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FEATURE ARTICLE

Polydopamine—a nature-inspired polymer coating for biomedical science

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Polymer coatings are of central importance for many biomedical applications. In the past few years, poly(dopamine) (PDA) has attracted considerable interest for various types of biomedical applications. This feature article outlines the basic chemistry and material science regarding PDA and discusses its successful application from coatings for interfacing with cells, to drug delivery and biosensing. Although many questions remain open, the primary aim of this feature article is to illustrate the advent of PDA on its way to become a popular polymer for bioengineering purposes.

Introduction

Polymer coatings have long been recognized as a powerful tool to control and steer cell responses—adhesion, proliferation and differentiation.[1–3] For instance, the use of poly(ethylene glycol) (PEG)-based coatings to prevent non-specific protein binding or uncontrolled cell adhesion for biosensing or tissue engineering applications is one of the best-studied and widely used approaches.[4] More recently, polymer thin films assembled *via* the sequential deposition of interacting polymers (layer-by-layer, LbL) have provided an almost unique potential in controlling and guiding cell/surface interactions. This approach has been particularly successful in the context of surface-mediated drug delivery.[3] Overall it is flexible in terms of the substrates used, allows post-modification with biomolecules, or drug loading within the assembled film. The method, however, involves multiple deposition steps using interacting polymers and requires modification of polymers for crosslinking or (bio)functionalization, thus rendering it relatively slow and labor intensive.

An alternative approach for forming polymer coatings which has attracted considerable interest in the last few years is the use of poly(dopamine) (PDA). PDA is a dopamine derived synthetic eumelanin polymer. Melanins represent a broad variety of pigments found in nature, both of natural (biopolymers) and synthetic origin. Eumelanins are, more specifically, pigments derived from tyrosine or *levo*-3,4-dihydroxyphenylalanine (DOPA). Whilst the chemistry of melanins does not entirely overlap with that of dopamine, they are worth noting, since they have such an ubiquitous existence in nature and diverse functions in various organisms. Plant melanins, on the other hand, which are mostly based on catechol are distinct since they seem not to be derived in any way from catecholamines.[5] Melanins play the role of protecting microorganisms, such as bacteria and fungi, against stresses that can cause cell damage. This includes solar UV radiation, reactive oxygen species, high temperatures,

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chemical stresses (*i.e.* heavy metals, oxidizing agents), and biochemical threats (*e.g.* host defenses against invading microbes).[6] In many pathogenic microbes, melanins appear to protect the microbe against immune responses of its host. Some melanotic radiotrophic fungi are believed to use melanin as a photosynthetic pigment which allows them to capture gamma rays, and through electron-transfer, convert it to chemical energy for growth.[7] In invertebrates, a major aspect of their innate immune defense system against invading pathogens involves melanin. Within minutes after infection, the microbe is encapsulated within melanin (melanization), and the generation of toxic benzoquinone intermediates during the formation of this capsule is thought to aid in killing them.[8] Melanin has also been found in the ink used by many cephalopods as a "smoke screen" defense mechanism against predators. It has been suggested that the melanin acts as a carrier for dopamine, DOPA and tyrosinase which are "alarm signals" that elicit escape responses in conspecifics.[9] In humans, melanin pigments (eumelanin, pheomelanin) are found predominantly in skin and hair, but they are also present within the pigment epithelium and *uveal melanocytes* of the eye, the *stria vascularis* of the inner ear and tissues including the medulla and *zona reticularis* of the adrenal gland.[10,11] In the brain, neuromelanins are found in catecholaminergic neurons within areas of the midbrain, such as the *substantia nigra* and the *locus coeruleus* in the pons, with lesser concentrations found in other brainstem nuclei.[12] The function and properties of melanin pigments in nature are vast and beyond the scope of this feature article. Reviews focussing specifically on melanin pigments and their surface chemistry and structures include those by Simon *et al.*[13,14] and on the chemical and structural diversity of eumelanins are covered by d'Ischia *et al.*[15]

The aim of this feature article is to outline the recent progress and developments in the use of the synthetic melanin-like polymer, PDA, as a (surface coating) material for biomedical applications. In the first part of this review, we will summarize the most relevant fundamental aspects of eumelanins with a focus, from a chemical standpoint, on PDA. We will highlight what is known regarding the mechanism of self-polymerisation and the structure of this material, as well as the properties of the coatings which are particularly interesting for biomedical applications *e.g.* conductivity, stability, *etc.* The (bio)conjugation of PDA will be discussed since the controlled modification of the outermost layer of the coating is of upmost importance to steer biological responses. The second part of the review will highlight successful applications reported to date involving PDA. We will discuss two particularly interesting substrates for PDA coatings: carbon nanotubes (CNTs) and colloids for the creation of PDA capsules. The subsequent section will address findings involving the interaction of PDA coated substrates with mammalian cells, bacteria and fungi, followed by a survey of biosensing applications making use of PDA. In doing so, we hope to illustrate the fast pace and increasing impact that PDA is having on a variety of biomedical areas. Further, we believe that advancing the understanding in the fundamental aspects of PDA and its unique characteristics will have a direct effect on potential biomedical applications such as tissue engineering or biosensing.

Material properties

Fundamental aspects

A peculiar property of PDA is its ability to deposit *via* the oxidative self-polymerisation of dopamine at slightly basic pH onto virtually any type and shape of surface. This wide applicability, as well as the simplicity, largely explain the growing interest in this material. However, a fundamental understanding regarding the mechanism of formation is still lacking.

