivo and *in vitro* evidence points to an interaction between Pax9, Msx1 and Bmp4 during early tooth development. *In vivo* experimentation showed that Bmp4 from dental epithelium (DE) induces Msx1 in dental mesenchyme (DM) which consequently with the help of unknown co-factor(s) leads to the expression of Bmp4 in DM. Pax9 was found to be expressed in DM independently from Msx1 and Bmp4 but to be necessary for the expression of both, Msx1 and Bmp4 in DM.
Our earlier *in vitro* investigations seemed to provide a straight forward explanation for the *in vivo* observations: We found that wild type Pax9, but neither Msx1 nor hypodontia-causing mutants of Pax9, can activate gene transcription from proximal *Bmp4* and *Msx1* promoter constructs. Pax9 and Msx1 co-immunoprecipitate and low (but not high) concentrations of co-transfected Msx1 potentiate the Pax9-induced activation of *Bmp4* and *Msx1* promoters. This synergism between Msx1 and Pax9 has so far been the only mechanism that could explain the *in vivo* observed dependence of Bmp4 expression on Msx1 in tooth bud mesenchyme.
Our more recent *in vitro* studies show that

1. Pax9 can only be co-immunoprecipitated (Co-IP) with Msx1 not with Msx2, Dlx1, Dlx2 and Dlx5.The physical interaction during Co-IP is not mediated by the homeodomain of Msx1 and is not related to the functional interaction with Pax9 during promoter activation.
2. Potentiation of the Pax9-induced Bmp4 activation is achieved by both, Msx1 and Msx2 but not by Dlx proteins. It must be mediated either by the homeodomains of Msx1 and Msx2 or by the conserved C-terminal amino acid sequence common to both, Msx1 and Msx2.
3. Potentiation of the Pax9-induced promoter transactivation by Msx2 is consistently stronger with the human *Bmp4* proximal promoter than with an equivalent of the mouse *Bmp4* promoter.
4. Mutant forms of Pax9 that cause human tooth agenesis lead to a loss of DNA binding ability which correlates with the severity of the patient phenotype.
5. Most mutant forms of Msx1 that cause human tooth agenesis do not affect the transcriptional cooperation with Pax9 in the activation of proximal *Bmp4* or *Msx1* promoters.
6. The transcriptional activity of more than 70kb of 5'extended *Bmp4* gene sequence is repressed by Msx1 except for one short area which shows slight activation. We will test if this area contains the enhancer which controls Bmp4 expression in tooth bud mesenchyme (Chandler et al. 2009)

These data indicate that:
The protein interactions between Msx1 and Pax9 seen in Co-IP can explain the decreased efficiency of the Msx1/Pax9 synergism which is observed with higher Msx1 concentrations: Large amounts of Msx1 are likely to sequester Pax9 by forming inactive complexes.
Msx2 may play a more important role in human odontogenesis as compared to mouse.
Pax9's involvement in human tooth agenesis appears to be primarily a consequence of the loss of DNA-binding capacity.
Msx1's ability to cause human tooth agenesis is largely independent from its potentiation of Pax9 induced Bmp4 activation suggesting that there must be other mechanisms for Msx1 to activate Bmp4 such as *Bmp4* enhancer engagement or a different pathway for Msx1 to cause hypodontia.

Poster Abstracts

P1

Timing of cranial neural crest cell migration is critical for tooth type formation in mice

Zhang L.[1], Li L.[1], Yuan G.[1], Yang G.[1], Zhang Q.[1], Chen Z.[1], KLOBM
[1]*Wuhan University, Key Lab of Oral Biomedicine of Ministry of Education, Wuhan, China*

Background: It was suggested the CNC cells that migrate into the first arch between the 10- and 12-somite stage have odontogenic potential to initiate tooth development, While the correlation between different timepoint of CNC cell migration and tooth type forming capability in mices is still unclear. The objective of the present study is to explore the correlation between different timepoint of cranial neural crest (CNC) cell migration and tooth type forming capability in mice.
Methods: The first arches (E8.5-E10.0) or mandibular arches (E10.5, E11.5) were dissected out and were grafted for subrenal culture for 2 and 4 weeks, respectively. Explants were dissected, processed for Hematoxylin-eosin stainning and Heidenhain Azan staining to examine tooth formation. Tooth width and cusp number were measured by stereomicroscope with a digital camera.
Results: The shape and size of formed teeth were analyzed. The first arch before E9.0 yielded only membranous bone. The first arch from E9.0 and E9.5 produced one multicuspid molar. The first arch from E9.75 (24-25 somites) or older embryos possessed the capability to form both molar and incisor. The size and cusp number of the molars increased from E9.0 to E11.5 grafts. Moreover, association of CNC cell number with tooth-forming capability was examined by dissecting the first arch of E9.75 and E9.5 into two halves. The grafts of half-arches of E9.5 and E9.75 failed to form tooth. When the number of CNC cells increased by transplanting the E9.5 first arch with its dorsally adjacent tissue, it was able to form one molar and one incisor.
Conclusion: These results suggested that the timing of CNC migration is critical for dental patterning.
This research was supported by the National Natural Science Foundation of China (Grant No.30700947) and Research Fund for the Doctoral Program of Higher Education of China (Grant No.20060486038).

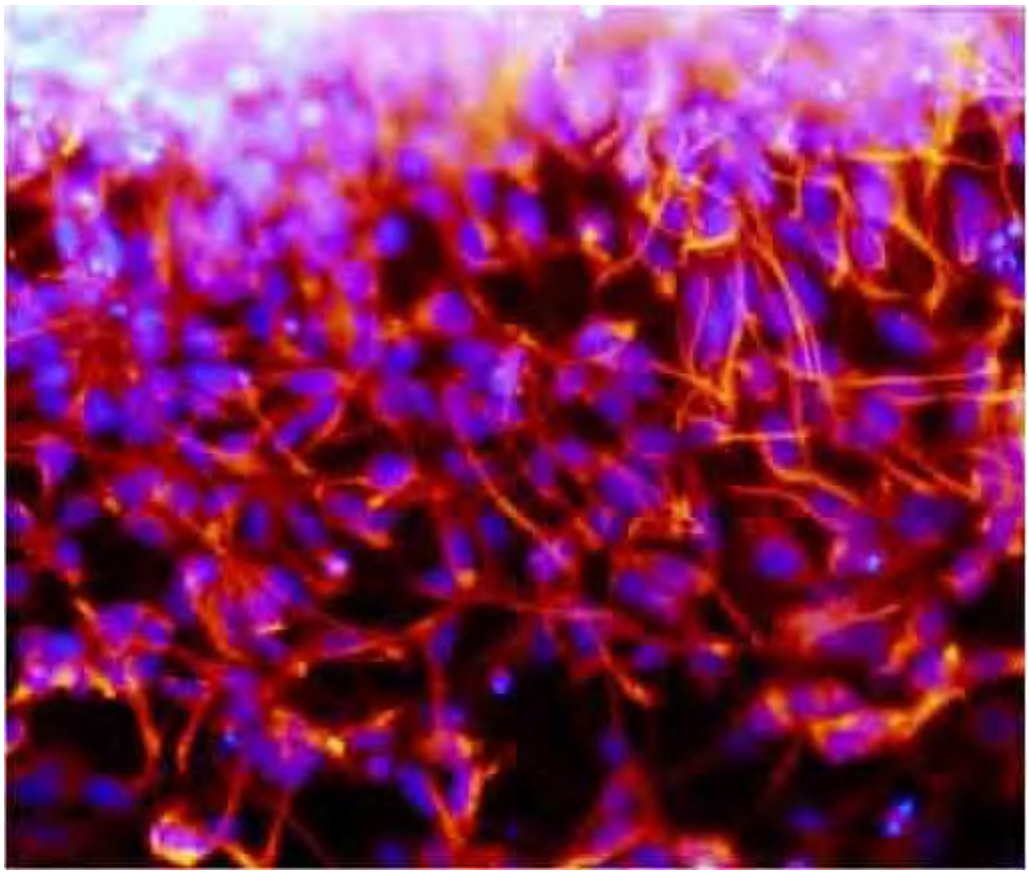
P2

Neural crest like cells from induced pluripotent stem cells for tooth regeneration

Kishigami R.[1,2], Otsu K.[1,3], Fujiwara N.[1], Harada H.[1]

[1]Iwate Medical University, Division of Oral Histology and Developmental Biology, Department of Oral Biology, Morioka, Japan, [2]Iwate Medical University, Division of Maxillofacial Surgery, Department of Oral and Maxillofacial Surgery, Morioka, Japan, [3]Iwate Medical University, Advanced Oral Health Science Research Center, Morioka, Japan

Induced pluripotent stem cells (iPS cells) are candidate to serve as a valuable source in regenerative medicine, because they can differentiate into various cell types of the body upon proper induction as well as embryonic stem cells. However, it has not been reported that iPS cells differentiate into odontogenic cells for tooth regeneration. In this study, we have designed to produce the neural crest like cells (NCLC), which could be an origin of odontogenic mesenchymal cells, using iPS cells. We maintained mouse iPS cells under the culture medium including leukemia inhibitory factor and the feeder cell layer. For NCLC differentiation, iPS cells formed spheroid body under suspension culture using HydroCell culture dish (CellSeed, Tokyo) for 4 days. Then, the spheroid bodies were seeded on fibronectin coated culture plates and were cultured in NCLC differentiation medium including B-27 for 6-9 days. The spheroid bodies spontaneously attached and expanded, and furthermore gave rise to stellate-morphology migratory cells. Immunofluorescence staining revealed that their cells prominently expressed neural crest marker, nestin (>90%, Fig.). Real time RT-PCR showed that mRNA of neural crest cell markers dramatically increased compared with that of undifferentiated iPS cells. These data suggest this culture condition could induce NCLC from iPS cells efficiently. Furthermore, to confirm whether NCLC can differentiate into odontogenic mesenchymal cells, we are planning to transplant them with dental epithelial cells.



[Fig.]

P3

Patterning of the heterodont dentition in the house shrew (*Suncus murinus*)

Yamanaka A.[1], Yasui K.[2], Uemura M.[1]
[1]*Department of Oral Anatomy, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan,* [2]*Marine Biological Laboratory, Graduate School of Science, Hiroshima University, Hiroshima, Japan*

Introduction: The most commonly upheld criteria for tooth class (type) homology have focused on the position of teeth in the upper jaw. Upper incisors are the teeth anchored in the premaxilla; the canine is the first tooth behind the premaxillary-maxillary (incisive) suture, behind which is some number of premolar, then molar teeth. The permanent dentition of the house shrew (*Suncus murinus*) comprises all tooth classes without diastema region (I3/1 C1/1 P2/1 M3/3＝30). During the dentition development of the house shrew, dental epithelium of upper deciduous first incisor originates from the frontonasal process, whereas that of second incisor originates from the maxillary process [1]. In this study, using the house shrew, we investigated (1) the expression pattern of the several genes (*Shh, Bmp4, Fgf8, Msx1, Barx1*) in the developing jaw, and (2) the positional relationship between the premaxillary-maxillary suture and the developing upper dentition. We discuss the patterning of the heterodont dentition in mammals both in terms of molecular control and the traditional criteria in comparative odontology.
Materials & methods: *Suncus murinus* orthologs of *Bmp4, Fgf8, Msx1* and *Barx1* were isolated from the house shrew embryos. Expressions of these genes in the developing jaws were detected by *in situ* hybridization. H-E stained serial histological sections of the head were prepared for each embryonic age. Three-dimensional morphology of developing dentition and bone in the upper jaw was reconstructed from these serial sections.
Results: In the upper jaw primordia prior to morphological initiation of tooth development, *Bmp4/Msx1* was expressed across the fusion site of the frontonasal and maxillary processes. *Bmp4/Msx1* was also expressed in the mesial part of the mandibular process. *Fgf8/Barx1* was detected in the distal parts of both upper and lower jaws.
On the other hand, the ossification in the jaws started 3 days behind the initiation of tooth development. The ossification center of the premaxilla appeared in the mesenchyme adjacent to the enamel organ of the upper first incisor. The premaxillary bone grew distally beyond the fusion site of the frontonasal and maxillary processes, and finally reached just mesial to the canine germ and formed the premaxillary-maxillary suture there.
Discussion: The model for tooth type determination is well established in the lower jaw of the mouse [2]. This model is applicable to both upper and lower jaws of the house shrew. The expression domain of *Bmp4/Msx1* corresponds to the incisor-forming region, whereas that of *Fgf8/Barx1* corresponds to the molar-forming region. Although the fusion site of the frontonasal and maxillary processes runs through between the first and second incisors, the premaxillary-maxillary suture is shifted distally to the canine-forming site. The above model is, therefore, not contradictory to the traditional criteria for tooth class homology in mammals.
References:
1. Yamanaka A, Yasui K, Sonomura T, Uemura M. (2007) Development of heterodont dentition in house shrew (*Suncus murinus*). *Eur J Oral Sci* 115: 433-440.
2. Tucker AS, Matthews KL, Sharpe PT. (1998) Transformation of tooth type induced by inhibition of BMP signaling. *Science* 282: 1136-1138.

P4

Development of the oral cavity: From gene to clinical phenotype in human

Laugel V.[1], Langer A.[1,2], Fraulob V.[1], Schuhbaur B.[1], Koch M.[1], Marrie J.[1], Ripp R.[1], Berthommier G.[1], Schmittbuhl M.[2,3], Choquet P.[4], Constentinesco A.[4], Dollé P.[1], Losson R.[1], Bloch-Zupan A.[1,2,3,5]

[1]IGBMC, Illkirch, France, [2]University of Strasbourg, Faculty of Dentistry, Strasbourg, France, [3]Reference Centre for Orodental Manifestations of Rare Diseases, Service de Soins Bucco-Dentaires, CHU, Strasbourg, France, [4]Biophysique et Médecine Nucléaire, CHRU, Strasbourg, France, [5]University College of London, Faculty of Dentistry, London, United Kingdom

Tooth development is under strict genetic control and is mediated by epithelio-mesenchymal interactions between first pharyngeal arch oral ectoderm and ectomesenchymal cells originating from cephalic neural crest. Signaling molecules of the main developmental pathways (Tgfβ, Shh, Wnt, Fgf, Notch) and transcription factors are involved. Oro-dental anomalies are one aspect of the 5000 known rare diseases or syndromes and 700 of these diseases have an oral phenotype. Our goal is to combine complementary approaches in developmental biology especially via the study of animal models and bioinformatics to improve the understanding of etiopathogenic mechanisms involved in oral development. We used two approaches to target relevant genes: (1) selection of known genes responsible for rare diseases but for which the expression and/or roles are not (or insufficiently) characterised; (2) identification of new candidate genes, through a systematic analysis of their craniofacial and dental expression patterns using the EURExpress mouse transcriptome-wide atlas (http://www.eurexpress.org/ee/). We then analysed by non radioactive manual or automated *in situ* hybridization the detailed expression patterns of these genes during mouse odontogenesis at E12.5, 14.5, 16.5, P0, P6. This method provides an accurate spatio-temporal description and cellular resolution of the expression of individual gene product.(1) *NSD1*, gene (nuclear receptor-binding SET domain protein 1 gene; locus 5q35), coding a retinoic acid co-regulator protein, when mutated is responsible for Sotos syndrome (OMIM #117550). This overgrowth condition combines excessive growth during childhood, macrocephaly, distinctive facial gestalt, various degrees of learning difficulty and variable minor features. An increased risk of tumors is reported. The oro-dental phenotype encompasses dental anomalies like hypodontia especially agenesis of premolars, supernumerary teeth, enamel hypoplasia, pulpal anomalies, premature tooth eruption, associated with high arched palate and prognathism. In the mouse, the localization of *nsd1* transcripts was detected throughout odontogenesis both in epithelial and in mesenchymal compartments. Transcripts were also detected in differenciating and differenciated cells (2) The screening of the EURExpress mouse transcriptome-wide atlas pointed towards the *Ap1m2* gene encoding the adaptor-related protein complex 1, mu 2 subunit. This protein, localized within the trans-golgi network clathrin coat and belonging to the adaptor complexes medium subunits family is capable of interacting with tyrosine-based sorting signals.The transcripts of *Ap1m2* were located within the enamel organ throughout odontogenesis and were also detected in differenciating and differenciated cells.To decipher further the role of these genes, interference with the function of these genes and associated proteins/pathways will be performed using *in vitro* siRNA silencing technics in tooth organ culture.

We acknowledge the University of Strasbourg, API HUS and IFRO for their support.

Huang N, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Losson R.Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. EMBO J. 1998 Jun 15;17(12):3398-412.

Laugel V et al., Bloch Zupan A, Développement de la cavité buccale : du gène à l'expression clinique chez l'Homme, IFRO, Cahiers de l'ADF, 2010 (in press).

P5

Molecular regulation of tooth replacement

Jussila M.[1], Järvinen E.[1], Thesleff I.[1]
[1]*University of Helsinki, Institute of Biotechnology, Helsinki, Finland*

The tooth replacement capacity of different species varies greatly. Fishes, snakes and amphibians can replace their dentition several times during their lifespan while most mammals, including humans, have two sets of dentitions. However the mouse has no tooth replacement. The molecular mechanisms regulating tooth replacement have long been poorly understood because of the limitations of the traditional mouse model. Roles for Wnt signaling have been indicated in congenital human disorders affecting tooth replacement and in transgenic mice with extensive formation of supernumerary teeth. We have studied the role of Wnt signaling using both the mouse and the ferret (Mustela putorius furo) as model animals. I will present our results showing that the initiation of the primary teeth and tooth replacement are controlled by both common and unique signaling patterns.

