**Final Abstract**:

Percutaneous Gene-Delivery Mediated Intervertebral Body Fusion

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**Introduction/Background:**

We have previously used adenovirus (Ad) to deliver bone morphogenetic protein (BMP) 2 and BMP7 to the rat intervertebral disk to drive bone formation and anterior spine fusion[1]. Fusion bone was induced external to the disk space, suggesting the disk to be hostile to neo-osteogenesis, and thus a barrier to engineered bone production. The current study was designed to assess: (1) if inducing a vascular supply inside the disk with gene-delivery of vascular endothelial growth factor (VEGF) would permit intervertebral disk osteogenesis with BMP co-treatment, and (2) if chemical destruction of the disk with purified chondroitinase ABC (chABC) would make the disk space osteogenesis permissive to BMP co-treatment.

**Methods/Study Design:**

All animal experiments were performed under an IACUC approved protocol. Using the previously described transperitoneal approach and technique for delivery of genetically modified cells[1], $10^6$ cells were delivered to each of the L4/L5 and L5/L6 disk spaces of male Lewis rats. Treatment groups were: no surgery (mock), delivery of naïve cells, delivery of VEGF expressing cells, delivery of BMP2/7 expressing cells, delivery of purified chABC (Sigma), and all combinations of the 3 treatments. Cells were delivered such that dosing of a given treatment was equal between groups, with $2x10^5$ VEGF expressing cells delivered for the VEGF groups, $8x10^5$ BMP2/7 heterodimer expressing cells delivered for the BMP groups, and the remainder of the cells delivered being un-infected to allow the total cells delivered to be constant at $10^6$. Cells delivered were isogeneic bone marrow stromal cells genetically modified with recombinant Ad vectors. Non-invasive spine mobility was tested preoperatively and at 4, 8, and 12 weeks using bending x-rays[2]. Animals were killed at 12 weeks for planned fusion assessments by palpation, and bone formation assessments by high resolution lateral X-rays, micro-CT, histology, and 4-point bending biomechanics.[1] X-rays were graded by 3 independent observers from 0-2, and scores were summed and used to define "abundant bone formation" for sums of 5 or more. Decalcified sections were cut sagittally at 5μm and processed for standard histological stains, and with vascularization assessed by V-cadherin immunostaining. Categorical data testing (fused or not) was performed by Fisher's exact test, continuous data was compared by ANOVA, and biomechanics data testing used the Kruskal-Wallis non-parametric test (SPSS, v16).

**Results**:

*Spine Mobility and Palpation*

Spine mobility assessed by serial lateral bending x-rays under anesthesia decreased 20% in all groups postoperatively, but progressive loss of mobility was most apparent in the BMP and BMP/VEGF treatment groups at 4, 8, and 12 wks. Fusion assessed by palpation after euthanasia demonstrated significant success in only the BMP and BMP/VEGF groups ($p<0.05$). These groups were not statistically different (37% versus

47% of attempted levels) and did not differ from the prior fusion success rate (53%, $p>0.05$)[1].

*Bone Production: High Resolution X-Ray and Micro-CT*

Qualitative scoring of X-ray images at L4/5 and L5/6 showed abundant bone production in only the BMP (11 of 30 levels), BMP/VEGF (12 of 30 levels), and chABC/BMP (2 of 30 levels) groups ($p=0.01$). As observed previously,[1] fusion bone for all treatment groups was located anterior and lateral to the disk space only, and not within the disk tissues (as assessed qualitatively by X-Ray, and quantitatively by micro-CT, data not shown). Micro-CT quantified bone production demonstrated more bone produced in the BMP and BMP/VEGF groups as compared to other treatments. The data also suggested that chABC interfered with bone production, as all treatment groups with chABC had lower new bone formation. Analysis of disk soft tissue volume demonstrated slight decrease in L4/5 disk space volume for the BMP and BMP/VEGF as compared to the naïve cells treatment group. No difference in volume was found between conditions for L5/6 and L3/4 (control) disk spaces.

*Histology and Biomechanics*

Endplate perforation alone resulted in intense central intra-discal V-cadherin immunostaining in 1 of 5 specimens (20%), chABC increased the finding to 40%, and the combined chABC/VEGF condition had 33%. In none of the other VEGF or BMP treated groups was there intense V-cadherin staining in the middle third of the disk space, suggesting that VEGF and BMP treatment interferes with the neoangiogenesis process at least to some extent. Histology also confirmed bridging bone external to and spanning the disk space in only the BMP and BMP/VEGF treatment groups. Biomechanical stiffness assessed by 4-point bending suggests a trend toward increased stiffness for the BMP and BMP/VEGF groups. Moment to failure assessment in extension did not demonstrate specific treatment effect.

**Discussion/Conclusions:**

Our results show that gene-delivery mediated tissue engineering of the rat intervertebral disk is possible, and that the avascular IVD can be made permissive to neoangiogenesis, but not to osteogenesis by the treatments utilized. Delivery of cells genetically modified to express heterodimers of BMP2/7 or VEGF in the presence or absence of chABC did not permit obvious bone formation inside the disk space, suggesting that neo-osteogenesis may be differently regulated in the IVD than is neoangiogenesis. These findings underscore the complex physiology of the IVD as regards maintenance of anti–angiogenesis and –osteogenesis homeostasis, and the issues to be addressed in the future for bone formation in the IVD.

We believe that chABC interferes with osteogenesis by its effect on early osteoid and matrix production. This effect may be avoided by using a smaller dose, using a different agent, or by delivering the chABC and BMP treatments at separate times. We believe that the lack of V-cadherin staining in the VEGF and BMP treated groups may be due to each of the factors delivering a trophic signal to the nucleus pulposus cells that allows them to be “healthy enough” to remain anti-angiogenic and maintain the avascular status of the disk. The presence of V-cadherin in the middle third of the chABC/VEGF treatment group but not the chABC/BMP treatment group may suggest that the BMP trophic effect on the cells is stronger than the VEGF effect. Further work on each of these hypotheses is planned.