In general, what is known for synthetic eumelanins is that they can be produced either by chemical oxidation of tyrosine, 5, 6-dihydroxyindoles (DHI) or 5,6 dihydroxyindole-2-carboxylic acid (DHICA) or by the enzymatic oxidation of tyrosine in the presence of tyrosinase or peroxidase. The process firstly involves oxidation of a catechol to a benzoquinone. Cyclization of the primary amine to DHI then leads to its oxidation to indole

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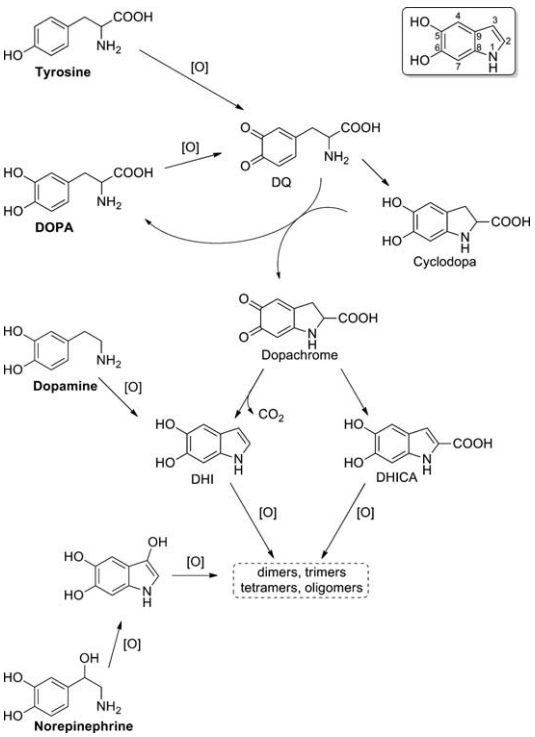
quinones (Scheme 1) and then to further self-condensation to dimers and trimers linked mainly through 2,4′- and 2,7′-bondings. These further condense to tetramers and oligomers through 2,3′-, 4,4′- and 7,7′-bondings.[15–18] It is proposed from the structure elucidation of *sepia* melanins[19–21] that these oligomers then aggregate through π–π stacking to form 1–2 nm sized plate-like aggregates, which further π-stack to second and third level aggregates of tens to hundreds of nanometres in diameter (Scheme 2). Despite several decades of work on melanins and melanogenesis, the complete picture of their macromolecular structure is still not clear, neither when they are found in their protein-bound natural state, nor in their isolated form or for those that are obtained from one of the many synthetic pathways. Due to the limited understanding to date regarding the complex chemical structure of melanins, these pigments are normally defined in the literature on the basis of their biogenetic origin.

The polymerisation of dopamine to PDA under slightly basic conditions should follow similar reaction pathways as the eumelanin synthesis, as indicated by initial mass spectrometry analysis. Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) analysis of a PDA coating on glass showed a M^+ mass peak of 445 *m*/*z*, corresponding to the mass of a DHI trimer structure and a fragmentation pattern suggesting liberation of two hydroxyls and a phenyl. Gel permeation chromatography (GPC) analysis of a 60 h incubated dopamine solution gave a broad molecular weight trace with a peak molecular weight of several million Daltons, as measured against a 1 MDa polyacrylic acid standard. Some oligomer peaks were also noted at higher retention times.[22] Polymerisation of DHI in the presence of transition-metal cations (*i.e.* Ni^{2+}, Cu^{2+}, Zn^{2+}) exerts regiochemical control over the formation of nearly exclusively the 2,2′-dimer. These and other chemistries could have interesting implications for controlling the structure, and thus the properties of the resulting materials. For instance, Wei *et al.*[23] looked at the growth of PDA coatings using several oxidants (potassium chlorate, ammonium persulfate, sodium periodate) to initiate the polymerisation. They found that the addition of the oxidizing agent increased the rate of PDA formation under basic conditions (pH 8.5) and allowed the polymerisation of dopamine to occur under neutral (pH 7) and under acidic (pH 4) conditions. This meant that PDA coatings could be formed on base sensitive materials (Al, glass, polyethersulfone, cellulose, nylon), extending the application range of this coating. Here, it would be interesting to know if PDA made under acidic conditions has different material properties to PDA formed under basic conditions.

In the case of DHICA, the polymerisation is limited in the choice of reactive sites for oxidative coupling as the carboxy group takes the 2-position on the indole ring. This limits the positional isomers of the dimers to mainly the 4,4′- and the 4,7′-positions. Readers are directed to the following reviews[13,15,24] for more in-depth discussions regarding chemical and structural elucidation of melanins.

Other catecholamine polymerisations

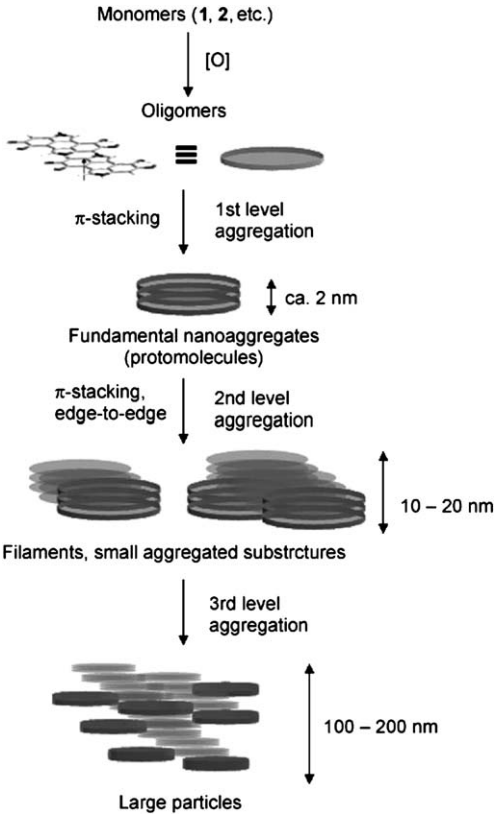
Other catecholamines also have the potential to undergo self-polymerisation allowing material independent surface



Scheme 1 Simplified schematic of the oxidative polymerisation of the catecholamines *levo*-tyrosine (tyrosine), *levo*-3,4-dihydroxyphenylalanine (DOPA), and dopamine into dimers, trimers, tetramers or oligomers. Readers are directed to the complete schematic of the Raper–Mason–Prota pathway of melanogenesis in ref. 13,14 and 121 and structures of the main oligomeric products of the oxidative coupling of 5,6-dihydroxyindole (DHI) and 5,6 dihydroxyindole-2-carboxylic acid (DHICA) in ref. 15–18. (Notes: DHI illustrates the standard numbering for bonding positions. The mechanistic aspects of the oxidative polymerisation of dopamine and norepinephrine are currently still in its infancy).