P6

Developmental analysis of limb development after temporal blocking of Hedgehog signaling

Kim E.-J.[1], Kwak S.[1], Cho S.-W.[1], Jung H.-S.[1]
[1]*Brain Korea 21 Project, Oral Science Research Center, Dental College of Yonsei University, Seoul, Korea, Republic of*

Background: The vertebrate limb skeleton develops through the endochondral bone formation, which involves in the substitution a cartilage primordium with the calcified bone matrix. The cartilage formation, called chondrogenesis, is crucial in determining the shape and size of definitive bones in vertebrate. During mouse limb chondrogenesis, mesenchymal progenitor cells are condensed differentiates into chondrocytes to form the cartilage primordium, and chondrocytes undergo sequential proliferation and differentiation along with secretion of extracellular matrix. Many studies have reported extensively the molecular mechanism on the endochondral bone formation rather than chondrogenesis. Especially, Indian hedgehog (Ihh) signaling is known to control both hypertrophy of chondrocytes and bone replacement which is particularly important in postnatal endochondral bone formation.
Methods: In this study, maternal transfer of 5E1 (a hedgehog-blocking antibody) to E12 mouse embryo causes an attenuation of the Indian hedgehog activity, and causes malformation of the mouse limb.
Results: We analysed genetic relationship during chondrogenesis in limb buds with mRNA microarray. As a result, we found some genes interacting with Ihh ; *Glo1, Dkk4, Mapk8, Ltbp3, Col1a2, Wasl, Dmkn, Flnc, Eltd1, Egfl7, Col22a1, Krt1, Col22a1, Krt1, Col19a1, Krt10, Hspa8, Fbxo2, Ocrl, map3k4, Garnl4, Fgf7, Krt4, Klf7* were up-regulated, and *Hhip, Hoxd13, Casp1, Nrg4, Alpl, Rassf8, Foxc2, Bmp5, Ddef1, Ptch1, Sdc4, Tbx5, Runx2, Ikkbkg, Ubc, Nog, Runx3, Fgfr1op2, Nfia, Gh* were down-regulated. Furthermore, we treated exogenous IHH and 5E1 protein into mesenchymal cells of limb bud in the micromass culture system. We suggested that Ihh increased condensation of mesenchymal cells and proliferation of prechondrocytes in early chondrogenesis.
Conclusion: Ihh signaling positively regulates early chondrogenesis, as well as hypertrophy of chondrocytes in the mouse limb development.

P7

Methylation status of the Runx2 P2 promoter in a family with ectopic maxillary canines

Camilleri S.[1], Scerri C.[2], Mcdonald F.[1]
[1]Dental Institute, Kings College London, Department of Orthodontics and Paediatric Dentistry, London, United Kingdom, [2]University of Malta, Department of Pathology, Msida, Malta

Aims and objectives: To determine the effect of methylation status of the Runx2 proximal promoter in buccal cell/lymphocyte DNA on ectopic eruption of the maxillary permanent canine (EC).
Introduction: Genetic factors contribute to the aetiology of EC, the inheritance pattern being autosomal dominant with variable expression and incomplete penetrance. However there is also an epigenetic component. The Runx2 gene is intimately involved in the mechanism of tooth eruption and mutations of this gene result in delayed and ectopic eruption of teeth. There is a large CpG island spanning its proximal promoter, first exon and part of the first intron. Similarities between the inheritance patterns of EC and other methylation disorders raises the hypothesis that differential methylation of the Runx2 promoter may contribute to EC.
Materials and methods: DNA from a 21 member, three-generation family, exhibiting 8 cases of EC was extracted from saliva (Oragene, DNAGenotek) using the phenol-chloroform method and converted with bisulphite (Methyleasy Xceed, Human Genetic Signatures) in order to determine 5mC content. Three primer pairs were designed, one being 2kb upstream of the transcription start site (TSS), one less than 1kb upstream of the TSS, within the promoter region and another in first intron. Polymerase chain reaction was used to amplify the relevant sites and the product directly sequenced using the Applied Biosystems 3130 Genetic Analyser. Analysis was carried out using the BIQ Analyzer program[1].
Results: There was no difference in the methylation status of the P2 promoter regardless of age or eruption status of the maxillary canines.
Conclusions: The Runx2 P2 promoter in buccal cells and lymphocytes is unmethylated regardless of age or eruption status of the teeth. As promoter methylation is tissue as well as age specific, further research should analyse dental tissues.
Reference: [1]Bock, C et .al. (2005). "BiQ Analyzer: visualization and quality control for DNA methylation data from bisulfite sequencing." Bioinformatics 21(21): 4067-8.

P8

Alteration of tooth development in two-phase organotypic cultures by transient Glycogen Synthase Kinase-3 (GSK-3) inhibition

Aurrekoetxea M.[1], Ibarretxe G.[1], García-Gallastegui P.[1], Unda F.[1]
[1]*Basque Country University (UPV/EHU), Cell Biology, Leioa, Spain*

Background: Tooth development depends on intercellular cross-talk signalling between dental epithelium and mesenchyme. Canonical Wnt pathway has been found to present an outermost importance to coordinate dental morphogenesis and differentiation. It activates when secreted Wnt proteins bind to their transmembrane receptors in the target cell. This inactivates a multiheteromeric destruction complex that, in absence of Wnt signals, triggers the degradation of β-catenin, a gene transcription cofactor. When Wnt pathway is active, β-catenin accumulates in target cells and is eventually translocated to the nucleus, where it induces transcription of Lymphoid Enhancement Factor (LEF) controlled genes. One of the main components of the β-catenin destruction complex is Glycogen Synthase Kinase-3 (GSK-3), which phosphorylates β-catenin, thereby sending it to degradation. Consequently, pharmacological inhibition of GSK-3 activates Wnt signalling, by preventing β-catenin elimination. The importance of Wnt pathway for dental development is underscored by the fact that transgenic mice that inactivate Wnt signalling fail to develop teeth past bud stage. On the contrary, Wnt overactivating mutants often present abundant supernumerary teeth. Genetically modified mouse models constitute an invaluable tool for the study of dental malformations, but since the majority of these reported modifications are also permanently expressed in the animal, it remains unclear at what specific stages of dental development is Wnt activation required, and for what specific functions, in the context of tooth development.
Methods: In order to specifically determine the role of an activation of Wnt pathway at different stages of dental development, we performed organotypic mouse tooth cultures extracted at E14.5 and E17.5, at the onset of cap and bell stages, respectively. In a first phase, we cultured these tooth germs in vitro for 6 and 12 days, in the absence or presence of pharmacological inhibitors of GSK-3 activity, such as Lithium Chloride (LiCl; 2mM) and 6-bromoindirubin-3´-oxime (BIO; 5 μM and 10μM). In a second phase, we used these in vitro cultured teeth to transplant them to testicles of surrogate male mice, to eventually achieve full-term tooth development. This two-phase procedure culminated with the production of a calcified mature dental piece.
Results: We assessed that GSK-3 inhibition effectively activated Wnt signalling in cultured teeth, by measuring increased β-catenin protein levels and nuclear translocation. Additionally, we analized different developmental parameters such as tooth morphogenesis (histology) cell proliferation levels (BrdU), apoptosis (active caspase 3), and differentiation of odontoblasts and ameloblasts (nestin and amelogenin). Treatment with GSK-3 inhibitors dramatically increased cell proliferation and delayed differentiation of dental cell types. Finally, these morphogenetical alterations induced the formation of abnormal teeth.
Conclusion: Our experimental model allows to perform transient activation of Wnt signalling at different tooth developmental stages, and assess the outcome of these manipulations in the fully developed dental piece. Understanding how Wnt activation affects dental development may help develop new strategies for prevention of developmental malformations, in tooth and other related ectodermal organs.
This work was funded by the Basque Country University (UPV/EHU), Jesús Gangoiti Foundation and Basque Government.

P9

Reduced expression of tight junction proteins ZO-1 and claudin-1 in ameloblasts and odontoblasts of Epiprofin/Sp6 deficient mice

Jimenez L.[1], Aurrekoetxea M.[2], Ibarretxe G.[2], Garcia P.[2], de-Vega S.[3], Unda F.J.[2]
[1]Ecole Polytechnique Fédérale de Lausanne (EPFL), Life Science Faculty, Lausanne, Switzerland, [2]University of the Basque Country, Cell Biology and Histology, Leioa, Spain, [3]National Institutes of Health, National Institutes of Dental and Craniofacial Research, Bethesda, United States

Background: Odontoblasts and ameloblasts are tall and columnar cells that characterize by a highly polarized distribution of cellular organelles. These cells differentiate terminally and deposit the organic matrix of dentin and enamel respectivelly. The contacts between odontoblasts are found at the junction area between the cell body and the odontoblast process in the form of a modified junctional complex. Cell adhesions between ameloblasts are present at the two junctional complexes between these cells, the proximal one that is adjacent to the stratum intermedium and the distal one that is located at their secretory pole. Junctional complexes and the cytoskeleton are important in maintaining cell polarity and cell-cell interactions. In addition, cell adhesión is a key regulador of cell differentiation. These interactions with neighboring cells and the extracellular matrix regulate gene expression, cell proliferation, polarity and apoptosis. Tight junctions are one type of such cell-cell junctions and several signaling complexes have been identified to associate with them.
Methods: In this work, we have studied the odontoblast and ameloblast polarization and differentiation in Epiprofin/Sp6 null mice and the correlation with the decreased expression of tight junctins proteins, such as zonnula ocludens-1 (ZO-1) and claudin-1.
Results: We found that odontoblast features were altered in Epiprofin/Sp6 null mice. Odontoblast differentiation was clearly delayed when compared to wild type mice. Both collagen I and DSPP dramatically decreased in E19.5 Epiprofin/Sp6 deficient first molars, whereas in normal molars these proteins were normally secreted. In adult mutant mice, some odontoblasts either lost the polarization or polarization was changed with the nuclei located at the secretory side facing dentin. Several layers of odontoblasts were formed, and the dentin layer was thin and incomplete. The pattern of dentinal tubules was altered. The inner enamel epithelium remained undifferentiated in mutant embryo mice teeth and polarized and differentiated ameloblasts were absent during odontogenesis. Consequently, no enamel was detected in Epiprofin/Sp6 -/- teeth.
In wild type mice, ZO-1 protein is expressed in preameloblasts and ameloblasts and localizes at the basal and apical sides of the cell membrane. In mutant mice, ZO-1 protein was barely detectable in the inner enamel epithelium, and its expression was decreased in odontoblasts and dental mesenchymal cells. Claudin-1 was expressed in the outer enamel epithelium and stratum intermedium in wild type molars, but its expression was clearly reduced in Epiprofin/Sp6 deficient molars.
Conclusion: We report defects in polarization and differentation of odontoblasts and ameloblasts in *Epiprofin/Sp6* (-/-) mice, that correlate with a decreased expresion of tight junction proteins. We hypothesize that this defective polarization is linked to an impaired formation of the tight junction adhesion complex, and therefore to a decreased membrane recruitment of signaling proteins triggering cell differentiation.
This work has been supported by the University of the Basque Country, the Basque Government and the Jesus Gangoiti Foundation. L Jimenez and M Aurrekoetxea contibuted equally to this work.

P10

Caspase knock-outs and dental apoptosis

Matalova E.[1,2], Vanden Berghe T.[3], Chlastakova I.[1,2], Doubek J.[2], Míšek I.[1,2], Tucker A.S.[4]
[1]Institute of Animal Physiology and Genetics CAS, v.v.i., Brno, Czech Republic, [2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]Ghent University, Department for Molecular Biomedical Research, Ghent, Belgium, [4]King's College, Guy's Hospital, Department of Craniofacial Development, London, United Kingdom

Background: Apoptosis (programmed cell death) has been shown to play an important role at a number of stages during tooth development. We are interested in the specific roles of caspases during odontogenesis. Caspases are a large protein family of cysteine aspartate proteases involved in inflammation or/and apoptosis. Initiator apoptotic caspases (caspase-8, -9, and -10) are activated via death adaptor molecules proximity and oligomerization induced proteolytic processing. Once activated, the initiator caspases cleave other members of the caspase family called effector caspases. Subsequently, effector caspases (caspase-3, -6, -7) cause degradation of several cellular polypeptides that are essential for cell survival (lamin, PARP) resulting to DNA fragmentation, cytoskeleton break-up, and cell death.
Methods: Mouse knock-out phenotypes for individual caspases were studied with a special focus on embryonic day E15.5. At this stage the primary enamel knot of the first mouse molar is eliminated by apoptosis and a typical cluster of apoptotic (TUNEL positive) cells is apparent. Hematoxylin-eosin staining was used to evaluate any alterations in morphology of the tooth germ, TUNEL assay to follow changes in the apoptosis pattern and PCNA immunohistochemical labelling to detect proliferating cells. Caspase-3 immunohistochemistry (Cell Signalling) was used to follow the temporospatial pattern of activation of this central caspase. Caspase-8 knock-outs die at E11.5 before morphological onset of odontogenesis, therefore an explants culture approach of pharmaceutical inhibition is necessary.
Results: In caspase-9 deficient mice, no TUNEL positive cells were detected in the primary enamel knot suggesting involvement of caspase-9 and the intrinsic pathway in dental apoptosis activation. The importance of this pathway was confirmed by the lack of TUNEL positive cells in mutant molars deficient in Apaf-1, another pro-apoptotic molecule in the intrinsic signalling. Proliferation in both mutants was not altered.Caspase-3 activation in odontogenesis correlates with apoptosis distribution based on TUNEL labelling. In caspase-3 deficient mice, temporal, strain-dependent alterations in tooth shape were found and TUNEL labelling confirmed a complete inhibition of apoptosis in the primary enamel knot of these mutants. Caspase-7 mutants are also under study to reveal further details related to the role of the caspase network in dental apoptotic machinery, while specific caspase-8 inhibition experiments are underway to investigate the role of the extrinsic apoptotic pathway.
Conclusion: Caspases play important role in the mechanism of apoptosis during death of the enamel knot. Loss of specific caspases results in loss of apoptosis and in some cases changes in tooth shape. However, compensatory mechanisms must be taken into account in the case of individual caspase inhibition and alternative cell death in the case of general caspase inhibition.
Supported by the Grant Agency of the Czech Academy of Sciences (KJB500450802 - primary enamel knot apoptosis research, IAA600450904 - embryonic apoptosis research). International cooperation runs under the Royal Society grant (JP080875) and the IAPG lab under IRP IPAG No. AVOZ 50450515.

P11

1,25α(OH)2 vitaminD3 regulate Msx1 antisense RNA expression in mouse incisor

Pinheiro Junior R.F.F.[1], Lezot F.[2], Hotton D.[2], Babajko S.[2], Berdal A.[2], Fernandes I.[2]
[1]Universidade Federal do Rio Grande do Norte, Departamento de Odontontologia, Natal, Brazil, [2]Universite Paris Diderot, Paris, France

Background: Clinical investigations report the existence of enamel and dentin alterations in vitamin D-related pathologies. Tooth morphogenesis results from the combinatorial action of different homeobox genes expressed in the facial neural-crest-derived mesenchyme. One of them, Msx1, is involved in cell proliferation and inhibits cell differentiation. A sense and a cis-antisens (AS) RNA are transcribed from Msx1 gene. They are present during tooth morphogenesis, but AS fonction is not clearly identified. It can modulate the distribution of the protein. Most of target organs of Msx1 are also known for their sensitivity to 1,25α(OH)2VitaminD3 (vitD). The aim of this study was to elucidate the influence of the vitamin D hormone on the sense and antisense Msx1 RNA expression pattern during dental cell differentiation.
Methods: For this purpose we used the mouse incisor continuous growing tooth. This model provides a continuous proliferation and differentiation of dental cells with well established zones of pre-secretion, secretion and maturation.
Results: For the first time the expression pattern of Msx1 sense and AS RNAs was investigated in mouse post-natal incisor, and the regulation of Msx1 AS expression in mice incisor by 1,25(OH)2D3 was shown. In vivo, Msx1 AS RNA expression is up-regulated in the microdissected epithelium from incisors of vitamin D-deficient mice, and rescued with 1,25(OH)2D3 repletion. In vitro, the treatment of ameloblast-like cell line (LS8) by 1,25(OH)2D3 decreased Msx1 AS RNA expression.
Conclusions: Our results suggest that the presence of Msx1 sense and AS RNAs in bone cells might be considered in the complex mechanism of vitamin D and bone physiology.

P12

Expression and function of microRNAs in tooth development

Oommen S.[1], Otsuka-Tanaka Y.[1,2], Imam N.[1], Mega J.[2], Hindges R.[3], Ohazama A.[1], Sharpe P.T.[1]
[1]Dental Institute, King's College, Guy's Hospital, Craniofacial Development, London, United Kingdom, [2]Nihon University School of Dentistry at Matsudo, Dentistry for the Disabled, Matsudo, Japan, [3]King's College, Guy's Hospital, MRC Centre for Developmental Neurobiology, London, United Kingdom

MicroRNAs are 19-25 nucleotide non-coding small signle-stranded RNAs that negatively regulate gene expression by binding target mRNAs. MicroRNAs have been implicated in numerous biological processes including development. The biological role of most individual mRNAs and the targets they modulate are largely unknown in tooth development. A comprehensive expression analysis of microRNAs in tooth development was therefore carried out as a starting point to understand the role of individual microRNAs. We have examined the temporo-spatial expression of 6 different microRNAs (miR-27560, miR-193, miR-378, miR-148a, miR-218 and miR-98) based on preliminary microarray data in murine tooth development. All 6 microRNAs show dynamic temporo-spatial expression in tooth development. MiR-27560 is expressed in both epithelium and mesenchyme throughout the early stages of tooth development. MiR-193 expression is observed in both epithelium and mesenchyme at initiation and bud stages whereas it could not be detected at the cap. MiR-378 and miR-148 show expression in presumptive tooth epithelium at initiation and basal epithelium at the bud stage whereas they are expressed in inner and outer enamel epithelium except the enamel knot at the cap stage. MiR-218 and miR-98 are strongly expressed in lingual cervical loops at the cap stage.
Dicer, an RNase III endonuclease, is the essential emzyme that cleaves microRNA precursors into 19-25 nucleotides. In order to investigate the role of miRNA in tooth development, we further examined mice with conditional Dicer deletion (BF1-Cre/Dicer$^{flox/flox}$). The collar of tooth germ epithelium is lacking in both incisors and molars in BF1-Cre/Dicer$^{flox/flox}$. These suggest a specific role for microRNAs in maintaining the epithelial collar during tooth development.