modifications to be performed using a mild aqueous dip-coating process. For natural eumelanins, the starting "monomer" is generally regarded to be tyrosine that is enzymatically converted to dopaquinone (DQ) *via* tyrosinase. DOPA is also converted by tyrosinase to DQ and through several biochemical pathways either starting point will lead to DHICA, or, after decarboxylation, to DHI, from which eumelanin forms. Tyrosine and DOPA have been shown to successfully polymerise under synthetic conditions to form melanin-like materials (Scheme 1).[25] It must also be noted that natural eumelanins are not homopolymers of DHI but copolymers of DHI and DHICA.[26] Further, pheomelanins are natural copolymers of DOPA/dopamine with cysteine. This form of melanin has its own set of interesting chemistry and material properties which are outside the scope of this review, but are comprehensively described in an account by Simon and Peles.[14]

Another recent example is the oxidative self-polymerisation of norepinephrine (noradrenaline), which in the presence of a variety of substrates shows the same material-independent coating-forming properties as dopamine (Scheme 1).[27] However, these poly(norepinephrine) (PN) coatings give access to additional surface chemistries not possible with PDA, *via* the alkyl hydroxyl group present in the PN coating. The resulting material



Scheme 2 Hierarchical aggregation as proposed for sepia eumelanins. Reprinted with permission from ref. 15.

has been shown to strongly activate surface-initiated, ring-opening polymerisation of a biodegradable polymer (ε-caprolactone) through the surface hydroxyls,[27,28] and also serves as a platform for trypsin bioconjugation through the use of the residual amine reactive quinone groups.[27]

Properties

The properties of PDA have yet to be fully explored and significant overlap should be expected between the characteristics found for PDA and synthetic and natural eumelanins. However, some caution needs to be employed here since it is known that even PDA synthesized under a different set of conditions can give rise to chemically and therefore structurally different materials.[13,15,29]

The three main biological functions of melanins are generally regarded to be photoprotection, reactive oxygen species scavenging (ROS) and metal cation sequestering. All these characteristics are relevant for coatings for biomedical applications and will be briefly discussed.

Photoprotection. The photoprotective properties of eumelanins are due to its rather unique monotonic and broad band absorbance from the ultraviolet (UV) through to the low frequency visible wavelengths, and its ability to dissipate (non-radiatively) over 99% of the absorbed photons as heat within 50 ps.[30] The photophysical properties of PDA would be expected to be similar to eumelanins, however, work by Bernsmann *et al.* showed that the UV-visible spectra of dopamine/melanin deposited on a quartz slide did not show a truly featureless, monotonic absorption.[31] This possibly indicates less molecular heterogeneity in the formation of films from a dopamine solution than what is typically found for films formed by casting solutions of solubilised natural eumelanins. These properties of eumelanins have been reviewed by Meredith and Sarna in detail.[32]

Redox characteristics. Eumelanin has the remarkable redox characteristic of behaving as an oxidizing and reducing agent, having both the oxidized *o*-quinone and the reduced *o*-hydroquinone as subunits (as well as a semi-oxidized/semi-reduced form). This material can therefore undergo electron-transfer reactions and the pigments serve as a reactive oxygen scavenger.[32–34] The reductive nature of the *o*-quinone units in PDA allows reactivity towards amines and thiols (*vide infra*) and also permits electroless metallization of easily reduced metal salt solutions without the use of an exogenous reducing agent to form metal films on a range of substrates (macroscopic substrates,[22] nanocables,[35] silica microspheres,[36] Al microspheres,[37] or Au films on CNTs[38]). This permits the easy formation of substrates for catalysis.

Radical nature. The free radical/paramagnetic nature of melanins has been known for some time[39] and it was one of the first biological materials studied by electron spin resonance (ESR) spectroscopy. The ESR spectra of both natural and synthetic melanins are quite similar, with a featureless signal, devoid of hyperfine coupling, a line width of 4–6 G, and a steady-state concentration of free radicals at a relatively low 10^{17} to 10^{18} spins g^{-1}.[15,34] Melanins exhibit quite an unusual type of stable radical and experimental evidence suggests that more than one type exist: a more labile *o*-benzosemiquinone anion radical and a radical associated with defects in the polymer backbone.[15,40] The physiological role of these highly diffusive radicals is photoprotective, forming radical traps for photoinduced radicals. Like melanin, PDA has also been shown to exhibit radical scavenging behaviour.[41] This free radical nature of PDA could have consequences when it is paired with controlled free radical techniques like atom transfer radical polymerisation (ATRP) or reversible addition–fragmentation chain transfer (RAFT) polymerisation.[42–44]

Recently, some preliminary research by Cano *et al.* has shown that synthetic eumelanins exhibit superparamagnetic properties.[45]

Metal cation sequestering. Dopamine, catecholamine analogues,[46,47] and other catechol-based materials are known to strongly bind to metals.[46] This has also been found for melanins in melanosomes which strongly sequester metal cations as a strategy to sequester potentially toxic transition metal cations in the *substantia nigra* and plays a part in Ca^{2+} homeostasis.[48] Eumelanin displays strong chelation to multivalent metal cations such as Fe^{3+}, Mn^{3+}, Zn^{2+} and Cu^{2+},[32,49–51] through the *o*-diphenol group, and additionally through the amine, imine, phenol, and in the case of melanin with a high DOPA content, the carboxy group. Melanin's stable metal ion binding nature has been explained to be due to these functional groups which show wide pH dependent association constants. An example of the use of

metal cation binding in PDA is the material-independent hydroxyapatite formation by the enrichment of Ca^{2+} ions on PDA-coated surfaces.[52]

Other properties. Electrical properties have been noted for melanins since the early days of research into organic electronics.[53,54] The electrical conductivity of solid state DOPA–melanin materials has been studied and was found to be dependent on its temperature and hydration state, *e.g.* the conductivity varying between 10^{-13} and 10^{-5} S cm^{-1} for a change in relative humidity of 0–100%.[25,55] Although the exact conduction mechanism in melanin films has yet to be fully described, there is sufficient evidence to show that it is similar to that of standard conductive organic materials based on conjugated aromatic systems which aggregate through strong π–π interactions.

Natural melanins and synthetic diethylamine melanins have been reported to efficiently absorb ultrasonic sound waves (1 MHz) through a proposed electron–phonon interaction.[56] This interesting property could allude to the function of melanin found in the *stria vascularis* of the inner ear.

For many biomedical applications, biodegradable materials are required since accumulation of foreign materials in the body is highly undesirable. The biodegradation of melanin is still not well understood and most work has been focused on melanosome degradation. The initial *in vitro* degradation of melanosomes starts with degradation of the associated lipids, carbohydrates and proteins while the melanin remains and is only degraded under oxidative attack. The enzyme (phagosomal NADPH oxidase) involved in the biotransformation of polycyclic aromatic hydrocarbons would be a strong candidate for the biodegradation of melanin *in vivo* due to the structural similarity of these two materials.[57] Despite its relevance, the closest to a PDA biodegradation study was undertaken by the Langer group[58] and is discussed in more detail in this paper.

PDA films

The qualitative presence of PDA films has been reported on a diversity of different materials. Some common characteristics which have been identified using a variety of surface characterization methods *e.g.* X-ray photoelectron spectroscopy (XPS), ellipsometry, quartz crystal microbalance, or atomic force microscopy will be briefly summarized in this section. XPS, due to its ability to determine both adsorption kinetics and atomic composition of the films, has often been used to confirm the PDA formation using two distinct properties: (a) the theoretical ratio of dopamine for $N/C = 0.125$ and (b) the C–O and C=O confirm the presence of catechol and quinone groups, respectively. The latter ones are important since they allow for the subsequent modification *via* thiols and amines. A PDA growth rate on silica of ~3.6 nm h^{-1} has been confirmed by XPS and other techniques when the standard conditions (tris(hydroxymethyl)aminomethane (TRIS) buffer, pH 8.5) are used.[31,59,60] Further, the water contact angle of PDA films independent of the underlying substrate has been measured to be 50–65°.[22,23,61] Also worth mentioning are the following five properties/observations: (i) there is a plateau effect for the growth rate observed when the dopamine solution is not replaced after ~30 min, probably due to the depletion of the monomers,[31] (ii) the deposition of PDA aggregates from solution which can be reduced by stirring the solution or placing the sample vertically, (iii) the PDA film growth depends on the used oxidant *e.g.* Cu^{2+} *vs.* O_2,[62] (iv) PDA films can be removed from silica surfaces using strong alkaline solutions (pH > 13), while acidic pHs were found to leave the films unaffected,[31] and (v) polymer multilayer films of poly(L-lysine) and hyaluronic acid incubated in dopamine solution could become free-standing membranes upon incubation in acidic solution.[63]

(Bio)conjugation of dopamine and PDA

(Bio)conjugation is a crucial aspect of biomaterials and coatings for tissue engineering and biosensing applications allowing controlled interactions with cells or biological tissue and target molecules, respectively. Some of the central aspects in terms of pre-modification and post-modification of dopamine and PDA, respectively, are reviewed in the following section.

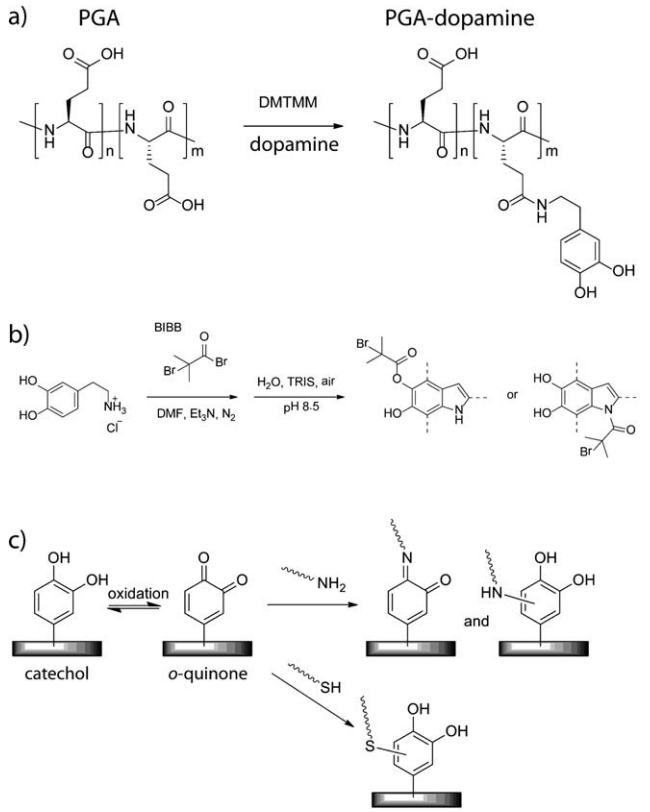
Pre-modification of reactants

The pre-modification of the reactants is a possibility to implement and characterize the conjugation prior to the film assembly. While knowing the properties of the starting material(s) is clearly beneficial, the modified reactant can potentially affect the deposition process and the subsequent properties of the coating. Ochs *et al.*[64] conjugated dopamine from 7 to 25 mol% to poly(L-glutamic acid) (PGA) by amide bond formation using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as the coupling reagent (Scheme 3a). The dopamine functionality on this polymer was initially used to bind the polymer to the surface of a silica colloid and subsequently drive the polymer layer growth *via* crosslinking through polydopamine formation. In a similar approach, a dopamine-modified poly(aspartamide) was used for surface modification through surface adhesion and crosslinking of the pendant dopamine units.[65] These types of strategies have seen prior use in cross-coupling of peptide chains to mimic *Mytilus edulis* foot protein adhesive bonding and crosslinking under aqueous conditions, and the sclerotization of insect cuticles.[66,67]

Zhu *et al.* have recently used non-specific *in situ* conjugation of an ATRP initiator (2-bromoisobutyryl bromide) onto either the catechol hydroxyl or the amine, just prior to changing the conditions to initiate PDA formation (Scheme 3b).[42] The resultant modified PDA films were found to grow at a comparable rate to unmodified PDA and the presence and viability of the initiator groups were confirmed.