P13

Ultrastructural localization of osteoadherin during early tooth development

Nikdin H.[1], Hultenby K.[2], Olsson M.-L.[1], Sugars R.V.[1]
[1]Karolinska Institute, Department of Dental Medicine, Stockholm, Sweden, [2]Karolinska University Hospital, Centre for Clinical Research, Stockholm, Sweden

Background: The small leucine-rich proteoglycans (SLRP) have been extensively implicated in the biomineralization process of bone and dentin, albeit the complex mechanism of mineralization remains to be fully elucidated. Specifically, biglycan, decorin, and fibromodulin have been shown to be involved in dentinogenesis, and ultrastructural immunohistochemistry has shown that they are differently distributed across the mineralizing tooth, from the odontoblast cell layer to pre-dentin, pre-dentin/dentin interface and dentin. Furthermore, keratan sulphate, the glycosaminoglycan associated with the class II family of SLRPs, demonstrated an increasing gradient towards the mineralization front. Osteoadherin (OSAD), a keratan sulphate-substituted SLRP is also reported to be present in the tooth and has been postulated to be involved in dentinogenesis. However, the exact role and distribution of OSAD during tooth formation remains to be fully determined.
Aims: This study aimed to clarify the ultrastructural localization of OSAD within the developing tooth; cell layer, pre-dentin, pre-dentin/dentin interface and dentine, and enamel, with a view to provide further evidence for the role of OSAD during dentinogenesis.
Methods: Mouse mandibles were collected at different time points; embryonic day 15 (E15), newborn (NB), postnatal day 5 (d5) and adult tissue (A), and prepared for transmission electron microscopy (TEM). Ultrathin sections were probed with an antibody against OSAD and the signal detected using an immunogold-conjugated secondary antibody. Controls were performed by blocking the primary antibody with recombinant OSAD. Immunogold-labeled particles were counted and expressed as the number of gold particles/μm^2. These were scored per different compartment (cell layer, pre-dentin, pre-dentin/dentin interface and dentin, and enamel).
Results: Quantification of OSAD density revealed considerable differences between the different developmental stages. At all developmental stages, few OSAD-labeled gold particles were detected in the cell layer and the enamel. With early dentinogenesis of the incisor at E15, OSAD was localized in the developing pre-dentin. However, by d5, OSAD was localized across all compartments, demonstrating an increase from the pre-dentin to pre-dentin/dentin interface and dentin. A similar pattern of OSAD localization was observed in molars from all developmental stages, with the highest number of immuno-gold-labeled OSAD particles observed in the pre-dentin and dentin layers. Of note, OSAD was found in close localization to collagen fibers in the pre-dentin and dentin layers of d5 mice, supporting the notion that OSAD, maybe involved in collagen fibrillogenesis, although this remains to be determined.
Conclusion: These novel data for the first time demonstrate the ultrastructural localization pattern of OSAD throughout the tooth. The gradient expression follows that previously reported for other SLPRs in dentinogenesis and provides useful information to aid in the elucidation of the role of OSAD in this dynamic mineralization process.

P14

c-Myb in prenatal odontogenesis: a pilot study in mouse and minipig models

Matalova E.[1,2], Buchtova M.[1,2], Bender T.P.[3], Tucker A.S.[4], Janeckova E.[1,5], Lungova V.[1,2], Smarda J.[5]
[1]Institute of Animal Physiology and Genetics CAS, v.v.i., Brno, Czech Republic, [2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]University of Virginia Health System, Charlottesville, United States, [4]King's College, Guy's Hospital, Department of Craniofacial Development, London, United Kingdom, [5]Masaryk University, Faculty of Science, Brno, Czech Republic

Background: The c-*myb* gene encodes a transcription factor involved in control of cell proliferation, differentiation, and survival. It is essential for control of hematopoiesis in vertebrates, however, its role in non-hematopoietic organs is less clear. There are multiple data suggesting participation of c-Myb in control of developmental processes. c-Myb mRNA was found in mouse embryos and there is one report indicating the presence of c-Myb mRNA in mouse incisors at E14.5 (Ess et al. 1999, Oncogene 18: 1103-1111). This study aims to further investigate c-Myb expression pattern and potential role of this protein in odontogenesis based on c-Myb immunohistochemistry and temporo-spatial correlation with proliferation and apoptosis in two animal models: the mouse as a monophyodont species and the minipig as a diphyodont species.
Methods: Cell morphology was analysed in serial histological sections after hematoxylin eosin staining. c-Myb expression was examined by immunohistochemistry (anti-c-Myb antibodies purchased from Abcam and Epitomics) and *in situ* hybridisation. Cell proliferation was addressed by immunohistochemistry of PCNA protein (DAKO antibody). Apoptotic cells were labeled by TUNEL assay (Chemicon). The study was supplemented by analysis of c-Myb in floxed mutant mice.
Results: The c-Myb protein was found both, in epithelial and mesenchymal parts of the mouse and pig tooth germs. Analysis of cell proliferation showed that c-Myb expression is linked to proliferation, however, c-Myb positive cells were abundant to PCNA positive cells. Apoptotic cells were c-Myb negative. In the minipig, interestingly, an asymmetric c-Myb expression was found in the epithelial part of the tooth germ. Moreover, c-Myb-positive cells in the minipig degrading dental lamina were situated on the side facing the tooth germ.Absence of proper c-Myb function is lethal; c-Myb deficient mice die *in utero* at day 14.5 from failure of fetal hematopoiesis. Compared to the wild type, the tooth mutant phenotype at E14.5 did not display any morphological alterations in budding or in the presence of proliferating and apoptotic cells.
Conclusion: Distribution pattern of c-Myb during tooth development positively correlates with proliferation, negatively with apoptosis and differentiation. c-Myb seems to have a specific role in diphyodont species related to the asymmetric growth of the tooth germ and dental lamina degradation. As c-Myb expression remains compatible with differentiation, the apparent lack of the first molar disturbance in mutant mice may reflect the fact that such events would arise after day 14.5.
Supported by the Grant Agency of the Czech Republic (524/08/J032 and 304/08/P289), the Grant Agency of the Czech Academy of Sciences (KJB500450802) and Ministry of Education (MSM0021622415). International cooperation runs under the Royal Society grant (JP080875) and the IAPG lab under IRP IPAG No. AVOZ 50450515.

P15

Roles of Wnt/ß-Catenin signaling in the formation of dentin and periodontium

Lim K.-H.[1], Baek J.-A.[1], Yang X.[2], Taketo M.M.[3], Cho E.-S.[1]
[1]Laboratory for Craniofacial Biology, Chonbuk National University School of Dentistry, Institute of Oral Bioscience and BK21 Program, Jeonju, Korea, Republic of, [2]Genetic Laboratory of Development and Disease, Institute of Biotechnology, Beijing, China, [3]Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Background: Wnt/ß-Catenin signaling is essential mediator of epithelial-mesenchymal interactions during tooth development. It has been well known that Wnt/ß-Catenin signaling is critical for the normal tooth morphogenesis in early tooth development. However, its roles in the formation of dentin and cementum are not completely understood. The objective of present study is to understand the roles of Wnt/ß-Catenin signaling in the crown and root formation.
Methods: We generated and analyzed the mice with tissue-specific activation of *ß-Catenin*. The key mediator of Wnt signaling, *ß-Catenin*, was tempospatially activated by *OC-Cre* with the activity of *Cre* recombinase under the control of *OC* promoter.
Results: Mutant mice exhibited prominent tooth phenotypes. In the crown of mutant molar, well-differentiated odontoblasts were observed but mineralized dentin was thinner than that of wild type. Instead of mineralized dentin, wide predentin was found underneath the dentin, which showed globular mineralized pattern. Root of mutant molar was more severely affected than its crown. Roots were shorter than those of wild type and the most part of roots were atypically surrounded by the cellular cememtum. In addition, periodontal space between cementum and alveolar bone was widened between the mutant molars.
Conclusions: These results indicated that appropriate *ß-Catenin* mediated signaling is essential for the mineralization of dentin and periodontium formation. Therefore, it is strongly suggested that Wnt/ß-Catenin signaling may be important in the formation of dentin and periodontium as well as in the early tooth development.
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (M10646010003-06N4601-00310 and 2009-0085733)

P16

Smad4 mediated signaling is essential for dentin formation

Ko S.-O.[1], Lee J.-Y.[2], Yoon C.-Y.[2], Chai Y.[3], Cho E.-S.[2]

[1]Department of Oral and Maxillofacial Surgery, Chonbuk National University School of Dentistry, Institute of Oral Bioscience and BK21 Program, Jeonju, Korea, Republic of, [2]Laboratory for Craniofacial Biology, Chonbuk National University School of Dentistry, Institute of Oral Bioscience and BK21 Program, Jeonju, Korea, Republic of, [3]Center for Craniofacial Molecular Biology School of Dentistry University of Southern California, Los Angeles, United States

Background: Accumulating evidences indicate that TGF-β/BMP signaling play key roles in the early tooth morphogenesis. However, their roles in the differentiation of odontoblasts and dentin formation are not clearly understood. The objective of this research is to understand the roles of TGF-β/BMP signaling in vivo during dentin formation.

Methods: We generated and analyzed the mice with odontoblast-specific inactivation of *Smad4*. The key mediator of TGF-β/BMP signaling, Smad4, was inactivated by three kinds of *Cre* mice lines (*Col1a1-Cre, Dmp1-Cre,* and *Dspp-Cre*) with the activity of *Cre* recombinase under the control of each matrix protein promoter.

Results: All of three mutant lines exhibited similar tooth phenotype, particularly in coronal dentin of molars. Although no differences in the size and shape of molars, dentin formation was severely affected in mutants. The coronal dentin of mutant molars was thinner than those of wild type littermate whereas the radicular dentin was normal. In addition, number of differentiated odontoblasts was apparently reduced in mutant molar and some odontoblasts were included in the dentin matrix. In some case, ossifying masses and inflammatory cells were observed in the pulp cavity. In other case, pulpal degeneration and periapical invasion of inflammatory cells were also observed.

Conclusions: These results indicated that *Smad4* mediated signaling is essential for dentin formation. Therefore, it is strongly suggested that TGF-β/BMP signaling may play as an essential regulator in the dentin formation.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2007-313-E00487 and M10646010003-06N4601-00310)

P17

Expression patterns of the *Fam83h* gene during murine tooth development

Lee M.-J.[1], Kim J.-W.[2], Jung H.-S.[1]
[1]*Department of Oral Biology, Research Center for Orofacial Hard Tissue Regeneration, Brain Korea 21 Project, Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Korea, Republic of,* [2]*Department of Cell and Developmental Biology, School of Dentistry, Seoul National University, Seoul, Korea, Republic of*

Background: Recently a novel gene, *FAM83H*, was identified by a genetic linkage study in the hypocalcified form of the amelogenesis imperfecta family with an autosomal dominant hereditary pattern. Little is known about this novel gene, and so we investigated the expression pattern of *Fam83h* in murine tooth development using serial sectional *in situ* hybridisation.
Methods: Using mandibles of ICR mouse at specific developmental stages, *in situ* hybridisation was performed by DIG-labeled RNA probe.
Results: Faint expression was detected in limited cells at embryonic day 14 (E14) in the molar. At the bell stage, E16, *Fam83h* was localized in the outer and inner enamel epithelium, as well as dental papilla. *Fam83h* expression begins on E15 in the developing incisor. At E18, *Fam83h* was expressed in the inner enamel epithelium of the apical bud, ameloblasts and odontoblasts. The expression was stronger in the presecretory stages than the secretory stages.
Conclusion: *Fam83h* was detected in the ameloblasts from the presecretory to the secretory stage, and also the odontoblasts layer and surrounding alveolar bone.

P18

Expression of prion gene and presence of prion protein during development of mouse molar tooth germ

Khan Q.-E.-S.[1], Press C.M.[2], Sehic A.[1], Risnes S.[1], Osmundsen H.[1]
[1]*University of Oslo, Department of Oral Biology, Oslo, Norway,* [2]*Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, Oslo, Norway*

Expression of Prnp (encoding the Prion protein) in the mouse first molar tooth germ increased several-fold during the secretory phase of odontogenesis. A similar time-course of expression was found for genes coding for enamel matrix proteins (Amelx, Ambn, Enam) and for Aplp1, Clstn1, and Clu. Western-blot analysis suggested that the amounts of Prion protein (PrP) and Amyloid beta (A4) precursor-like protein 1 (APLP1) in the tooth germ followed similar time-courses to those of expression of the corresponding mRNAs. Immunohistochemical studies of PrP in cells of mouse molar and incisor tooth germs at E18.5 suggested this protein to be located in the cervical loop, outer enamel epithelium, and in preameloblasts. Immunolabelling for PrP was also observed in the dental papilla, initially at the base of the tooth germ, later more centrally. Different immunolabelling of preameloblasts on the mesial and distal aspects of a lower molar cusp may be related to different enamel configurations on the two aspects. The results suggest that PrP, as well as other extracellular proteins showing similar expression profiles, may have functions during secretory odontogenesis and amelogenesis.

P19

Early odontogenesis of *Chamaeleo calyptratus*

Buchtova M.[1,2], Balková S.[1], Zahradníček O.[3], Tucker A.S.[4]
[1]Academy of Sciences of the Czech Republic, Institute of Animal Physiology and Genetics, v.v.i., Brno, Czech Republic, [2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]Charles University, Department of Zoology, Praha, Czech Republic, [4]King's College London, Department of Craniofacial Development and Orthodontics, London, United Kingdom

Background: Veiled chameleons (*Chamaeleo calyptratus*) are omnivores with a diet consisting of insects, leaves and plants. They have monophyodont dentition in contrast to most toothed reptiles with polyphyodont dentition. Furthermore, chameleons possess a heterodont dentition with monocuspid and multicuspid teeth acrodontly ankylosed to jaw bones. The aim of recent study is to investigate odontogenesis in the chameleon, with specific focus on the comparative morphology of the dental lamina, the mechanism of transition from polyphyodont to monophyodont dentition and cusp formation.
Methods: Embryos of veiled chameleon were collected during the first six months of incubation (from 5th to 24th week) over 7 day intervals. The *in ovo* development of this species lasts from 6-8 months depending on incubation conditions. Serial histological sections were prepared and alternative slides were used for immunohistological analysis (PCNA, TUNEL) and *in situ* hybridization. MicroCT analysis was performed on one and half month old specimen to analyze shape of individual teeth along the jaw. *In situ* hybridization was performed using a python *Shh* probe.
Results: After five weeks of incubation, the dental lamina was present as a shallow epithelial thickening protruding into the mesenchyme. A week later the epithelium elongated more deeply into the mesenchyme to form the dental lamina. Dental lamina growth was lingually angled. The formation of tooth germs was initiated in close proximity to the oral epithelium in the 7th week of incubation. From early stages, tooth germs developed as asymmetrical structures with a large cervical loop on the lingual side. In contrast to gecko, the stellate reticulum contained numerous cells. Transversal sections revealed small clusters of cells with few apoptotic cells situated in the inner enamel epithelium that resembled the enamel knot area in mouse. Dentin and enamel production was well on the way by the 11th week. Development of a small successional dental lamina was initiated in the 14th week and overgrew the tooth germ in the lingual direction. Proliferation activity was high in the cervical loops and successional dental lamina. *Shh* expression was present in the inner enamel epithelium of cup and bell stages. Furthermore, *Shh* transcript was localized asymmetrically in the oral epithelium on the lingual side of lamina. MicroCT analysis showed the presence of heterodont dentition in young chameleons with tricuspid teeth in the more proximal jaw area. These tricuspid teeth were composed of the massive central cusp and two lateral smaller cusps localized in mesio-distal direction. In the distal jaw area, teeth were unicuspid. One and half month old animals showed twelve erupted teeth. New teeth were initiated in the caudal areas as the jaws became elongated during the post-hatching period.
Conclusion: Reptiles exhibit large variability in the type of dental attachment from acrodont to pleurodont, in the shape of teeth from unicuspid to multicuspid as well as in the number of replacement teeth generations from monophyodont to polyphyodont replacement. This high variability enables us to study evolutionary and developmental mechanisms of odontogenesis and compare differences in developmental processes in relation to mammalian species.
Acknowledgements: MB and OZ are supported by GAASCR (grant KJB601110919) and IRP IPAG No. AVOZ 5045015, AST is supported by an International Joint grant from the Royal Society (JP080875).

P20

Mechanism of dental lamina degradation

Buchtová M.[1,2], Štembírek J.[1,3], Matalová E.[1,2], Míšek I.[1,2]
[1]Academy of Sciences of the Czech Republic, Institute of Animal Physiology and Genetics, Brno, Czech Republic, [2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]University Hospital Ostrava, Department of Oral and Maxillofacial Surgery, Ostrava, Czech Republic

Background: Minipig has diphyodont and heterodont dentition resembling that of human. Elongation of the dental lamina into the mesenchyme is necessary process for replacement teeth formation. Successional lamina overgrows the deciduous anlagen in the lingual direction when primary dentition reaches the late bell stage. At the same developmental stage as the second generation is initiated, the dental lamina is disconnected from oral epithelium and fragmentizes into several pieces. However, nothing is known about mechanism of dental lamina regression during development of diphyodont dentition. Here, we aim to evaluate possible processes during early stages of dental lamina regression by using immunohistochemical detection of candidate molecules.
Methods: Minipig embryos and fetuses were obtained from Liběchov animal facility (Czech Republic, strain LiM). They were collected between the embryonic day (E) 19 and 67 and fixed in 4% neutral formaldehyde. After paraffin processing, 5 mm serial tissue sections were prepared, stained with Hematoxylin-Eosin and alternative slides were used for immunohistochemical analysis. The apoptotic DNA breaks were labeled by the TUNEL method (Chemicon). To study early apoptotic markers in the dental lamina we detected Fas (Santa Cruz Biotechnology), Fas-L (Santa Cruz Biotechnology) and FADD (Santa Cruz Biotechnology). As markers for the epithelial-mesenchymal transition (EMT) we chose E-cadherin (Abcam), MMP2 (Abcam) and Slug (Abcam). Primary antibody was incubated at room temperature in a humidified chamber for 1hour. Counterstaining with Hematoxylin was performed.
Results: The first sign of the lamina degeneration and loss of its connection to the oral epithelium became obvious at E50. Between E56 and E67 the dental lamina fragmentized and lost the body mass. There were morphological differences between both sides of the dental lamina where the side facing the tooth anlagen became degraded as the first. We detected the presence of apoptotic cells during the regression of dental lamina. We expected to see condensation of apoptotic cells at area of connection the lamina to the oral epithelium and the differences in distribution of TUNEL positive cells on both sides of lamina. However, only few TUNEL positive cells were evident in the dental lamina at these developmental stages. To test the presence of pro-apoptotic markers we detected Fas, Fas-L and FADD at the late stages of lamina development when the signs of regression were already obvious. All markers were situated on the side of lamina facing the teeth. Next we tested the presence of epithelial-mesenchymal transformation markers during the degradation of lamina. MMP2 and Slug were increased in the side facing the tooth germ in E56 compared to E67 while E-cadherin was decreased in the whole mass of dental lamina.
Conclusion: Based on TUNEL analysis results we propose that early disconnection of dental lamina with oral epithelium is not occurring by massive death of dental lamina cells and thus apoptosis does not seem to be the main mechanism involved in the dental lamina fragmentation. The localization and dynamics of EMT markers during the dental lamina degradation correspond to their pattern in the secondary palate seam disintegration and our results demonstrate the EMT as the process involved in the lamina breakup.
Acknowledgements: This research was supported by the GACR (grant 304/08/P289) and the lab runs under IRP IPAG No. AVOZ 5045015.