Post-modification of PDA coatings

The post-modification of PDA films allows employing similar protocols for *e.g.* protein conjugation to coatings which are deposited onto different types of surfaces. This approach is particularly powerful since it enables modification *via* thiols and amines, sites which are often used in (bio)conjugation. Under slightly basic conditions, the catechol groups (in PDA) equilibrate to *o*-quinones that are extremely reactive to both amines and thiols *via* Schiff base or Michael addition reactions (Scheme 3c). The reaction of a thiol compound to give the thiol adduct proceeds extremely fast, in the case of cysteine (pH 7) with rate



Scheme 3 (a) Process used in conjugating dopamine in varying amounts to PGA *via* amide formation using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as a coupling reagent.[64] (b) Two step reaction of dopamine with 2-bromoisobutyryl bromide (BIBB) and the subsequent deposition of initiator functionalised PDA onto silicon wafers.[42] (c) Aqueous chemical equilibrium between catechol and *o*-quinone, shifting towards *o*-quinone under basic conditions. Quinone Schiff base reaction (left) and/or Michael addition (right) of amines, Michael addition of thiols.[24,122,123,69]

constants ranging from 4×10^5 to 3×10^7 M^{-1} s^{-1} (increasing with the electron withdrawing capacity of the substituent group).[68] The reaction with amines proceeds slower and either forms an amine adduct (aminochome) or condenses to an *o*-quinonimine, which is controlled by the intrinsic chemical reactivity of the *o*-quinone. In contrast, the intramolecular cyclization to form the cyclic amine adduct, in the case of dopaquinone to cyclodopa, is fast proceeding at a rate of 7.6 s^{-1}.[24]

The post-modification of PDA surfaces through the reaction of a thiol or amine has already been demonstrated by Lee *et al.*[22] *via* the formation of alkanethiol monolayers on PDA films, and the post-conjugation of thiolated methoxy-PEG, aminated methoxy-PEG and 50% thiolated hyaluronic acid. This technique was later utilized for bioconjugation of the enzyme trypsin onto PDA surfaces *via* the amines present on the enzyme.[69] In this work, they also compared the stability and chemical reactivity of trypsin *via* amine coupling to PDA and *N*-hydroxysuccinimide (NHS)-functionalised surfaces. Unlike for NHS-functionalised surfaces, they found that upon pre-incubation in alkaline buffer the PDA surface showed undiminished reactivity. Further examples of the use of the reactivity of PDA surfaces to amines or thiols inherent on biomolecules are the immobilisation of bovine serum albumin (BSA),[70] thermolysin enzyme,[71] amphotericin B (AmB),[72] concanavalin A-RNase B,[73] or poly(L-lysine).[74] Additionally, it has also been suggested that apart from the covalent binding, electrostatic interactions between proteins and PDA coatings are involved in protein adsorption.[75]

Non-planar substrates

There would be a variety of substrates of different materials and shapes which could potentially benefit from a PDA coating beyond bio-related applications, but in this review we are focusing on CNTs and colloids due to their relevance in biomedical research.

Carbon nanotubes (CNTs)

Due to their unique electrical and mechanical properties, carbon nanotubes (CNTs) are considered in a variety of different fields, from CNT-based electronic components, catalysis, sensing to filtration or biomedical applications, recently reviewed by Schnorr and Swager.[76] Although the surface modification of CNTs is a crucial step required to ensure their wide application, it often involves harsh conditions and/or multiple reaction steps. PDA coatings have been considered as an alternative, resulting in CNTs wrapped with a polymer shell (CNT_{PDA}), with the shell thickness controllable *via* the pH, time or temperature.[38] Due to this polymer shell, it is now possible to perform secondary reactions using the CNT_{PDA}. For instance, by dispersing them in aqueous solution of chloroauric acid, a few gold particles were found to adhere to the surface of the CNT_{PDA}s. In another report, CNT_{PDA}s were functionalized with a mercapto-ATRP initiator which supported the further polymerisation of dimethylamino-ethyl methacrylate, which resulted in the formation of poly(dimethylamino-ethyl methacrylate) (PDMAEMA) brushes on the CNT_{PDA} surface.[43] The PDMAEMA-modified CNT_{PDA}s were quaternized and the quaternized polymer brushes were used to bind charged metal complexes, in this case palladium, confirmed *via* the adherence of palladium nanoparticles on their surface. Both of these reports demonstrate that CNT_{PDA}, due to the option of a secondary modification, can be modified with metal nanoparticles toward catalysis applications or for electrochemical devices.

PDA capsules

Polymer capsules are considered as drug delivery vehicles or for encapsulated catalysis for a variety of reasons.[77] These capsules, mostly assembled *via* the LbL technique followed by the removal of the sacrificial template, owe their popularity to the simplicity and flexibility in assembly, yielding tailor-made carriers. Since their colloidal stability remains an issue, they are often rather large in size (typically 3–5 μm). However, they have demonstrated potential for intracellular drug delivery *i.e.* toward vaccination applications,[78–80] or encapsulated catalysis[81–84] toward cell mimicry.[85]