P21

Dynamics movement of dental tissue during tooth development

Rothova M.[1,2,3], Peterkova R.[2], Tucker A.S.[1]
[1]King's College London, Craniofacial Development, London, United Kingdom, [2]Academy of Sciences of the Czech Republic, Department of Teratology, Prague, Czech Republic, [3]Charles Uninversity, Department of Developmental Biology, Prague, Czech Republic

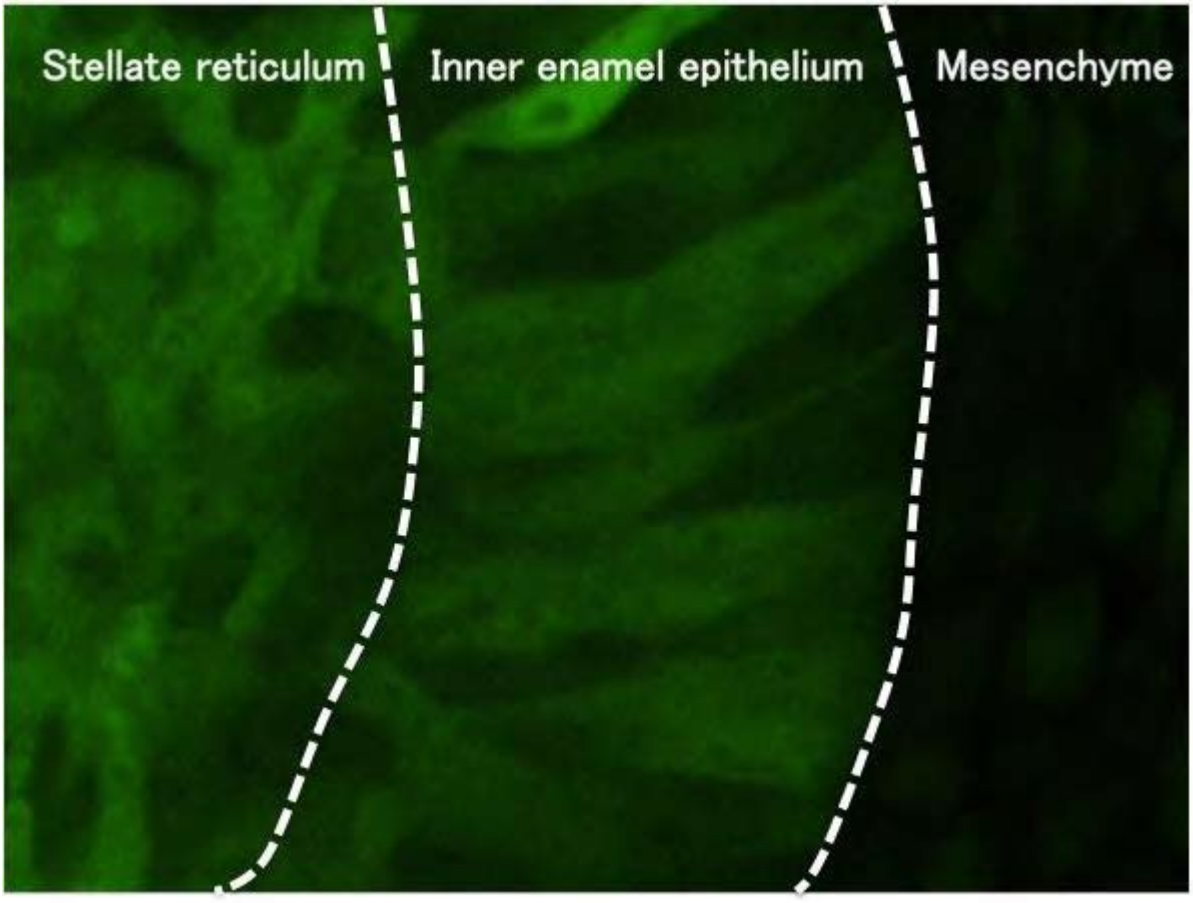
Background: Teeth develop from epithelium and neural-crest derived mesenchyme via a series of reciprocal epithelial-mesenchymal interactions. Previous studies have shown the dynamic movement of cells in the dental follicle and their contribution to the periodontium. In this project we focus on the composition of the developing dental papilla, and movements in and out of this structure as it develops from the cap to the late bell stage.
Methods: Using a slice culture technique to visualise the developing tooth germ we have followed the movement and fate of dental cells during tooth development. Our research combines cell labeling, using DiI and DiO, and the use of transgenic reporter mice, to follow cell movement and the origin of dental tissues.
Results: Our results show that the tooth is a dynamic organ with dramatic movement of cells. We show the timing of migration of mesoderm cells into the tooth and the changing pattern of cells involved with epithelial-mesenchymal interactions.
Conclusions: This work lays the ground work for our understanding of the tooth, and shows the contribution of a variety of cells to dental development.
This work is funded by an Erasmus agreement between Charles University and King's College London and a grant from the European Science Foundation.

P22

Visualizing cellular dynamics of tooth development

Otsu K.[1,2], Fujiwara N.[2], Harada H.[2]
[1]Iwate Medical University, Advanced Oral Health Science Research Center, Morioka, Japan, [2]Iwate Medical University, Division of Oral Histology and Developmental Biology, Morioka, Japan

Rationale: Most of studies for tooth development have been mainly done with histological methods and cell culture. However, it has been difficult to observe the morphological changes and cellular dynamics properly during the organogenesis. We have therefore thought it´s been needed to design a new experimental system which can observe living cells keeping in vivo kinetics. In this study, we show the real time imaging system for a living tooth using the slice culture.
Material & methods: We dissected the apical end of postnatal 2-4 days mouse lower incisors. The apical ends were embedded in a low melting point agarose. The embedded samples were then sliced to 150-200 mm thickness with vibratome and transferred to the culture chamber on confocal microscopy. The sliced sample was maintained at 37°C and exposed moisturized 5% CO_2 gas. Time-lapse 3D imaging was performed in the xyz-t mode using FV300 multiposition stage system. Data analysis and creation of the movies were performed using Metamorph software.
Results: Time-lapse images of GFP mouse apical bud containing dental epithelial stem cells (Fig.), revealed that the stellate reticulum cells facing inner enamel epithelium were dividing. Regarding the divided cells, one daughter cell stayed on-site and the other one migrated into a layer of basal epithelial cells. These observations were in line with dental epithelial stem cell kinetics we proposed before (Harada, et al, JCB, 1999). Furthermore, we used Fucci mouse to determine the cell-cycle dynamics in apical bud. The cells with green fluorescence, which represents S/G_2/M phases, were mainly located in the inner enamel epithelium and the peripheral stellate reticulum cells facing inner enamel epithelium. The location of the cells was consistent with that of dental epithelial stem cells, suggesting that this imaging system could allow chasing dental epithelial stem cells in real time.
Conclusion: This imaging system for living tooth we established will be powerful tool to help us understand spatial and temporal phenomena during tooth development.



[Otsu Fig.]
Appearance of the apical bud of sliced GFP mouse incisor. Three layers were distinguished with the morphology.

P23

Morphogenesis of the mouse third molar (M3)

Chlastakova I.[1,2], Radlanski R.J.[3], Wells K.[4], Tucker A.S.[4], Lungova V.[1,2], Misek I.[1,2], Matalova E.[1,2]
[1]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [2]Institute of Animal Physiology and Genetics CAS, v.v.i., Brno, Czech Republic, [3]Freie Universität Berlin, Center for Dental and Craniofacial Sciences, Berlin, Germany, [4]King's College, Guy's Hospital, Department of Craniofacial Development, London, United Kingdom

Background: In humans the third molar (M3) is the most commonly missing tooth type. All three molars in a quadrant (M1, M2, M3) develop from a single molar placode. Formation of the three molars has been followed in the mouse. M1 and M2 form during embryonic development, while M3 develops largely postnatally, once growth of the jaw has reached a size to accommodate all the teeth. Therefore, the mouse M3, unlike the M1 or M2, develops surrounded by fully mineralized bone. The aim of this study was to supplement our poor knowledge of M3 development and osseointegration in the mouse, with a special focus on the stages of enamel knot formation.
Methods: M3 development was evaluated by analysis of histology, proliferation (PCNA - proliferating cell nuclear antigen), apoptosis (TUNEL) and *in situ* hybridisation (*Shh, Fgf4*) from postnatal (P) 0 to P9.
Osteoclasts were identified in the surrounding bone using tartrate resistant alkaline phosphatase activity (TRAP staining).
The mouse mandibular molar dental primordia from P0-P20 were visualized in serial sections and reconstructed in 3D together with their surrounding bony structures. Regions of bone apposition and resorption were included.
Results: The M3 is first visible as a bud extending from the M2 at P0. A cap stage forms by P3, at which stage the primary enamel knot undergoes apoptosis. The secondary enamel knots are visible at P5.
Apoptosis in the surrounding bone showed a lingual-buccal asymmetry, correlating with the growth of the cervical loop as shown by PCNA staining.
While in P0 the M3 is not completely surrounded by bone, it becomes gradually more encapsulated towards stage P20. The occlusal surface, however, remains free of bone. The bony crypt is characterized by bone resorption activity, while the crestal, the interdental and the periradicular bone is characterized by apposition.
Conclusions: The morphology of the developing M3 and the surrounding bone were investigated by a variety of methods. The M3 primary enamel knot is visible at P3, while the secondary enamel knots form at P5. P3 and P5 for M3 therefore correspond to Embryonic day (E)15 and E17 for M1. Although M3 develops at a time of extensive jaw bone development, we have been able to show that it initiates budding in an area at the edge of the bone, and later becomes encapsulated.
Supported by the Grant Agency of the Czech Republic (524/08/J032), the Deutsche Forschungsgemeinschaft (Ra 428/1-9), and a Royal Society Joint International Grant (JP080875). The IAPG lab runs under IRP IPAG No. AVOZ 50450515.

P24

The developmental stage-dependent increase in expression of soluble guanylate cyclase during mouse tooth development

Korkmaz Y.[1], Beikler T.[1], Klinz F.-J.[2], Schneider K.[1], Behrends S.[3], Addicks K.[2], Bloch W.[4], Raab W.H.-M.[1]
[1]Heinrich-Heine-University, Düsseldorf, Department of Operative Dentistry, Periodontics and Endodontics, Düsseldorf, Germany, [2]University of Cologne, Department I of Anatomy, Cologne, Germany, [3]Technical University of Carolo-Wilhelmina at Braunschweig, Institute for Pharmacology, Toxicology and Clinical Pharmacy, Braunschweig, Germany, [4]German Sports University, Cologne, Germany

The nitric oxide-receptor enzyme soluble guanylate cyclase (sGC) contains one prosthetic heme group and two heterodimer isoforms ($\alpha_1\beta_1$, $\alpha_2\beta_1$) whose enzymatic activity have been characterized. There is no information available regarding the expression of sGC during tooth development. In order to clarify the expression of sGC during mouse tooth development, localizations of the α_1-, β_1- and α_2-subunit of sGC were examined in cells of the molar germs at prenatal (E20.5), postnatal (P3) and in adult (3 months) stages. In ameloblasts and odontoblasts of the molar germ at E20.5, a weak staining for α_1-, β_1- and α_2-subunit was detected. The localization of the β_1-subunit was identified in the nucleus of odontoblasts. In ameloblasts and odontoblasts of the molar germ at P3 stage, the staining intensities for the α_1- and β_1-subunit were increased. There were no changes in staining intensities for the α_2-subunit between E20.5 and P3 stages. In comparison to P3, higher staining intensities of the α_1-, β_1- and α_2-subunit of sGC were detected in adult molar odontoblasts. These data indicate that ameloblasts and odontoblasts may be regulated by the $\alpha_1\beta_1$-heterodimer of sGC during tooth development, while adult odontoblasts are regulated by $\alpha_1\beta_1$- as well as by $\alpha_2\beta_1$-heterodimer isoforms of sGC. We conclude that there is a developmental stage and cell type dependent expression of sGC during tooth development.

P25

Functional isolation of dental stem cells

Pang Y.W.Y.[1,2], Mantesso A.[1], Wall I.[3], Sharpe P.[1]
[1]Kings College London, Craniofacial Department- Dental Institute, London, United Kingdom, [2]University College of London, Biochemical Engineering, London, United Kingdom, [3]University College of London, Advanced Centre for Biochemical Engineering - Regenerative Medicine Bioprocessing, London, United Kingdom

Background: One of the main challenges in making biological tooth replacement a reality is the isolation of potential stem cells for therapeutic application. Although many stem cell populations have been identified in anatomically distinct regions of the human tooth and its surrounding tissues, there is currently no reliable marker to isolate these cells. Markers used in literature reveal different populations with diverse stem cell properties which compromises broad clinical application of these cells due to heterogeneity.
Methods: Rather than detecting cellular markers, our approach was to identify mesenchymal stem cells in rodent incisors using physical properties to isolate different cell populations. We aimed to identify the stem cells by performing growth curve, differentiation and migration experiments on cells isolated from cervical loop and body region of the rodent incisor and from the molar tooth. To determine whether a privileged population of very small embryonic-like (VSEL) stem cells also exists in the tooth, we have adopted a modified cell migration approach. Using invasion chambers with 8μm and 4μm pore sizes, cells which migrated through were assessed in terms of differentiation capacity. Growth curves were conducted to compare the proliferation rates of the different cell types.
Results: Our results have demonstrated that the cells isolated from mesenchyme close to the cervical loop region are distinctly different from those isolated from the bone marrow or molar dental pulp. These differences are evident in terms of size, morphology and greater proliferation capacity of cervical loop cells. In addition, the migratory capacity is greatly altered indicating that these cells are a unique, privileged population that requires further characterisation to determine their clinical potential.
Conclusion: In summary, identifying dental stem cells based on migration may enable isolation of a purer population with relative ease. In terms of future bioprocessing and translation to a reproducible therapy, the migration procedure is economical and quick to perform. The results obtained to date from rodent incisors suggest that it may be possible to obtain from adult dental tissue suitable stem cells for dental tissue engineering.

P26

Characterization of the putative epithelial stem cells involved in continuous tooth replacement in Atlantic salmon (*Salmo salar*)

Vandenplas S.[1], Willems M.[1], Soenens M.[1], Witten P.E.[1], Huysseune A.[1]
[1]*Ghent University, Biology Department, Ghent, Belgium*

With the exception of mammals, vertebrates have the ability to replace their teeth throughout life. It has been suggested that adult epithelial stem cells are required for continuous tooth replacement (Huysseune & Thesleff 2004). The dentition of Atlantic salmon (*Salmo salar*), a teleost fish and member of the Salmonid family, has been well characterized (Huysseune et al. 2007). Similar to other salmonids (e.g. rainbow trout, *Oncorhynchus mykiss),* replacement teeth develop without the involvement of a successional dental lamina. Presumably epithelial stem cells reside in a cell layer between the inner and outer dental epithelium, termed the middle dental epithelium (Huysseune & Witten 2008). Their progeny is assumed to translocate to the outer dental epithelium to form a transient amplifying cell (TAC) population that eventually gives rise to a new tooth bud.
To better understand the spatial relationship between the potential stem cell niche and the developing replacement tooth, we made 3-D reconstructions based on histological sections. To characterize cell dynamics within the different epithelial cell layers (including the putative stem cell compartment), we performed cell proliferation assays. Different stages of tooth replacement were labelled for proliferating cells in late G1-phase and S-phase of the cell cycle by means of PCNA (Proliferating Cell Nuclear Antigen) immunostaining. High numbers of labelled cells were observed in the local thickening (placode) of the outer dental epithelium of the functional predecessor, representing the initiation stage of the replacement tooth. At the morphogenesis stage, labelled cells were observed at the border of the middle dental epithelium and the outer dental epithelium of the predecessor tooth, in contrast to the inner dental epithelium and the bulk of the middle dental epithelium, which showed no labelling. Labelling in the replacement tooth was mostly restricted to the lingual side of the outer dental epithelium at the level of the cervical loop. The numbers of labelled cells in the different epithelial layers of the replacement tooth germ decreased during cytodifferentiation.
These results are consistent with the behaviour of putative epithelial stem cells in the middle dental epithelium as hypothesized above. Ongoing experiments to further characterize putative stem cells include BrdU (Bromodeoxyuridine) proliferating assays to investigate label retention.

Huysseune, A. and Thesleff I. (2004). Continuous tooth replacement: the possible involvement of epithelial stem cells. BioEssays **26**(6): 665-671.
Huysseune A. , Hall B. K., Witten P. E. (2007). Establishment, maintenance and modifications of the lower jaw dentition of wild Atlantic salmon (*Salmo salar* L.) throughout its life cycle. Journal of Anatomy **211**: 471-484.
Huysseune, A. and Witten P.E. (2008). An evolutionary view on tooth development and replacement in wild Atlantic salmon (*Salmo salar* L.). Evolution & Development **10**(1): 6-14.

P27

Cell culture of human dental epithelial cells and its approach for regenerative dentistry

Mayama H.[1], Fujiwara N.[2], Sasaki A.[2], Otsu K.[2,3], Sakuraba H.[1], Miura H.[1], Harada H.[2]
[1]Iwate Medical University, Division of Orthodontics, Morioka, Japan, [2]Iwate Medical University, Division of Histology and Development Biology, Morioka, Japan, [3]Iwate Medical University, Advanced Oral Health Science Research Center, Morioka, Japan

Teeth regeneration is an ideal treatment for patients with congenitally missing teeth. In the current state, teeth regeneration has been studied using animal model, however, human tooth regeneration has not yet been reported. Because it is known well that tooth development proceeds through epithelial-mesenchymal interactions, in the human tooth regeneration we also requires both of epithelial and mesenchymal stem cells derived from human tissues. In recent years, the human mesenchymal stem cell has been discovered from pulp, periodontal membrane, impacted third molar germs and exfoliated deciduous tooth, otherwise human dental epithelial stem cells has not been reported. Therefore, in this study, we have challenged an exploration of human dental epithelial stem cells and tried to culture the cells, and furthermore examined the possibility of human tooth regeneration using these dental epithelial stem cells and mesenchymal stem cells.
Because some recent studies using a variety of animals suggested the possibility that dental lamina includes the dental epithelial stem cells, we examined if human dental lamina cells exist in enucleated third molar germs. In this study, we utilized impacted third molar germs extracted from patients (8-12 years old) undergoing orthodontic treatments, under sufficient informed consent in accordance with the Ethics Committee of School of dentistry, Iwate Medical University. The third molar germ was cut into two parts, an upper part including dental lamina and dental papilla. And we made paraffin embedded sections and carried out hematoxylen-eosin staining and Immnunostaining. The results showed that fragmented dental lamina epithelial cells existed in the tissues and were positive for cytokeratin14, E-cadherin and p63.
Next, the upper part was digested by collagenase/dispase, and used for primary culture. The cells were successfully cultured under DMEM/hamF12 serum-free culture condition in the presence of EGF and FGF2, and at second passage dental lamina epithelial cells successfully purified from mesenchymal cells by the method utilizing difference of cell-dish adhesion property between these cells. Immunophenotypic analyses of cell surface antigens by FACS showed that more than 90% of the cells were positive for E-cadherin. Furthermore, these cells were positive for cytokeratin14, CD133, CD49f, and p63. The result was identical with that of human dental lamina epithelium, in vivo.
In order to research the possibility of tooth regeneration using these cells, we transplanted the recombination of the dental lamina epithelial cells and dental papilla into abdominal cavity of SCID mice. 0ne month later, the epithelial cells developed dental epithelium of bud stage in the recombinant tissue. Taken together, the results suggests that human dental lamina cells are useful as a source of dental epithelial stem cells for human tooth regeneration.

P28

Complexity of chiropteran dentition: the GIS approach

Spoutil F.[1], Jernvall J.[2], Evans A.[3]
[1]University of South Bohemia, Deparment of Zoology, Ceske Budejovice, Czech Republic, [2]University of Helsinki, Institute of Biotechnology, Helsinki, Finland, [3]Monash University, Deaprtment of Zoology, Malbourne, Australia

Background: Chiroptera represents the second largest mammalian order. Yet, most of its members bears the most primitive molar type, the tribosphenic one, which is the best type for insectivory (except myrmecophagy). Nevertheless, differences have occurred in the clade in various aspects of the molar shape during its evolutionary history: from differences in size and cusp height to finer changes, i.e. reorientation of shearing crests. The impact of these characters on the final dietary niche is not always easy to estimate.
Methods: To attempt to do so, we have used GIS techniques (already tested on Rodentia and Carnivora) to describe overall complexity of molar rows in bats and so to quantify the impact of the differences in molar crown shape and size. In addition, great morphological similarity among various molar rows of bat species can help us to test limitations of this new method.

P30

Exploring the roles of core-binding factor β (CBFB) in tooth development and ameloblast physiology

Kurosaka H.[1], Kuremoto K.[2], Hayano S.[1], Nurul I.[1], Harada H.[3], Yamashiro T.[1]
[1]Okayama University, Department of Orthodontics, Okayama City, Japan, [2]Osaka Dental University, Department of Removable Prosthodontics and Occlusion, Osaka City, Japan, [3]Iwate Medical University, Department of Oral Anatomy II, Morioka-City, Japan

Core-binding factor β (CBFB), a cofactor of RUNX gene family had attracted broad interest in recent years because of its critical roles in various developmental events. In the present study we investigated the possible roles of CBFB at the dental epithelium, using conditional null mutant mice (K14-Cre;RUNX1 flox, K14-Cre;CBFB flox).
Rodent incisor featured with continuous elongation throughout life. This mechanism is support by labial cervical loop epithelium which contains proliferative cells and ameloblast precursor cells. CBFB deficiency in epithelium resulted in shorter incisor with poor enamel formation, non-polarized incisor ameloblast and less proliferating cells in labial cervical loop epithelium. In addition, FGF3 mRNA expression decreased at the mesenchyme which underlies the labial cervical loop. These results suggest CBFB contribute to ameloblast differentiation, cell proliferation of incisor cervical loop epithelium and FGF expression in underlying mesenchyme.

P31

Involvement of micro-RNAs in tooth morphogenesis and ameloblast differentiation

Michon F.[1], Juuri E.[1], Tummers M.[1], Kyyronen M.[1], Frilander M.[1], Thesleff I.[1]
[1]*Institute of Biotechnology, University of Helsinki, Finland*

Background: Teeth form as appendages of the ectoderm and their morphogenesis is regulated by tissue interactions mediated by networks of conserved signal pathways. The micro-RNA (miRNA) pathway has emerged as an important regulator of various aspects of embryonic development but its function in odontogenesis has not been elucidated.
Methods: In order to examine the functions of miRNA during odontogenesis, we have used qPCR, miRNA microarray profiling and transgenic mouse lines.
Results: The expressions of several RNAi pathway effectors and miRNAs were dynamic during tooth morphogenesis and epithelial cell differentiation. The conditional deletion of Dicer-1 in the dental epithelium in transgenic mice led to aberrations in the molar shape and cusp pattern as well as defects in the structure of enamel. Moreover, epithelial cell proliferation was increased in the continuously growing incisor and defects in ameloblast differentiation appeared in the pups and increased with the time.
Conclusion: Our findings indicate that the miRNA pathway regulates several aspects of odontogenesis and plays important roles in epithelial morphogenesis and cell differentiation. The aberrations seen in molar cusp patterning in the Dicer1 mutant mice suggest roles for miRNAs in the evolution of the tooth crown.

P32

Distribution and structure of the initial dental enamel formed in incisors of young wild-type and Tabby mice

Sehic A.[1], Peterkova R.[2], Lesot H.[3], Steinar R.[1]
[1]University of Oslo, Department of Oral Biology, Oslo, Norway, [2]Institute of Experimental Medicine, Academy of Sciences, Department of Teratology, Prague, Czech Republic, [3]University of Strasbourg, UMR INSERM 977, and Dental Faculty, Strasbourg Cedex, France

Mouse incisor enamel can be divided into four layers: an inner prism-free layer; an inner enamel with prism decussation; outer enamel with parallel prisms; and a superficial prism-free layer. We wanted to study how this complex structural organization is established in the very first enamel formed in wild-type mice and also in Tabby mice where enamel coverage varies considerably. Unworn incisors from young female wild-type and Tabby mice were ground, etched, and analyzed using scanning electron microscopy. In both wild-type and Tabby mice, establishment of the enamel structural characteristics in the initially formed enamel proceeded as follows, going from the incisal tip in an apical direction:
(i) a zone with prism-free enamel,
(ii) a zone with occasional prisms most often inclined incisally, and
(iii) a zone where prism decussation was gradually established in the inner enamel.
The distribution of enamel in Tabby mice exhibited considerable variability. The sequence of initial enamel formation in mouse incisors mimics development from a primitive (prism-free) structure to an evolved structure. It is suggested that genes controlling enamel distribution are not associated with genes controlling enamel structure. The control of ameloblast configuration, life span, organization in transverse rows, and movement is important for establishing the characteristic mature pattern of mouse incisor enamel.

P33

Fine structural and immunohistochemical observations of the collar enamel in Lepisosteus and Polypterus, actinopterygian fish

Sasagawa I.[1], Ishiyama M.[2], Yokosuka H.[2], Mikami M.[3], Uchida T.[4]
[1]Nippon Dental University, Advanced Research Centre, Niigata, Japan, [2]Nippon Dental University, Histology, Niigata, Japan, [3]Nippon Dental University, Microbiology, Niigata, Japan, [4]Hiroshima University, Oral Biology, Hiroshima, Japan

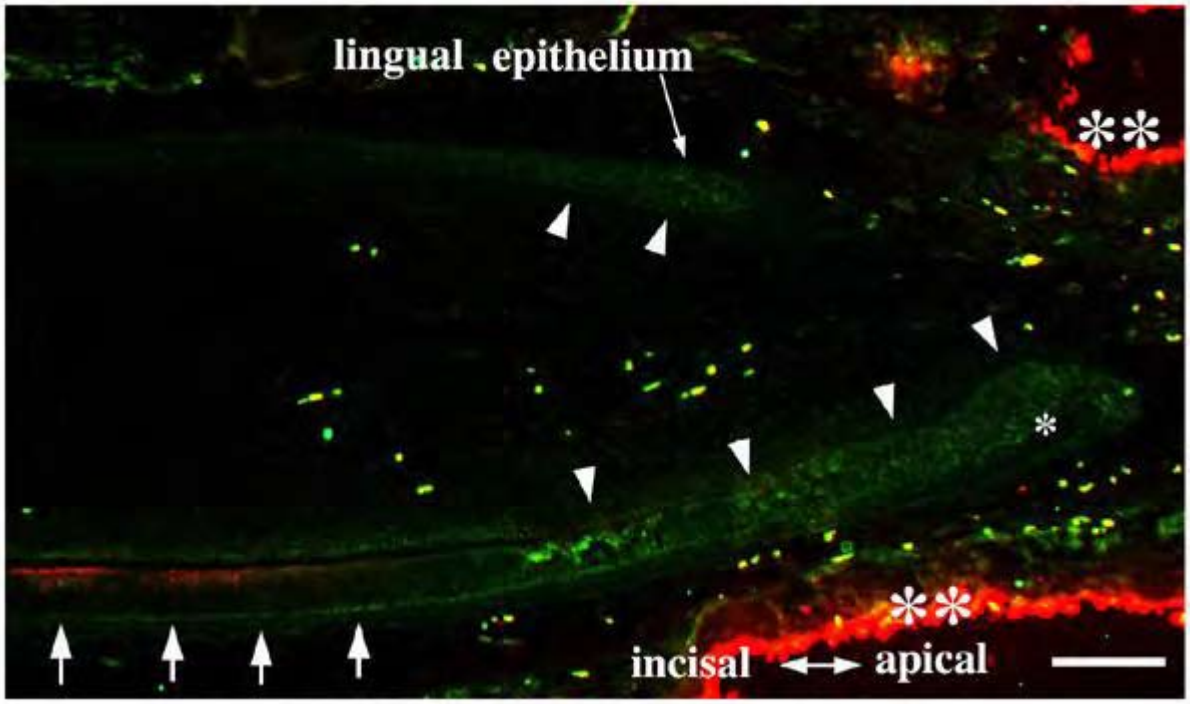
Background: It is commonly accepted that ectodermal enamel covers the tooth crown in reptiles, amphibians and sarcopterygian fish, Latimeria and lungfish. A few actinopterygian fish, Lepisosteus and Polypterus, demonstrate collar enamel on the surface of the tooth shaft [1, 2, 3]. The structural features of collar enamel in Lepisosteus and Polypterus resemble those of the enamel in sarcopterygians and amphibians. The organic matrix of the collar enamel in Lepisosteus and Polypterus shows marked mammalian amelogenin immunoreactivity [2, 4]. Collar enamel in actinopterygians is thought to be a precursor of ectodermal enamel in higher vertebrates. However, the genes related to enamel protein that are present in amphibians, reptiles and mammals, are not found in fish. It is proposed that secretory calcium-binding phosphor-protein (SCPP) genes are involved in producing enamel-like tissues in teleost fish. A recent molecular genetic study reported that the strong expression of the odontogenic, ameloblast associate (ODAM) gene, which might be involved in the hypermineralization process at the late stage of enameloid maturation, was detected in the inner dental epithelial (IDE) cells of zebrafish [5]. In Lepisosteus and Polypterus, both enameloid and collar enamel are found in the same tooth. Therefore, they are suitable materials to study developmental relationships between enameloid and enamel. In this study, we focus on the morphological features of collar enamel, and on the chemical composition of collar enamel.
Methods: Collar enamel and dental epithelial cells in Lepisosteus oculatus and Polypterus senegals were observed by light and transmission electron microscopy, and by light and electron microscopic immunohistochemistry using crude antiserum against porcine 25 kDa amelogenin, region-specific antibodies or antiserum against the C-terminus and central region of the porcine 25 kDa amelogenin, respectively [6].
Results and conclusion: The enamel layer, 600nm-5um thick, containing amorphous fine organic matrix was located between the dentin and IDE cells in the secretory stage. The layer also continues to the surface of enameloid near the dentin - enameloid junction, suggesting that the enamel covers both dentin and mature enameloid. It is assumed that the collar enamel in Lepisosteus and Polypterus is produced as a terminal product of the IDE cells at the late stage of enameloid maturation.
References:
[1]Prostak et al. (1989) In, Fearnhead R. W. (ed) Tooth Enamel V. 188-192, Florence.
[2]Ishiyama et al. (1999) Arch Histol Cytol, 62, 191-197.
[3]Sasagawa I. et al. (2008) Front Mater Sci China, 2, 134-142.
[4]Sasagawa I. et al. (2009) Front Mater Sci China, 3,174-182.
[5]Kawasaki K. (2009) Dev Genes Evol, 219. 147-157.
[6]Uchida et al. (1991) Histochemistry, 96, 129-138.
Acknowledgments: This study was supported in part by a Grant-in-Aid for Scientific Research, 16591844, from the Ministry of Education, Science, Sports and Culture, Japan, and by a Research Promotion Grant (NDUF-09-18, 08-19, 07-12) from Nippon Dental University.

P34

Identification of suppressor element in the amelogenin promoter.

Matsumoto A.[1,2,3], Xu L.[1,4], Harada H.[5], Taniguchi A.[1,3]
[1]National Institute for Materials Science, Biomaterials Center, Tsukuba, Japan, [2]Kyokuto Pharmaceutical Industrial CO. LTD., Takahagi, Japan, [3]University of Tsukuba, Tsukuba, Japan, [4]National Institute for the Control of Pharmaceutical & Biological Products, Beijing, China, [5]Iwate Medical University, Department of Operative Dentistry and Endodontics, Morioka, Japan

Background: Amelogenin expression is regulated at both the transcriptional and post-transcriptional levels. Previously, we demonstrated that the reuptake of amelogenin protein results in increased levels of amelogenin mRNA through enhanced mRNA stabilization (1,3). Amelogenin expression is also regulated in a cell type-specific manner. Investigators have previously identified an enhancer element by using the 5' flanking sequence of the amelogenin promoter (4). However, the cell type-specific regulation of the amelogenin gene remains poorly understood. In some genes, the first intron regulates tissue-specific expression. We hypothesized that intron 1 is important for the cell type-specific regulation of amelogenin expression.
Results: To understand the molecular mechanisms involved in the cell type-specific expression and developmental regulation of the amelogenin gene, we analyzed the effects of intron 1 on the amelogenin promoter activity in HAT-7 cells. We identified a suppressor element between -74 and -464 in amelogenin promoter. We also found enhancer activity in intron 1. Additionally, we found that the suppressor element in the promoter region suppresses intron 1 enhancer activity. The suppressor and the enhancers acted in an ameloblast-like cell line HAT-7, but not in HeLa cells. Mutation of the Oct-1 binding sites reversed the suppressor activity, suggesting that Oct-1 sites are essential for suppression. These results indicate that Oct-1 and intron 1 contribute to amelogenin expression. We performed immunostaining of Oct-1 and amelogenin in serial sections of PN d7 mouse incisors. The Oct-1 signal is observed in the inner enamel epithelium and is localized to the nuclei. The amelogenin signal is strongly observed in secretory ameloblasts concomitantly with the decrease in the Oct-1 signal (Figure 1). These findings suggest that Oct-1 as a cofactor might directly or indirectly collaborate in a cell type-specific manner with the intronic enhancer in the amelogenin gene (5).



[Fig.1]
Figure 1. Double immunostaining of Oct-1 (green) and amelogenin (red) in the mouse incisors at PN d3.

Conclusion: We identified a suppressor element in the promoter region and enhancer elements in intron 1. The suppressor and enhancers acted in an ameloblast-like cell line, HAT-7, but not in HeLa cells, suggesting that these elements act in a cell type-specific manner.
References:
1. Xu L, Harada H, Tamaki T-Y, Matsumoto S, Tanaka J, Taniguchi A. (2006) J Biol Chem. 281: 2257-2262
2. Xu L, Harada H, Taniguchi A. (2006) J Biol Chem. 281: 32439-32444.
3. Xu L, Harada H, Taniguchi A. (2008) J Biochem. 144: 531-537.
4. Zhou YL, Snead ML. (2000) J Biol Chem. 275: 12273-12280.
5. Xu, L., Matsumoto, A., Sasaki, A., Harada, H., Taniguchi, A. (2010) J. Dent. Res., 89, 246-251.

P35

Loss of transglutaminase 2 in the mouse results in an accelerated enamel mineralization

Lampé I.[1], Sarang Z.[2], Papp T.[3], Hegedűs C.[1], Szondy Z.[2], Felszeghy S.[3]
[1]University of Debrecen, Medical and Health Science Centre, Department of Prosthetic Dentistry, Debrecen, Hungary, [2]University of Debrecen, Medical and Health Science Centre, Department of Biochemistry and Molecular Biology, Research Center of Molecular Medicine, Debrecen, Hungary, [3]University of Debrecen, Medical and Health Science Centre, Department of Anatomy, Histology and Embryology, Debrecen, Hungary

Tissue transglutaminase (TG2) is an ubiquitously expressed member of the large TG family exerting a number of physiological and/or pathological functions. The involvement of extracellular TG2 in cell adhesion, extracellular matrix assembly or bone ossification has been demonstrated previously. However, there are no earlier *in vivo / in vitro* studies to assess the role of TG2 in tooth development. Therefore we studied TG2 null mice to characterize its tooth phenotype. Mice were sacrificed at different developmental stages as follows: intaembryonic developmental stages (E 14, 15, 16), prior to tooth eruption (postnatal day 1), and adulthood (2 months postnatal). During early tooth development, when the tooth germs were going through bud, cap and bell morphogenetic stages, no obvious morphological alteration were observed due to the loss of TG2. The first significant differences in the hardness of the enamel, which showed accelerated enamel mineralisation, became apparent at postnatal stages. Furthermore, additional 3D analysis of incisors from the knockout mice revealed that they were slightly longer with a thicker enamel layer than their wild-type controls. Our findings reveal that TG2 may play a regulatory role in the enamel bio-mineralization processes.