PDA coating of (nano)particles[60,86–88,90] or emulsion droplets[89] followed by the dissolution of the template offers an alternative polymer capsules fabrication approach with the advantage that the polymer film deposition is far less labour intensive, while varying the polymerisation reaction time controls the properties

of the film *i.e.* its thickness. PDA, being an ampholytic or zwitterionic polymer (containing both protonatable amines and deprotonatable phenolic hydroxyls), has been shown to display reversible, pH-switchable perm-selectivity to both cationic and anionic probe molecules.[90] Yu *et al.* reported an interesting unidirectional loading/release behaviour of PDA capsules in certain solvents when the cationic dye rhodamine 6G (Rh6G) was used as probing agent.[88] The loading with Rh6G increased with increasing pH of the buffer solution, while it is almost completely hindered in ethanol. On the other hand, the Rh6G release was fast in ethanol but very slow in solvents of different pH. The authors suggest that these permeability properties are largely due to a chemical potential gradient present in aqueous solutions, and absent in solvents like ethanol due to a low dielectric constant. Down the same line, Liu *et al.* confirmed the pH dependent loading of Rh6G and also reported the inverted effect for an anionic dye, methyl orange.[87] In the latter case, decreasing the pH showed increasing loading efficiency. This observation could be explained by the fact that at pH 7 the PDA membrane was found to be partially net negatively charged due to the deprotonated phenols, suggested by a ζ-potential of −2.26 mV.[75] Further, Szpoganicz *et al.*[50] performed potentiometric titrations on DHI–melanin and assigned a pK_a of 6.3 to the quinonimine groups and pK_a values of 9.4, 10.6, 11.7 and 12.8 to dimers of catechol groups. This pH dependent loading/release behaviour could potentially proof highly valuable for drug delivery applications *e.g.* to facilitate intracellular drug release.

Alternatively, it has been shown that emulsion droplets, loaded with magnetic nanoparticles, quantum dots or a small hydrophobic drug prior to the PDA deposition, yield ~1 μm sized cargo-loaded PDA capsules.[89] Although these loaded capsules show promise, their potential in *in vitro* assays remains to be determined. The only cell experiment so far conducted with PDA capsules was a cell viability assay, showing that the 1 μm capsules at the tested concentrations are not inherently cytotoxic for LIM1215 cells.[60] Degradation ability of the capsules is an important property when they are planned to be used as drug delivery vehicles. This aspect has been considered in a follow-up work by Ochs *et al.*[64] They modified PGA with dopamine and upon deposition on sacrificial templates, PGA capsules cross-linked with PDA were yielded. Further, the authors suggest degradation of the capsules (*i.e.* PGA part) upon exposure to protease due to the observed loss of encapsulated fluorescently labeled lysozyme.

Recently, pristine PDA[91] or organic (PDA)–inorganic hybrid[92] capsules assembled using calcium carbonate templates were employed to convert starch to isomaltooligosaccharides in a three-step enzymatic conversion (Fig. 1). The three enzymes were immobilized at different positions *i.e.* α-amylase on the surface, β-amylase in the PDA membrane and glucosidase in the lumen of the capsule. There are only very few other reports which demonstrate an encapsulated three-step cascade reaction, demonstrating that PDA thin films, probably largely due to their simple deposition procedure, are well suited for use as building blocks in complex functional nano-bio assemblies.

Although not capsules, an alternative approach to synthesise melanin-like nanoparticles without the need for template particles reported by Ju *et al.*[41] is worth mentioning in the context of this review. They modified >100 nm particles with thiol–PEG and demonstrated no effect on the viability of HeLa cells, and efficient free radical scavenging activity, representing a biological function of eumelanin.

PDA coatings and their interaction with cells

Mammalian cells

The purpose of this part of the review is to outline the multifunctionality of PDA coatings deposited *via* the self-polymerisation of dopamine in alkaline pH in the context of controlled response of mammalian cells. Already Lee *et al.*[22] assessed the adhesion of fibroblasts and megakaryocytes (bone marrow cells) to PDA modified surfaces in their initial report. Fibroblasts were shown to easily adhere and proliferate in the same magnitude on PDA coatings as on bare glass whereas limited megakaryocytic adhesion was observed on pristine PDA. Megakaryocytic cell adhesion was only achieved upon modification of the PDA film with hyaluronic acid (HA). This improved adhesion was ascribed to a HA receptor found in megakaryocytes. These early results already demonstrated that PDA films can be cytophobic or cytophilic, depending on the cell line and that PDA films allow for the covalent attachment of biomolecules. The latter aspect has been further considered by Poh *et al.*, who assessed the possibility to immobilize vascular endothelial growth factors (VEGFs) to a PDA modified titanium alloy substrate without losing the protein's activity.[93] Successful immobilization of VEGFs could be utilized to modify implants for bone regeneration where the stimulating properties of VEGF to promote revascularization are important for a quick healing process. A thin film of PDA was applied to a titanium alloy onto which VEGF was able to be immobilized *via* covalent coupling to the functional groups of PDA. The preserved functionality of VEGF was proven by the beneficial response of human dermal microvascular endothelial cells (HDMECs) and human mesenchymal stem cells (HMSCs) seeded on these modified surfaces. The stimulated proliferation of HDMECs was ascribed to the functional VEGF, and HMSCs were found to differentiate into endothelial cells, an effect known to be stimulated by VEGF. The ability to immobilize functional VEGF *via* PDA onto a metal substrate is thus hypothesized to be an important parameter for revascularization in angiogenesis. In a different report, Lai *et al.* investigated the influence of bone morphogenetic protein 2 (BMP2) on the proliferation and differentiation of rat MSCs when conjugated to titanium oxide nanotubes with varying topography using the PDA coating as an easy linker for protein conjugation (Fig. 2a).[94] The cell viability and proliferation were found to be increased on the modified substrate. Further, the cells were able to differentiate into osteoblasts when grown on the BMP2 modified surface, an effect ascribed to a synergy between the topography and the presence of BMP2. In another example, PDA coatings conjugated with BSA have also been shown to significantly reduce platelet adhesion.[70] Apart from the conjugation with biomolecules, the modification of PDA coatings with PEG has been considered with the aim to yield protein and cell resistant surfaces. The two reported approaches consider either the post-modification with amine- or thiol-terminated methoxy-PEG[22] or a one-step reaction by mixing the copolymer