P37

A frameshift mutation in DSPP associated with dentinogenesis Imperfecta type II

Tallón-Walton V.[1], Nieminen P.[2], Arte S.[3], Aluusua S.[3], Chimenos-Küstner E.[4], Carvalho-Lobato P.[1], Manzanares-Céspedes M.-C.[1]

[1]University of Barcelona, Human Anatomy and Embryology Unit, Exp. Pathology and Therapeutics Dept, Hospitalet, Barcelona, Spain, [2]University of Helsinki, Institute of Dentistry, Helsinki, Finland, [3]Helsinki University Central Hospital, Department of Oral and Maxillofacial Diseases, Helsinki, Finland, [4]University of Barcelona, Odonto-Stomatology, Hospitalet, Barcelona, Spain

Background: Dentinogenesis imperfecta (DI) types I, II and III, is a congenital rare disease which affects the organic dentine matrix. Its etiology has been associated with defects in the dentin sialophosphoprotein (DSPP) gene (chromosome 4) (Kim et al, 2007, Barron et al, 2008). The aim of the present work was to study the phenotype and the genotype of three generations of a family affected by DI type II, showing a dominant transmission pattern.
Methods: The genomic DNA was isolated from peripheral lymphocytes or buccal swabs. The PCR product had to be inserted into pJET plasmid (Fermentas, Burlington, Canada) and cloned into Stbl2 cells (Invitrogen, Carlsbad, USA) to detect the mutation. The results were compared to the previously published human DSPP genomic sequence.
Results: Amber colour and total obliteration of the pulps and root canals in both dentitions were observed. Attrition was slight. Shortened and abnormally thin roots were observed in some permanent teeth. Periapical infections and dental fractures had caused several dental extractions. A heterozygous frameshift mutation (c.2349delT) causing a deletion in the region coding for DPP was identified.
Conclusion: We concluded that the identified mutation was the etiological cause of DI type II, suggesting that its location could be reflected in the phenotypic features as a severity gradient from dentine anomalies.

P38

Fragile fracture of dental pulp chamber: a new method to obtain dental pulp stem cells

Manzanares-Céspedes M.-C.[1], Paganelli C.[2], Laffranchi L.[3], Laffranchi A.[4], Porta F.[4]
[1]University of Barcelona, Human Anatomy and Embryology Unit, Exp. Pathology and Therapeutics Dept, Hospitalet, Barcelona, Spain, [2]Università degli Studi di Brescia, Orthodontics, Brescia, Italy, [3]University of Torino, Torino, Italy, [4]Az Spedali Civili di Brescia, SSVD OncoEmatologia Pediatrica, Brescia, Italy

Objective: The traditional technique to obtain Dental Pulp Stem Cells DPSC, with rotary tools for opening pulp chamber has been compared with a new fragile tooth fracture technique in order to improve dental pulp collection.
Methods: We have obtained a brittle fracture by an unstable propagation of a concentrated stress by the application of a calibrated increasing load on the teeth surface. Moreover we tested different notch techniques adapted to the different types of tooth morphology. The improvement in the cell collection has been tested by:
1) Identification of human dental stem cells using appropriate cell markers (STRO-1, CD34, CD44, CD106, CD146 and c-kit) assayed by cytofluorimetric analyses.
2) Assessment of the consequences of the pulp Collection timing, by the measurement of differences between DPSCs obtained immediately after tooth extraction and DPSCs from teeth cryopreserved following several protocols reported in literature.
3) Assessment of the effects on the vitality and the DPSC differentiation level due to different pulp extraction methods.
Results: We obtained a significant increase in cell vitality with our fragile fracture method when compared with the previously described technique.
Conclusion: The lessening of the thermal stress on the DPSC because of the reduction of temperature when opening the pulp chamber for stem cells extraction is probably the main cause of the improvement the dental pulp collection as well as the lowering of the differentiation induction.
This study was supported by a Spain-Italy Integrated Research Action Grant HI2007-0089.

P39

The vascularization of embryonic mouse molar papilla evaluated by CD34, CD31 antigen expression and morphological arrangement

Yuan G.[1], Yang G.[1], Zhang L.[1], Zhang Q.[1], Chen S.[2], Chen Z.[1]
[1]Wuhan University, Key Lab of Oral Biomedicine of Ministry of Education, Wuhan, China, [2]The University of Texas Health Science Center at San Antonio, Pediatric Dentistry, San Antonio, United States

Background: Mouse teeth develop as a result of sequential and reciprocal interactions between oral epithelium and cranial neural crest-derived mesenchyme. The vasculature is very important for embryonic organ development, for it is in charge of maintaining metabolic homeostasis by supplying oxygen and nutrients as well as excreting waste products. Although the fine structures and three-dimensional organization of the capillaries in the enamel organ, the dental epithelial structure, have been previously investigated, the process of vascularization of the tooth mesenchyme remains poorly understood.
Methods: In the hope of better understanding the mechanism of formation of tooth mesenchyme capillaries, mouse mandibular molars during embryonic (E) 13.5 to E16.5 were harvested and processed for CD34, CD31 immunohitochemistry and conventional transmission electron microscopy (TEM).
Results: At E13.5, molar tooth germ comprises condensed mesenchyme and a typical epithelial bud. At this stage, CD34- and CD31- positive cells are distributed in the prospective dental follicle around the tooth epithelium and condensed mesenchyme; By E14.5, the bud epithelium progressively takes the form of the cap configuration and develops into the internal and the external enamel epithelium, while the mesenchyme develops into the dental papilla and follicle. At this stage, in addition to the localization of CD34- and CD31- positive cells in the follicle, a few positive cells appeared within the follicle at the bottom of the tooth germ sprouting toward the dental papilla underlying the cap epithelium; From E15.5 to E16.5, molar tooth germ develops into early bell stage. And during this period, the CD34- and CD31- positive cells have entered the dental papilla, some of which are even progressively close to the prospective odontoblasts. Negative staining was found in the dental epithelium during the stages investigated. For TEM, abundant ribosomes, mitochondria, rough endoplasmic reticulum (RER), Golgi complexes, pinocytic vesicles, glycogen granules are present in the cytoplasm of the endothelial cells. The basal lamina of most capillaries was discontinuous or absent. The presence of cells containing vacuoles suggests vasculogenesis is taking place; Loss of basal lamina, presence of filopodia and lateral sprouting suggests angiogenesis is also occurring.
Conclusion: Neoformation of capillaries of molar mesenchymal papilla seems to occur simultaneously by mechanisms of vasculogenesis and angiogenesis.
This research was supported by the National Natural Science Foundation of China (Grant No. 30572043).

P40

Non-invasive imaging of human dental pulp stem cells

Struys T.[1], Gervois P.[1], Martens W.[1], Bronckaers A.[1], Politis C.[1,2], Lambrichts I.[1]
[1]Hasselt University, Department of Morphology, Diepenbeek, Belgium, [2]Ziekenhuis Oost-Limburg, Department of Maxillo-Facial Surgery, Genk, Belgium

Stem cells, with the capacity to differentiate into different types of cells and finally tissue, raise the hope of cell-based regenerative medicine for many diseases such as ischemic heart disease, neural disease, cancer, and many other disorders. Especially, adult mesenchymal stem cells, which have been described to be present in almost every organ including teeth, are gaining more interest because of their ease of isolation and elaborate differentiation potential and plasticity. As determining the fate of stem cells or their lineage committed progeny transplanted in vivo will be of utmost importance, stem cell labeling and non-invasive imaging will play a crucial role in determining the possible therapeutic potential of these stem cells. Magnetic resonance imaging (MRI) is one of the most powerful diagnostic imaging techniques currently available for in vivo diagnosis. Unfortunately, the excellent spatial resolution and detailed structural information obtained with this technique has to be paid with a decrease in sensitivity. Furthermore, diffusion of contrast agent out of labeled cells can provide non-specific signals. In contrast, optical imaging making use of bioluminescence offers a methodology which is highly sensitive and specific but lacks any structural 3D information. Therefore, a combination of both MRI and bioluminescence imaging will give detailed information on the in vivo location/migration of stem cells thereby providing both a high resolution and high sensitivity.
As little is known about the applicability of both MRI and optical bioluminescence imaging on human dental pulp stem cells, this study will investigate the efficacy by which these postnatal stem cells can be labeled and imaged making use of both techniques. For MRI, cells will be labeled with a commercially available MRI contrast agent named Endorem® which in fact are iron oxide based nanoparticles. Cells will be analysed using transmission electron microscopy (TEM), high resolution MRI and inductively coupled plasma spectrometry in order to quantify the amount of endocytosed iron. For bioluminescence imaging, cells will be transduced with a lenti-viral vector containing both GFP and Luciferase. Cells will be analysed using an IVIS optical imaging system. For both techniques, the optimal labeling strategies will be discussed. The effect of both labeling techniques on cell viability will be assessed by an MTT assay.

P41

Dental pulp progenitor / stem cells - dentin interactions in vivo

Kodonas K.[1], Gogos C.[1], Papadimitriou S.[2], Korpeti I.[3], Kouzi-Koliakou K.[3], Tziafas D.[1]
[1]School of Dentistry, Aristotle University of Thessaloniki., Endodontology, Thessaloniki, Greece, [2]Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Companion Animals Clinic, Thessaloniki, Greece, [3]Medical School, Aristotle University of Thessaloniki, Laboratory of Histology-Embryology, Thessaloniki, Greece

Introduction: Dentinogenesis, represents a complex sequence of biological processes, including the proliferation, migration and differentiation of pulpal progenitor cells into a new generation of dentin-forming cells. To date, numerous experimental approaches have demonstrated that isolated Dental Pulp Stem Cells (DPSCs) can be directed to differentiate into cells of the odontoblastic lineage forming dentin-like structures and associated pulp tissue in transplants.
Objectives: The purpose of the present study was to approach interactions between dentin matrix and dental pulp progenitor / stem cells seeded on organic or synthetic scaffolds and implanted as hybrid root implants in the jaw bone of mini pigs.
Methods: All animal and stem cell experiments were approved by the National Institute of Animal Care and the Ethic Committee of Aristotle University of Thessaloniki. Immature permanent incisor teeth and unerupted premolars at the early root forming stage of three, 6-8 month-old miniature pigs were extracted and a number of teeth were evaluated histologically. The expression pattern of the extracellular matrix protein tenascin and the cell surface proteoglycan syndecan was evaluated using immunohistochemistry. Mesenchymal Stem / Progenitor Cells were isolated from Dental Pulp. Cells were cryopreserved at -196^{0}C. The expression of cell surface antigen markers for mesenchymal stem cells STRO-1, CD90, CD105, and CD146 was examined using flow cytometric analysis before and after the cryopreservation procedure. A year later, autologous cryopreserved DPSCs were implanted into the jaw bone of the adult mini pigs using organic (collagen) and synthetic (PLGA) scaffolds in a new hybrid root implant model. In detail, pieces of root canals containing scaffolds seeded with the autologous DPSCs were implanted into the fresh extraction socket of the mini pigs. The resulting constructs were harvested after 2, 6 and 12 weeks and evaluated by x-ray, histological and mmunohistochemical analyses.
Results: 9 - 15 x 10^6 cells were obtained from each tooth. Porcine pulp Stem Cells were positive for CD90 (strong expression), CD105 and CD146 (slight expression) and negative for STRO-1. Similar results were obtained before and after the cryopreservation procedure. Immunohistochemical analysis showed the specific distribution pattern of these cells both in pulp and apical papilla tissues. Upon histological examination of the root implants, new extracellular matrix had been deposited in a polar predentin-like pattern on the canal dentinal walls by cuboidal non-polarized cells.
Conclusions: The interactions of dental pulp progenitor cells with the dentin matrix may provide evidence to investigate stem cell-mediated dentin regeneration. Since the unique odontoblast phenotypic expression is defined by specific epithelio-mesenchymal interactions that are difficult to replicate during experimental conditions, dentin- or pulp- like tissue formation resulting from stem cell-mediated tissue engineering approaches should be further characterized for its specific nature.

P43

Implantation of odontoblast progenitors in the rat molar pulp leads to the formation of reparative osteodentin

Harichane Y.[1], Dimitrova-Nakov S.[1], Kellermann O.[1], Goldberg M.[1], Poliard A.[1]
[1]*Universite Paris Descartes, Paris, France*

The presence of mesenchymal-like stem cells has been demonstrated in the human dental pulp. Stem cell based therapies may be promising treatments for tooth injury since ectopic implantation of these cells have been shown to induce the formation of a dentin-like mineralized tissue. Yet their capacity to readily contribute to the formation of dentin, in a tooth lesion has never been directly explored. The aim of our study was to evaluate the feasibility of a cellular therapy for pulp injury. This was achieved by implanting pulpal progenitor cells into an injured pulp of a rat first molar and analyzing the histology of the implanted tooth 7, 15 and 28 days after treatment,. We used clonal dental pulp progenitors derived from the first molar of ED 18 mouse embryo (A4 cell line). These cells can be recruited alternatively towards the odonto/ osteoblast, chondrogenic or adipogenic differentiation programs depending on the type of induction and thus, behave as multipotent progenitors *in vitro*. Rats were randomized in two experimental groups: 1) controls, in whom only the tooth cavity was performed 2) treated rats, in whom 10^5 odontoblast progenitor cells were implanted in the cavity. Our data show that implantation of these progenitor cells lead to lesion repair through the formation of an osteodentin bridge. Inflammation can be evidenced during the first week. At day 15, the formation of a dentinal barrier starts to be noticed. At day 28, the bridge completely fills the implantation site. The treated teeth keep their vitality since infection, necrosis or pulp retraction is not observed. To determine whether the grafted cells were directly responsible for the neodentin formation or whether they provided signals recruiting local progenitors, GFP-transfected A4 cells were implanted and followed after 3 and 7 days. GFP positive cells could not be revealed neither by fluorescence nor by immunohistochemistry using a GFP specific antibody. These results could be explained by a destruction of the implanted cells due to an immune reaction or the inflammatory process present in the first week post-implantation. Dental pulp stem cells, as other mesenchymal stem cells, have been described as immunotolerant. Rat/mouse and human/mouse xenotransplantations are documented in bone and muscle but nothing is known on the behaviour of pulpal progenitors in the tooth microenvironment. Experiments in immunodepressive and/or anti-inflammatory conditions are in progress to understand the cellular mechanisms underlying the formation of a reparative dentin after stem cell implantation. Altogether, our study paves the way for the future development of stem cell based therapies of tooth injuries.

P44

Expression pattern of Apin and Amelotin during formation and regeneration of the junctional epithelium

Nishio C.[1], Wazen R.M.[1], Kuroda S.[1], Moffatt P.[2], Nanci A.[1]
[1]Université de Montréal, Stomatology, Montreal, Canada, [2]Shriners Hospital for Children / McGill University, Montreal, Canada

Background: The junctional epithelium (JE) is the component of the dentogingival junction that adheres to the tooth surface, and seals off periodontal tissues from the oral environment. This unique, incompletely differentiated epithelium is formed by the fusion of the reduced enamel organ (REO) with the oral epithelium. Attachment of the gingiva to the enamel surface is provided by a structural complex called the epithelial attachment. This complex consists of an inner basal lamina (BL) formed and maintained by the superficial JE cells to which they are attached by hemidesmosomes. This BL is considered atypical because it contains laminin-5 but not other typical components, such as γ1 chain-containing laminins, and type IV and VII collagens. However, the exact mechanisms by which the JE maintains its adhesive relationship with the tooth surface through the BL and establishes its unique incompletely differentiated cellular status are still not known. Efforts to identify the secretome of the epithelial cells responsible for creating tooth enamel, have led to the identification of genes encoding for two novel proteins called Apin (APIN) and amelotin (AMTN). Unexpectedly, they are also strongly expressed in the JE. The objective of this study was therefore to investigate the presence and distribution of APIN and AMTN at various stages of tooth eruption and in regenerating JE following gingivectomy.
Methods: Immunohistochemistry was carried out on erupting rat molars, and in samples of regenerating JE following gingival wound healing. Cell proliferation activity involved in these two processes was also examined by immunolabeling for the Ki67 marker.
Results: During tooth eruption, both APIN and AMTN were immunodetected at the REO-tooth interface. APIN was also distinctively expressed by cell clusters present between the REO and oral epithelium. In established JE, APIN and AMTN were likewise found at the cell-tooth interface, where the inner BL is found, but APIN was additionally observed among JE cells. During early JE regeneration, only APIN was detected in association with cells at the leading wound edge. At the later phase, both APIN and AMTN were present at the interface with the enamel surface, but only APIN was observed among cells of the reforming JE. Cells associated with JE formation and regeneration exhibited higher cell division activity than adjacent epithelial cells.
Conclusion: The dual localization of APIN is consistent with the idea that it may be implicated in both cellular activities and in the molecular mechanisms that allow the JE to adhere to tooth surface. It may influence the apical extension of the gingival wound edge to the reestablishment of a functional JE and may play a role in modulating the cell differentiation status of the JE. Instead, the late appearance of AMTN when the reforming JE reestablishes contact with the tooth, and its conspicuous localization in the area of the inner BL suggest that the role of AMTN in the JE is restricted to events taking place at the epithelium-tooth interface. As components of the inner BL of the JE, both APIN and AMTN may contribute, directly or indirectly by interacting with other components of the BL, to the attachment mechanism of the JE.
Supported by the Department of Foreign Affairs and International Trade, Government of Canada, and by the Canadian Institutes of Health Research.

P45

Cellular events in tooth root morphogenesis

Choi M.-A.[1], Sohn W.-J.[1], Yamamoto H.[2], Lee S.[1], Ryoo Z.Y.[1], Shin H.-I.[1], Jung H.-S.[3], Kim J.-Y.[1]
[1]Kyungpook National University, Daegu, Korea, Republic of, [2]Nihon University School of Dentistry at Matsudo, Histology, Cytology, and Developmental Anatomy, Chiba, Japan, [3]Yonsei University, Oral Biology, Seoul, Korea, Republic of

Objectives: Most of studies in tooth development have been restricted in and focused on the early development of crown formation. However, developmental mechanisms and cellular events were not examined properly in tooth root formation, known as a following event after the completion of the crown formation. In this study, morphogenesis and cellular events such as cell proliferation, apoptosis and cytoskeletal formation have been examined using mice lower molar development.
Methods: Localization patterns of pan-cytokeratins, as an epithelial marker and Ki-67, cell proliferation marker showed the distinguished boundary of a root and a bifurcation forming regions of developing molar tooth. In addition, treatments of pharmacological inhibitors including cytochalasin D and nocodazole, inhibitors of actin filaments and microtubules respectively, while in vitro tooth root culture.
Results: In tooth root development at PN3, PN5 and PN8, showed the specific localization patterns of cellular events markers with the specific morphological changes. In vitro tooth root culture could confirm that tooth root formation would be modulated by the fundamental mechanisms of cellular events regulated by signalling molecules.
Conclusion: These dynamic morphological alteration patterns of tooth root formation suggest that region specific cellular events, regulated by signalling molecules, would determine the multiple rooted tooth formation.

P46

Genome-wide screening of key molecules for tooth root development

Date Y.[1,2], Ota M.S.[3], Yokoyama Y.[1], Iseki S.[3], Kasugai S.[1,2]

[1]Tokyo Medical and Dental University, Section of Oral Implantology and Regenerative Dental Medicine, Tokyo, Japan, [2]Tokyo Medical and Dental University, Administration of Global Center of Excellence (GCOE) Program, Tokyo, Japan, [3]Tokyo Medical and Dental University, Section of Molecular Craniofacial Embryology, Tokyo, Japan

Background: The tooth root is one of the most critical parts that support the force of tribosphenic functions, however, even molecular mechanisms of the root development remains unknown. Although various studies on tooth regeneration have been carried out, the tooth root structure remains to be difficult to regenerate. We tried to identify specific molecules involved in root morphogenesis to present basic informations as a first step to approach to regenerate tooth root structure.
Methods: Laser capture micro-dissection was employed to isolate cementoblasts and PDL cells from undecalcified frozen sections of murine mandible, and primary culture of neural crest cells(NCC) and bone marrow stroma cell (BMSC) were performed to collect odontogenic mesenchyme. The expression level of mRNA was compared between these tissues by genechip. We compared each sets of transcripts and isolated cementum(+)/PDL(-)/NCC(+)/BMSC(+) genes as candidate molecules involved in root development. Then, we examined the expression of these candidates by real-time PCR and *in situ* hybridization.
Results: A comparative analysis of mRNA expression by genechip showed that about 67 genes were differentially expressed between cementoblasts and PDL cells. By the further analysis, the cementum(+)/PDL(-)/NCC(+)/BMSC(+) genes were remained as 19 candidates. In the molar roots, Chd3 was specifically expressed in the Hertwig's epithelial root sheath (HERS) which is recently thought to differentiate into cementoblasts. In the incisor roots, Chd3 expression was identified until adult stage, suggesting that Chd3 play unknown function in developing roots. Next, we examined the expression of Chd3 at embryonic stage, the signal was very weak as background level. These expression pattern suggests that Chd3 is particular important for root formation process. We hypothesized that this molecule may also play a important role in transcriptional regulation during the process of root formation. It is reported that chd3 form the nucleosome remodeling deacetylation (NuRD) corepressor complex that mediates posttranslational modifications of histones and nonhistone proteins resulting in transcriptional repression in drosophila. We think that in mouse NuRD complex would repress the proliferation and differentiation of HERS during root formation. Further study will focus on the biological function of Chd3 in HERS cells.In preliminary experiment, we identified that Chd3 express in the HERS derived cells. We are studying gene function by using knockdown system with siRNA to examine molecular functions in vitro using HERS derived cells.
Conclusion: We isolated candidates involved in tooth root morphogenesis by genechip. Although further functional analysis is required, chd3 might contribute to the apical extension of tooth root and might be a specific factor for the initiation of cementogenesis.

P47

Establishment of immortalized Hertwig's epithelial root sheath cells and character of cell line

Fujiwara N.[1], Akimoto T.[1], Sasaki A.[1], Otsu K.[2], Mayama H.[3], Harada H.[1]
[1]Iwate Medical University, Department of Oral Biology, Morioka, Japan, [2]Iwate Medical University, Advanced Oral Health Science Research Center, Morioka, Japan, [3]Iwate Medical University, Department of Developmental Oral Health Science, Morioka, Japan

Tooth root formation starts after the completion of crown morphogenesis. The transition from crown to root is seen as the formation of Hertwig's epithelial root sheath (HERS), consisting of two epithelial layers. HERS development plays a key role of inducing odontogenesis in the root formation. We developed the original organ culture system to observe postnatal tooth development, and have studied the relationship between HERS development and the regulations by growth factors. From the studies, we reported that epidermal growth factor (EGF) signaling regulated transition from crown to root in mouse molars (Fujiwara, et al.,2009), and insulin-like growth factor (IGF)-I accelerated HERS elongation during early root development (Fujiwara, et al., 2005). However, it is difficult to analyze the characteristics of HERS cells by the culture method. Here, to discover the problem, we planed the establishment of HERS cell line and examined the characteristics of HERS cells. HERS cells were isolated from mandibular first molar of a PN 6d mouse and immortalized naturally under serum-free culture condition, and a cell line, HERS01a is cloned by a cloning cylinder. HERS01a proliferated actively in the presence of EGF, IGF-I, hepatocyte growth factor (HGF). Next, we examined the gene and protein expression of HERS01a, and compared with that of HERS in vivo. Real-time PCR showed that HERS01a expressed growth factor receptors of EGF, IGF-I, and HGF. Interestingly, HERS01a expressed mesenchymal markers, vimentin and N-cadherin, as well as cytokeratin 14, E-cadherin and p63 of epithelial stem cell markers. Immunohistochemical staining also showed that HERS01a expressed both of cytokeratin 14 and vimentin. We speculate that HERS01a cells have unique feature associated with epithelial-mesenchymal transition. Accordingly, these data suggested that HERS01a reflected the characteristic of intact HERS cells *in vivo*, and is useful for analyzing the biological characteristic of HERS and for elucidating the mechanisms of root development and the molecular mechanisms of EMT.

References:

1. Fujiwara, N., Tabata, M.J., Endoh, M., Ishizeki, K., and Nawa, T. (2005). Cell Tissue Research *320*, 69-75.
2. Fujiwara, N., Akimoto, T., Otsu, K., Kagiya, T., Ishizeki, K., and Harada, H. (2009). J Exp Zoolog B Mol Dev Evol *312B*, 4886-4494.

P48

Biological effects of cyclic diarylheptanoids on tooth root formation

Lee J.-W.[1], Ota M.S.[2], Fujiwara N.[3], Yonezawa T.[1], Date Y.[2], Harada H.[3], Iseki S.[2], Seo H.-J.[4], Jeon W.-B.[4], Cha B.-Y.[1], Nagai K.[1], Woo J.-T.[1]

[1]Chubu University, Research Institute for Biological Functions, Aichi, Japan, [2]Tokyo Medical and Dental University, Section of Molecular Craniofacial Embryology, Tokyo, Japan, [3]Iwate Medical University, Iwate, Japan, [4]Daegu Gyoengbuk Institute of Science and Technology, Daegu, Korea, Republic of

The mastication is one of the essential factors for maintenance of better quality of life. Healthy tooth roots and periodontal tissues are important for support the applied force and to keep the function of tooth. As tooth roots are fundamental structures for tooth development, it is important to find compounds that specially target functional tooth root formation to be used as ideal candidates for subsidiary agents for clinical applications. Therefore, we study about the biological effects of natural products on tooth root formation *in vitro* and *in vivo*.

Bone diseases such as osteoporosis and periodontitis result from an imbalance in bone remodeling caused by excessive bone resorption relative to bone formation. There are two potential categories of pharmacological treatments for bone diseases: Anti-resorptive agents that inhibit osteoclast differentiation and bone resorption, and anabolic agents that stimulate osteoblast differentiation and bone formation. In the screening for anabolic agents from natural compounds, we found acerogenin, cyclic diarylheptanoids isolated from stem barks of *Acer nikoense*, have the biological activities to induce the differentiation of mouse MC3T3-E1 osteoblast cells into mature osteoblasts by evaluating alkaline phosphatase (ALP) activity and mineralization activities by the Alizalin Red S staining. In this study, we applied these compounds to developing tooth buds of post-natal 7 days pups and performed the kidney capsule grafting[1)]. We found that these compounds stimulated tooth root formation by micro CT analysis. Then, we performed HE staining and immuno-histochemistry of anti-keratin antibody for the histological analysis of the samples, suggesting that acerogenin maintained HERS cell population. Next, we examined growth stimulation activities of acerogenin on HERS derived cell line by MTT assay and observed unique activities. In addition, acerogenin stimulated ALP activities and mineralization activities of periodontal ligament primary cells derived from Sostdc1-/- mutants comparing to wild type, suggesting functional relationship between acerogenin and BMP signaling pathway.

Acknowlegement: This study was supported by a Grant for Supporting Project for Strategic Research of Nihon Univ. Sch. Dentstry at Matsudo by the Ministry of Education, Culture, Sports, Science and Technology, 2008-2012 (Team: Dental morphogenesis; to MO) and partially funded by a grant for Biodefense Programs of the Ministry of Education, Science and Technology of the Republic of Korea.

References: 1) J. Oral Tissue Engin 2007; 4(3):137-142

P49

Essential roles of *Osx/Sp7* in the root dentin formation

Kim T.-H.[1], Cho E.-S.[1]
[1]*Chonbuk National University School of Dentistry, Institute of Oral Bioscience and BK21 Program, Laboratory for Craniofacial Biology, Jeonju, Korea, Republic of*

Background: Dentin, a major component of tooth, is formed by odontoblasts. Although dentin is physiologically different from bone, its matrix proteins show biochemical similarities with those of bone. Recently we found that an essential transcription factor for osteoblast differentiation, *Osterix/Sp7(Osx)* is also temporospatially expressed in the differentiating odontoblasts during tooth development. The aim of present study is to understand the roles of *Osx* in the odontoblast differentiation and dentin formation.
Methods: We generated and analyzed the mice with odontoblast-specific inactivation of *Osx*. *Osx* was inactivated in the odontoblasts by the transgenic *Cre* mice lines (*Col1a1-Cre* and *OC-Cre*) with the activity of *Cre* recombinase under the control of each matrix protein promoter.
Results: Tooth phenotypes of two independent *Osx* conditional knockout mice were very similar in each other. Incisors as well as mandibles of both mutant lines were short in their length. In both mutant lines, any remarkable phenotype was not found in the crown. However, roots of mutant mice were shorter than those of wild type littermates. In histological analysis, inter-radicular dentin of mutant molar was severely affected in contrast to the coronal dentin.
Conclusions: These results indicated that transcriptional regulation of *Osx* is necessary for the radicular dentin formation. Therefore, it is strongly suggested that *Osx* may play as an essential regulator in the differentiation of odontoblasts and dentin formation, particularly in root formation.
This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MEST) (R01-2007-000-20005-0)

P50

Formation of the maxillary alveolar bone in humans

Kalinke U.[1], Konietzny M.[1], Ditscher S.[1], Renz H.[1], Radlanski R.J.[1]
[1]*Charite, Campus Benjamin Franklin, Freie Universität Berlin, Craniofacial Developmental Biology, Berlin, Germany*

There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the maxillary bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the human maxillary bone together with the developing tooth primordia was examined for the prenatal stages of 19 to 270 mm CRL, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form.
Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft

P51

Formation of the mandibular alveolar bone in mice

Wehofsky R.[1], Mey R.[1], Matalova E.[2], Renz H.[1], Radlanski R.J.[1]
[1]Charite, Campus Benjamin Franklin, Freie Universität Berlin, Craniofacial Developmental Biology, Berlin, Germany, [2]Academy of Sciences of the Czech Republic, Laboratory of Animal Embryology, Brno, Czech Republic

There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the mandibular bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the murine mandibular bone together with the developing molar tooth primordia was examined for the prenatal stages of E13 to postnatal stages P20, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form.

Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft
Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde (DGZMK)
Czech Academy of Sciences GACR 524/08/J032

P52

Formation of the maxillary alveolar bone in mice

Han S.-M.[1], Leckzik V.[1], Matalova E.[2], Renz H.[1], Radlanski R.J.[1]
[1]*Charite, Campus Benjamin Franklin, Freie Universität Berlin, Craniofacial Developmental Biology, Berlin, Germany,* [2]*Academy of Sciences of the Czech Republic, Laboratory of Animal Embryology, Brno, Czech Republic*

There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the maxillary bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the murine maxillary bone together with the developing molar tooth primordia was examined for the prenatal stages of E13 to postnatal stages P20, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form.

Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft
Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde (DGZMK)
Czech Academy of Sciences GACR 524/08/J032

P53

Formation of the mandibular alveolar bone in humans

Tsengelsaikhan N.[1], Schuster F.[1], Zimmermann C.[1], Renz H.[1], Radlanski R.J.[1]
[1]*Charite, Campus Benjamin Franklin, Freie Universität Berlin, Craniofacial Developmental Biology, Berlin, Germany*

There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the maxillary bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the human maxillary bone together with the developing tooth primordia was examined for the prenatal stages of 19 to 270 mm CRL, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form.
Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft

P54

A new experimental model for monitoring bone neogenesis and osseointegration of implants in rats (OSSI model)

Blazsek J.[1], Dobó Nagy C.[2], Blazsek I.[3], Varga R.[4], Varga G.[1]
[1]Semmelweis University, Department of Oral Biology, Budapest, Hungary, [2]Semmelweis University, Independent Section of Radiology, Budapest, Hungary, [3]INSERM U602, ICIG and Universite de Paris-Sud, Paris, France, [4]Full-Tec Ltd Hungary, Budapest, Hungary

Background: The last decade promoted a tremendous development in bone and tooth reconstruction techniques including the increasing application of dental implants. During osseointegration of the implant, the quality of the newly formed and the timeframe of its formation is crucial. Therefore, it is important to develop new, well reproducible, low-cost experimental models to study bone regeneration and osseointegration of implant. The small size of rat maxillary and mandibular alveolar bones does not permit reproducible bone regeneration studies. But our structural analyses revealed that the rat tail bones are very similar to maxillary and mandibular bones but much more roboust.
Methods: Based on these observations, we decided to model dental implantation in the rat tail and developed a new model to study osseointegration. This novel method involves (1) implantation of titanium screw into the tail vertebrae, (2) followup of the integration process, and (3) quantitative biophysical measurements which mirror consolidation of implant, i.e. strength of fixation and changes in newly formed bone architecture using micro Computer Tomograph (mCT). The maximum force needed to extract the titanium implant is measured using a Tenzi TE 18.1 (TENZI Ltd. Hungary) apparatus and expressed in Newton (N).
Results: We observed that the one millimeter diameter titanium mini-implants integrated into the rat tail bone. The strength of osseointegration increased gradually by time as measured by the force needed to extract the implant starting at week 3. It reached a plato value after 36 weeks of implantation. mCT investigations confirmed these functional observations showing a gradual increase of bone density during the investigated period. When Zometa, an amino-bisphosphonate - a test material known to inhibit osteoclast, and stimulate osteoblast activity in healthy rats - was applied for 6 weeks following implantation, it significantly increased the force needed to remove the implant. Also, when the amino-bisphosphonate was applied, there was a three-fold increase in new bone volume around the implant by week 6, as observed by mCT.
Conclusions: We have developed a new, highly reproducible experimental model for studying bone regeneration and osseointegration of titanium implants in rats. This promising method is suitable to test various implant materials and surfaces, and also the effect of bioactive materials, scaffolds and even stem cells to investigate their action on the osseointegration process.
Supported by the Hungarian National Scientific Research Fund (CK 80928).