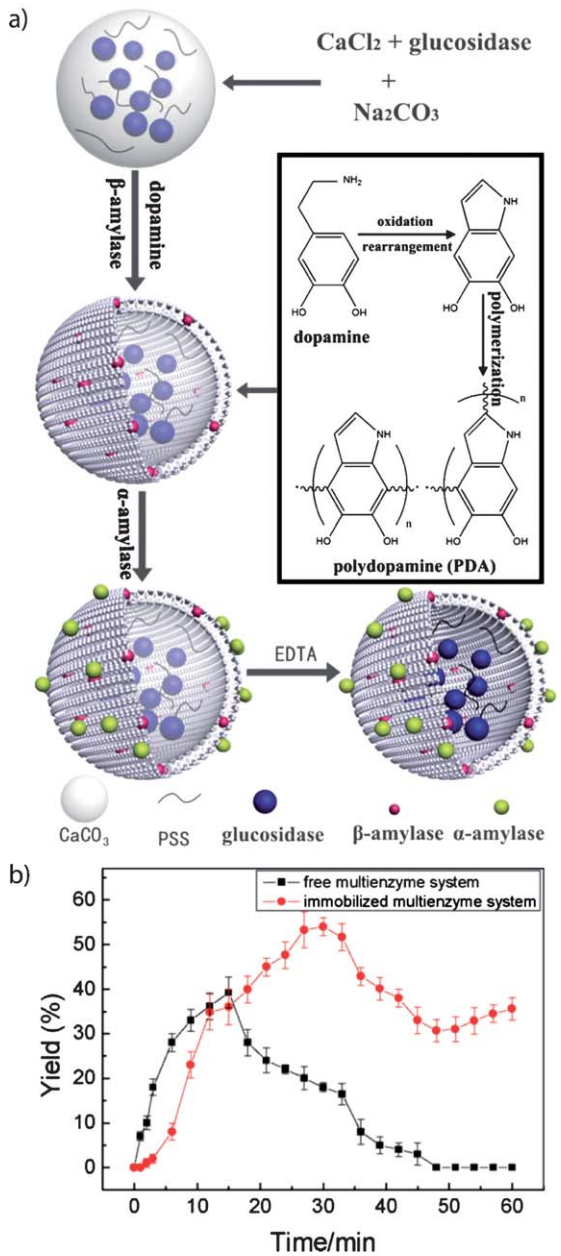


Fig. 1 (a) Schematic of the assembly which accommodates a three-step enzymatic cascade reaction using PDA capsules is shown. The enzymes are immobilized at different positions: α-amylase on the surface, β-amylase in the PDA membrane and glucosidase in the void of the capsules. (b) Starch conversion with reaction time comparing the free with the immobilized multi-enzyme system, showing the increased yield for the encapsulated approach. Reprinted with permission from ref. 91.

poly(ethylene imine)-*graft*-PEG with the dopamine solution prior to the coating deposition.[95] Both coatings, when deposited under the optimal conditions, were reported to allow for subsequent controlled cell adhesion.

Further, since PDA films can be deposited on virtually any type of substrate including non-wetting surfaces such as poly(ethylene) (PE), poly(tetrafluoroethylene) (PTFE), poly(dimethyl-siloxane) (PDMS) and silicone rubber, these types of surfaces can be equipped with an interface potentially compatible with cells and tissue in a simple and fast manner. For instance, on the mentioned non-wetting unmodified substrates, there was little to no mouse osteoblasts and rat pheochromocytoma adhesion, but when these cells were seeded on the PDA modified surfaces, similar adhesion and spreading as cells grown on glass were observed.[96] In comparison to unmodified surfaces, the total cell area was increased as high as 26 fold. Along the same lines, the Park group also reported the enhanced growth of human umbilical vein endothelial cells (HUVECs) on electrospun polycaprolactone nanofibers with a PDA coating in comparison to

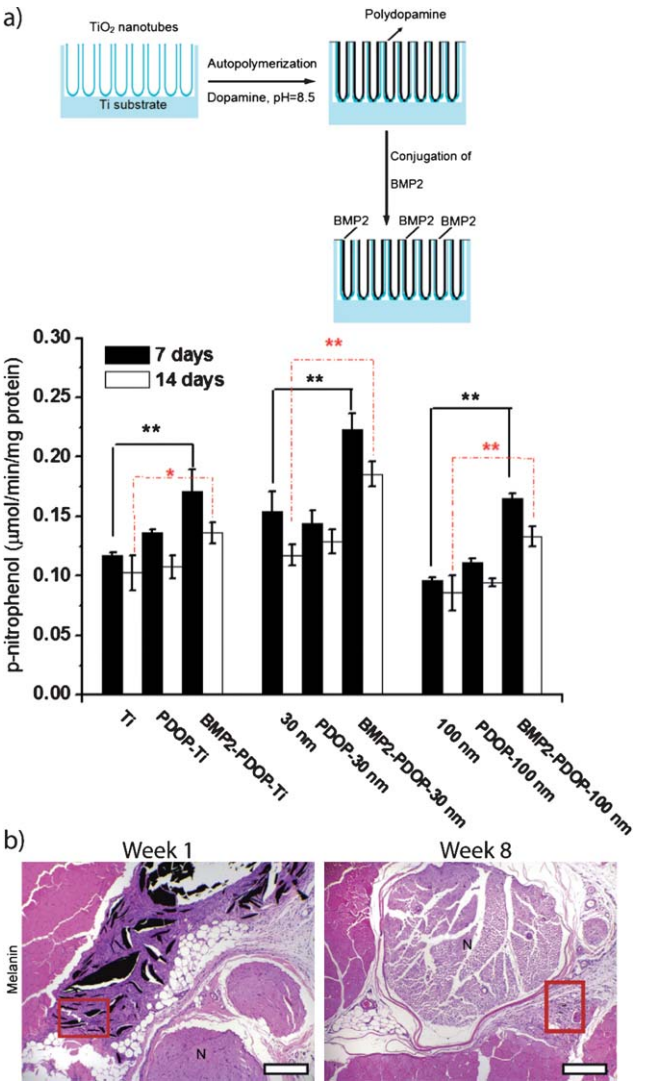


Fig. 2 (a) Top: schematic illustration of the immobilization of bone morphogenetic proteins (BMP2) to PDA modified TiO_2 nanotubes. Bottom: alkaline phosphatase activity of mesenchymal stem cells cultured onto different substrates showing higher activity on the PDA-BMP2 modified substrates than on Ti (left: unmodified Ti, middle: PDA coated Ti and right: PDA coated Ti modified with BMP2. The same modification using flat (left), 30 nm nanotubes (middle) or 100 nm nanotubes (right)). Reprinted with permission from ref. 94. Copyright 2011 American Chemical Society. (b) Histological examination of the *in vivo* response of nerve to melanin implants. After 8 weeks, most of the melanin implant appeared to be resorbed (left *vs.* right image). Scale bars represent 200 μm. Reprinted with permission from ref. 58.

unmodified or gelatin-coated nanofibers.[97] Nanofibers are considered as tissue engineering scaffolds due to their ability to mimic extracellular matrix structures, but they are often not capable of promoting cell adhesion/proliferation because of their hydrophobicity. Applying PDA to the surface of the nanofibers thus yielded a substrate which stimulated cell adhesion and considerably increased the cell number and improved cell survival. Furthermore, in order to determine cell activity, the cells were analysed for endothelial cell markers showing that these were well expressed in the cells grown on PDA, making PDA coated nanofibers a promising tool in vascular tissue engineering.