P55

Osteoblastic differentiation of transgenic mice overexpressing Apert syndrome-type mutant FGFR2 and its soluble form

Suzuki H.[1,2], Suda N.[1,2], Shiga M.[1,2], Tanimoto Y.[3], Nakamura M.[4], Iseki S.[5], Moriyama K.[1,2]
[1]Graduate School, Tokyo Medical and Dental University, Department of Maxillofacial Orthognathics, Tokyo, Japan, [2]Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan, [3]Helsinki University, Department of Orthodontics, Institute of Dentistry, Helsinki, Finland, [4]Human Gene Sciences Center, Tokyo Medical and Dental University, Tokyo, Japan, [5]Graduate School, Tokyo Medical and Dental University, Department of Molecular Craniofacial Embryology, Tokyo, Japan

Background: Apert syndrome is an autosomal dominant disease characterized by craniosynostosis and syndactyly. This syndrome is caused by one of two mutations (S252W or P253R) of fibroblast growth factor receptor 2, that leads to gain-of-function of FGF signal. Previous study reported that FGFR2IIIc-S252W induced the accelerated osteoblastic differentiation, and its soluble form (FGFR2IIIc-SOL), which lacks transmembrane and tyrosine kinase domains, inhibited the differentiation[1)]. Since it was the study using osteosarcoma cell line, particular interest is in the function of FGFR2IIIc-S252W and FGFR2IIIc-SOL on normal osteoblastic differentiation. To ask this question, we generated two lines of transgenic mice, S252W-Tg and SOL-Tg, which overexpress FGFR2IIIc-S252W and FGFR2IIIc-SOL, respectively.
Methods: Primary calvarial osteoblasts were isolated from postnatal 2-day old mice of wild-type (WT), S252W-Tg, SOL-Tg, and mice crossed with S252W-Tg and SOL-Tg (S252WxSOL-Tg). After culturing cells in the differentiation inducing medium (50 mg/ml ascorbic acid, 10 nM dexamethasone and 10 mM b-glycerophosphate) for 3 weeks, osteoblastic differentiation was examined by the alkaline phosphatase (ALP) activity, alizarin red staining, and expression of osteoblast-related genes. Next, phosphorylation of signaling molecules of FGF, such as MEK, ERK, PLC-γ, and P38 by Western blot analysis. Finally, osteoblasts were implanted subcutaneously into immunodeficient mice to examine the ectopic bone formation. Eight week after implantation, histological analysis was carried out.
Results:
1) Osteoblasts from S252W-Tg showed higher level of ALP activity, alizarin red staining, and expression of Runx2 and osteocalcin, than those from WT and SOL-Tg mice. Interestingly, all these levels were lower in osteoblasts isolated from S252WxSOL-Tg than those from S252W-Tg mice.
2) Osteoblasts from S252W-Tg showed higher phosphorylation of MEK, ERK, PLC-γ, and P38, whereas they were all suppressed in osteoblasts from S252W×SOL-Tg.
3) Osteoblasts from S252W-Tg formed much larger amount of bone-like tissue than those from WT mice, and osteoblats from S252W x SOL-Tg formed less amount of bone-like tissue than those from S252W-Tg.
Conclusion: These findings indicate that FGFR2IIIc-S252W promotes osteoblastic differentiation through MEK, ERK, PLC-γ, and P38 pathways, whereas SOL suppresses this process. Findings also suggest that SOL is an useful therapeutic target of Apert syndrome.
Reference: [1] Tanimoto Y, Yokozeki M, Hiura K, Matsumoto K, Nakanishi H, Matsumoto T, Marie PJ, Moriyama K (2004) A soluble form of fibroblast growth factor receptor 2 (FGFR2) with S252W mutation acts as an efficient inhibitor for the enhanced osteoblastic differentiation caused by FGFR2 activation in Apert syndrome. *J Biol Chem* 279; 45926-45934

P56

Characterizing the craniofacial and dental phenotype of Costello Syndrome patients

Goodwin A.[1], Oberoi S.[1], Charles C.[1], Groth J.[1], Fairley C.F.[1], Chen X.[2], Fagin J.A.[2], Rauen K.A.[3], Klein O.D.[1]
[1]University of California, San Francisco, Orofacial Sciences, San Francisco, United States, [2]Memorial Sloan Kettering Cancer Center, Human Oncology and Pathogenesis Program, New York, United States, [3]University of California, San Francisco, Helen Diller Family Comprehensive Cancer Center, San Fancisco, United States

Background: Costello Syndrome (CS) is a rare disease consisting of a wide range of craniofacial, cardiac, musculoskeletal, dermatological and functional abnormalities. Costello Syndrome is caused by a heterozygous de novo germline mutation in HRAS that results in a constitutively active Ras protein. Ras proteins function downstream of receptor tyrosine kinase (RTK) signaling, and RTK signaling is known to play an important role in tooth development. To date, no studies have systematically screened a cohort of CS patients for craniofacial and dental defects. Therefore, we set out to examine patients at the 2009 Costello Syndrome International Conference to comprehensively characterize the dental and craniofacial phenotype of CS.
Methods: We performed craniofacial and dental exams on 29 patients at the 2009 Costello Syndrome International Conference, including intra and extraoral photographs, clinical examinations, xray reviews and alginate impressions. We next studied the craniofacial and dental defects in the CS mouse model.
Results: The CS patients presented with a number of craniofacial findings, including relative macrocephaly, bitemporal narrowing, hyperteloric and telencanthic appearance, downslanting palpebral fissures, epicanthal folds, ptosis, short nose with depressed nasal bridge and anteverted nares, and low set, posteriorly rotated ears. During our craniofacial and dental examinations, we noted full cheeks with a large-appearing mouth and tongue and thick-appearing lips. Nearly 80% of patients presented with a convex facial profile, whereas 20% had a concave profile. There was a slight predilection towards Class II (35%) and III (35%) molar relationship compared to Class I (30%), and we detected a fairly high incidence of posterior crossbite (36%) and open bite (39%). In addition, delayed tooth development and eruption were noted. Many patients also presented with a high arched palate (81%) and thickening of the posterior alveolar ridge (32%). Patients also demonstrated habits including bruxism (45%), tongue thrusting (28%) and open mouth posture (39%). In light of the importance of RTK signaling in dental development, we were surprised to find that the morphology of teeth was normal. An interesting finding during our clinical examinations was enamel hypoplasia, which occurred in 84% of patients. Micro computed tomography (microCT) of exfoliated primary teeth from CS patients showed a significant decrease in enamel thickness compared to controls. The enamel defect was also seen in the CS mouse model, and further inspection revealed disorganization of the ameloblasts in the incisor. In order to determine the etiology of the ameloblast dysfunction and subsequent enamel defect, we are currently studying cell proliferation and polarity using incisors from mutant mice. In addition, we plan to transiently transfect an ameloblast-like cell line with plasmids encoding wild-type and mutant HRAS to determine how this affects signaling and behavior of the cells.
Conclusion: We systematically examined a cohort of 29 CS patients and characterized their craniofacial and dental phenotypes. One of our most striking findings was a pronounced enamel hypoplasia, and we were surprised to find normal dental morphology. Ongoing experiments are focused on understanding the etiology of the enamel defect and the role of Ras signaling in tooth development.

P57

miR-200b integrates Tgf-b signaling pathway in mouse palate development

Shin J.-O.[1], Lee J.-M.[1], Jung H.-S.[1]
[1]*Brain Korea 21 Project, Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Korea, Republic of*

Background: During palatogenesis, dynamic cellular and molecular events occur, including the elevation and fusion of the palate. These complex events are mediated by cell proliferation, cell death and epithelial-mesenchymal transformation (EMT). Failure of these processes can cause cleft palate, the most common birth defect in human. Epithelial-mesenchymal transformation (EMT) is a fundamental mechanism in many embryological processes. In palate formation, the epithelium of both sides palatal shelf adheres to form Midline Epithelial Seam (MES) and then disappear through EMT. Particularly, EMT cause seam to break away into small islands of epithelial cells that are in the process of transforming to mesenchyme. Many transcription factors, such as Snail, Slug, Zeb1, Zeb2 and Twist directly bind to the E-cadherin gene promoter to inhibit its transcription.
Methods: miRNAs are non-coding, endogenous single-stranded RNAs of about 22 nucleotides that negatively regulate gene expression, mainly through post-transcription repression.
Results: Particularly, we found predicted target genes of miR-200b on the miRGator website. Smad2 (factor of TGF-β pathway), Snail, Zeb1, Zeb2 were selected as a predicted target genes. We therefore hypothesized a role for regulation of EMT related genes and miR-200b. First of all, to see the expression of E-cadherin•Zeb1•Zeb2, we performed immunostaining and in situ hybridization. E-cadherin was observed in epithelium of palatal shelves. Where small islands were formed, there are the expressions of E-cadherin, miR-200b in small islands in the seam and epithelium of palatal region. Contrary to E-cadherin, ZEB1, 2, Snail was distributed in the mesenchyme cells according to in situ hybridization. In order to see effect of miR-200b during palatogenesis, miR-200b was transfected into palate shelves using miR-200b expressing lentivirus. Those genes were inhibited by miR-200b during palatogenesis but not E-cadherin expression.
Conclusion: These results indicate that miR-200b modulates Smad2, Snail, Zeb1, Zeb2 as regulator of key factor of the TGF-β pathway during palatal EMT process.

List of Presenters in alphabetical order

Surname	Name	Title	Country	Email	Abstract-No.
Acevedo	Ana		Brazil	acevpoppe@gmail.com	P58
Amendt	Brad	Professor	United States	bamendt@ibt.tamhsc.edu	010, 047
Aurrekoetxea	Maitane		Spain	maiac707@hotmail.com	P8
Balková	Simona		Czech Republic	s.balkova@seznam.cz	P19
Beikler	Thomas	Prof. Dr. med. Dr. med. dent.	Germany	beikler@med.uni-duesseldorf.de	P24
Berdal	Ariane		France	ariane.berdal@crc.jussieu.fr	046
Blazsek	József	Dr.	Hungary	blajozs@net.sote.hu	P54
Bleicher	Françoise	Professor	France	bleicher@univ-lyon1.fr	033
Bronckers	A.		Netherlands	a.bronckers@vumc.nl	029
Buchtová	Marcela	Dr.	Czech Republic	buchtova@iach.cz	P20
Camilleri	Simon	Dr	United Kingdom	simon.camilleri@kcl.ac.uk	P7
Catón	Javier	Doctor	United Kingdom	javicaton@gmail.com	031
Charles	Cyril		United States	cyril.charles@ucsf.edu	P29
Chen	Zhi	Prof	China	zhichen@whu.edu.cn	P1
Chen	Shuo	Assistant Professor	United States	chens0@uthscsa.edu	045
Chlastakova	Ivana		Czech Republic	ivanachlastakova@seznam.cz	P23
Cho	Eui-Sic	Professor	Korea, Republic of	oasis@chonbuk.ac.kr	P16, P49
Cho	Sung-Won	Dr	Korea, Republic of	chosome1@yuhs.ac	014
Chun	Yong-Hee Patricia	Assistant Professor	United States	chuny@uthscsa.edu	028
Churava	Svatava	MSc	Czech Republic	churava@biomed.cas.cz	03
Cobourne	Martyn	Dr	United Kingdom	martyn.cobourne@kcl.ac.uk	048
Date	Yuki		Japan	date.irm@tmd.ac.jp	P46
Delgado	Sidney		France	sidney.delgado@upmc.fr	056
DenBesten	Pamela	Professor	United States	pamela.denbesten@ucsf.edu	026
Descroix	Vianney	DDS FarmD PhD	France	vianney.descroix@crc.jussieu.fr	019
Deutsch	Dan	Professor	Israel	dddan@cc.huji.ac.il	030
Farges	Jean-Christophe		France	jean-christophe.farges@sante.univ-lyon1.fr	034
Felszeghy	Szabolcs		Hungary	szabi@chondron.anat.dote.hu	P35
Fujiwara	Naoki	Associate Professor	Japan	naokif@iwate-med.ac.jp	P47
Gadban	Naser		Israel	nasergadban@yahho.com	043
Ganss	Bernhard		Canada	b.ganss@utoronto.ca	025
Ghafoor	Sarah	Dr	United Kingdom	sarah.ghafoor@kcl.ac.uk	04
Goodwin	Alice		United States	alice.goodwin@ucsf.edu	P56
Harichane	Yassine		France	yassine.harichane@parisdescartes.fr	P43
Heikinheimo	Kristiina	DDS, PHD, MS	Finland	heikinhe@netlife.fi	051
Honda	Masaki	Associate Professor	Japan	honda-ms@dent.nihon-u.ac.jp	042
Hovorakova	Maria		Czech Republic	marhor@biomed.cas.cz	01
Hu	Bing		Switzerland	bing.hu@unil.ch	013
Huysseune	Ann	Prof. Dr.	Belgium	ann.huysseune@ugent.be	053
Ida-Yonemochi	Hiroko		Japan	hyone@dent.niigata-u.ac.jp	05
Jussila	Maria		Finland	maria.jussila@helsinki.fi	P5
Juuri	Emma	MSc	Finland	emma.juuri@helsinki.fi	P31
Khan	Qalb-E-Saleem		Norway	qsahmed@odont.uio.no	P18
Khonsari	Roman	Dr	United Kingdom	roman.khonsari@kcl.ac.uk	020
Kim	Eun-Jung		Korea, Republic of	blueleah@yuhs.ac	P6
Kim	Jae-Young		Korea, Republic of	jykim91@knu.ac.kr	P45
Kishigami	Ryota	PhD Student	Japan	ryokishi@iwate-med.ac.jp	P2
Kodonas	Kostantinos	DDS, MSc	Greece	kkodonas@dent.auth.gr	P41
Korneli	Nicole	Dr. med. dent.	Germany	nicole.korneli@uniklinikum-dresden.de	P42
Lähdesmäki	Raija	Assistant professor	Finland	raija.lahdesmaki@oulu.fi	049
Lambrichts	Ivo	Prof. Dr.	Belgium	ivo.lambrichts@uhasselt.be	P40
Lapthanasupkul	Puangwan	Dr	United Kingdom	puangwan.lapthanasupkul@kcl.ac.uk	012
Laugel	Virginie		France	laugel@igbmc.fr	P4
Lee	Min-Jung		Korea, Republic of	minjung78@empal.com	P17
Lee	Ji-Won		Japan	jwlee@isc.chubu.ac.jp	P48

Surname	Name	Title	Country	Email	Abstract-No.
Lesot	Hervé	Dr.	France	herve.lesot@odonto-ulp.u-strasbg.fr	O52
Lim	Ka-Hae		Korea, Republic of	dla2862@naver.com	P15
Lungova	Vlasta		Czech Republic	vlasta.lungova@upol.cz	P14
Lyaruu	Donacian	Dr.	Netherlands	dm.lyaruu@gmail.com	O27
Manzanares-Céspedes	Maria-Cristina	Profesor Titular de Universidad	Spain	mcmanzanares@ub.edu	P38
Matalova	Eva	Assoc. Prof.	Czech Republic	matalova@iach.cz	P10
Mayama	Hisayo	DDS Phd	Japan	hmayama@iwate-med.ac.jp	P27
Meredith Smith	Moya	Professor	United Kingdom	moya.smith@kcl.ac.uk	O59
Mina	Mina	Professor and Chair	United States	mina@nso1.uchc.edu	O37
Moffatt	Pierre	Assistant professor	Canada	pmoffatt@shriners.mcgill.ca	O50
Moustakas	Jacqueline	Postdoctoral Research Fellow	United States	jacqueline.moustakas@helsinki.fi	O57
Mues	Gabriele	Dr.	United States	gmues@bcd.tamhsc.edu	W62
Mutoh	Noriko	Dr	Japan	noriko@kdcnet.ac.jp	O40
Nakatomi	Mitsushiro		United Kingdom	nktm-emb@umin.ac.jp	O21
Napierala	Dobrawa	Assistant Professor	United States	dobrawan@bcm.edu	O35
Nieminen	Pekka	PhD, adjunct professor	Finland	pekka.nieminen@helsinki.fi	O7
Nikdin	Hero	PhD Student	Sweden	hero.nikdin@ki.se	P13
Ohshima	Hayato	Prof	Japan	histoman@dent.niigata-u.ac.jp	O39
Ota	Masato	Assistant Professor	Japan	seijin.emb@tmd.ac.jp	O41
Otsu	Keishi		Japan	kotsu@iwate-med.ac.jp	P22
Otsuka-Tanaka	Yoko	PhD	United Kingdom	tanaka.yoko@nihon-u.ac.jp	P12
Pang	Yvonne		United Kingdom	yvonne.pang.09@ucl.ac.uk	P25
Papagerakis	Petros	Assistant Professor	United States	petrosp@umich.edu	O9
Peterkova	Renata		Czech Republic	repete@biomed.cas.cz	O54
Peters	Heiko	Dr	United Kingdom	heiko.peters@ncl.ac.uk	O16
Pinheiro Junior	Roberto Flavio	PHD	Brazil	robertopinheirojr@hotmail.com	P11
Porntaveetus	Thantrira		United Kingdom	thantrira.porntaveetus@kcl.ac.uk	O18
Prochazka	Jan	MSc	Czech Republic	janproch@biomed.cas.cz	O55
Radlanski	Ralf	Prof. Dr. Dr.	Germany	ralfj.radlanski@charite.de	P50, P51;P52;P53
Renvoisé	Elodie	PhD	Finland	elodie.renvoise@helsinki.fi	O58
Rothova	Michaela		United Kingdom	rothovam@gmail.com	P21
Saito	Kotaro	Dr	Japan	kosaitou-nii@umin.ac.jp	O36
Sasagawa	Ichiro	Prof.	Japan	ichsasgw@ngt.ndu.ac.jp	P33
Sasaki	Ai	PhD Student	Japan	aisasaki@iwate-med.ac.jp	O32
Sehic	Amer		Norway	amer.sehic@odont.uio.no	P32
Seidel	Kerstin	PhD	United States	kerstin.seidel@ucsf.edu	O15
Shin	Jeong-Oh		Korea, Republic of	lajuls@yahoo.co.kr	P57
Spoutil	Frantisek	MSc.	Czech Republic	fanda-sp@prf.jcu.cz	P28, O60
Suda	Naoto	Ph.D., DDS	Japan	n-suda.mort@tmd.ac.jp	O44
Suzuki	Hiroyuki		Japan	h-suzuki.mort@tmd.ac.jp	P55
Takano	Yoshiro	Professor	Japan	takanoyh.bss@tmd.ac.jp	O23
Tallón-Walton	Victoria	Dr	Spain	vtallon@ub.edu	P37
Taniguchi	Akiyoshi	Director	Japan	taniguchi.akiyoshi@nims.go.jp	P34
Thesleff	Irma		Finland	irma.thesleff@helsinki.fi	O17
Tummers	Mark	Dr	Finland	mark.tummers@helsinki.fi	O2
Unda	Fernando	Doctor	Spain	fernando.unda@ehu.es	P9
Vandenplas	Sam		Belgium	sam.vandenplas@ugent.be	P26
Varga	Gabor	Professor	Hungary	varga-g@net.sote.hu	O38
Verstraeten	Barbara		Belgium	barbara.verstraeten@ugent.be	O6
Wang	Xiu-Ping	Dr.	United States	xiuping_wang@hsdm.harvard.edu	O22
Wazen	Rima		Canada	rima.wazen@umontreal.ca	P44
Willems	Maxime	Dr.	Belgium	maxime.willems@ugent.be	O61
Witten	P. Eckhard	Prof. Dr.	Belgium	peckhardwitten@aol.com	O8
Yamaguti	Paulo	MSc	Brazil	paulomarcio@unb.br	P36
Yamanaka	Atsushi	Dr.	Japan	yamanaka@dent.kagoshima-u.ac.jp	P3
Yamashiro	Takashi	Professor	Japan	yamataka@md.okayama-u.ac.jp	P30
Yuan	Guohua		China	yuanguohua@gmail.com	P39
Zahradnicek	Oldrich	M.Sc.	Czech Republic	o.zahradnicek@gmail.com	O11
Zhang	Yanding	Professor	China	ydzhang@fjnu.edu.cn	O24