Additionally, controlling the spatial distribution of cells *via* patterning of cell-adhesive structures in a non-interacting background is important when *e.g.* biochips[98] or advanced cell sheet engineering[99] are considered. To this end, PDA was deposited on a PDMS template in a pattern directed by PDMS microchannels yielding cell-adherent lines in a cytophobic background.[100] Human fibrosarcoma, mouse preosteoblasts and mouse fibroblasts were found to be able to adhere and proliferate on the PDA coated patterns on the surface while the cell adhesion was hindered on PDMS substrates. Furthermore, on the micropatterned PDA surfaces, the cells aligned in the direction of the PDA structure with more elongated nuclei in the micropattern direction as compared to cells grown on an unpatterned PDA surface.

The fact that melanin films are conductive makes them interesting to study their performance in the context of nerve tissue engineering. Recently, Kang *et al.* reported that the deposition of PDA followed by poly(D-lysine) immobilization on different substrates and microelectrode arrays allowed the culturing of viable neuronal networks.[74] In the latter case, spontaneous and evoked neural activities were observed, making this platform promising as an interface to record neural signals. Previously, Bettinger *et al.* examined the *in vitro* and *in vivo* response of Schwann and PC12 cells when incubated on melanin films (Fig. 2b).[58] Unlike all the other examples, not oxidation of dopamine but spin-coating of dissolved synthetic melanin was used to prepare the films in this case. When incubated *in vitro* Schwann cells showed a significantly more proliferated profile on melanin films as compared to uncoated or collagen coated substrates. PC12 cells grown in the presence of nerve growth factor (NGF) on melanin films exhibited neurites which have extended significantly more than on the other substrates. When tested *in vivo* in rats, the melanin implants were found to cause a foreign body response, but to have a benign effect on nerve tissue, both comparable to silicone implants. Further, unlike the silicone implants, the melanin implant almost completely degraded within 8 weeks *in vivo*. The authors do note that the implants were mechanically rigid and that gross disintegration could have played a large part in the erosion of the implant, with small melanin particulates being taken up by macrophages and giant cells being observed from the first week onwards. Complete degradation of the heteropolymer into its monomers had not been elucidated in this work; however it was hypothesized for it to degrade into its monomers (tyrosine, dopamine and their derivatives). Overall, the authors suggest that melanin is a promising compound to be used in nerve tissue engineering due to the observed biocompatibility, biodegradability as well as its physical, chemical and electrical properties.

Going a step further from using PDA prepared *via* the oxidation of dopamine to control cell adhesion and proliferation, we were recently the first ones to report the use of PDA coatings in the context of surface-mediated drug delivery. We embedded liposomes in a PDA coating and showed the ability of myoblast cells to take up fluorescent lipids from the surface as a model hydrophobic therapeutic (Fig. 3).[59] Liposomes are expected to serve as drug deposits in particular for small hydrophobic compounds or fragile biomolecules to allow for the controlled release and/or access of adherent cells to therapeutic molecules embedded in the polymer coating.

Bacteria and fungi

Bacteria and fungal adhesion to surface coatings is an important topic with impact on many fields from food industry to medicine. Most often the challenge includes the prevention of the adhesion of these microorganisms, but sometimes a bio-adhesive coating is required *i.e.* for the quantification and identification of bacteria in solution. To this end, Liu *et al.* considered a PDA coated electrochemical soft bilayer actuator made from polypyrrole (PPy) and gold.[101] When coated with PDA, the bacteria were shown to adhere to the actuator with a 10 times higher magnitude as compared to the bare gold/PPy actuator thus showing that PDA is bio-adhesive. The electrochemical actuation process was reported to be more efficient in "fishing out" bacteria when compared to dipping or mechanically stirring, leading to the conclusion that the positive potential of the actuator during cyclic voltammetry is electrostatically attracting the bacteria which then could adhere to the PDA surface. Down the same line to use PDA as a wet bio-adhesive, Kang *et al.* coated a tipless cantilever with PDA followed by the immobilization of a single bacterium for live single cell force measurements.[102] The bacterium cell immobilized on the PDA was still metabolically active which makes this approach superior over the use of *e.g.* glutaraldehyde for bacterium immobilization. When this live cell probe was used to measure adhesion and retraction forces, it was seen that there were either none or repulsive forces present when it comes in contact with a quartz surface. This was directly opposed to the earlier studies using glutaraldehyde which showed attractive forces which indicated that these previous force studies could have been affected by the immobilization compound. The pull-off force was also different between the two types of substrates showing a higher force was needed to retract the PDA-immobilized cell from the surface.

Although fungal infections are among the most common complications in hospitalized patients,[103,104] there are only very few approved compounds available,[105,106] which demonstrate the need for novel ways to implement them in the clinical setup *e.g.* as antifungal coatings. To this end, amphotericin B (AmB), a strong approved antifungal compound, was conjugated to the surface of silica nanoparticles (Si NPs) and those conjugated Si NPs were immobilized onto a glass substrate using PDA as an adhesive layer to create a non-leaching antifungal surface.[72] They demonstrated that these surfaces exhibited contact-mediated antifungal activity but were neither cytotoxic to mononuclear cells nor having hemolytic effect on red blood cells.

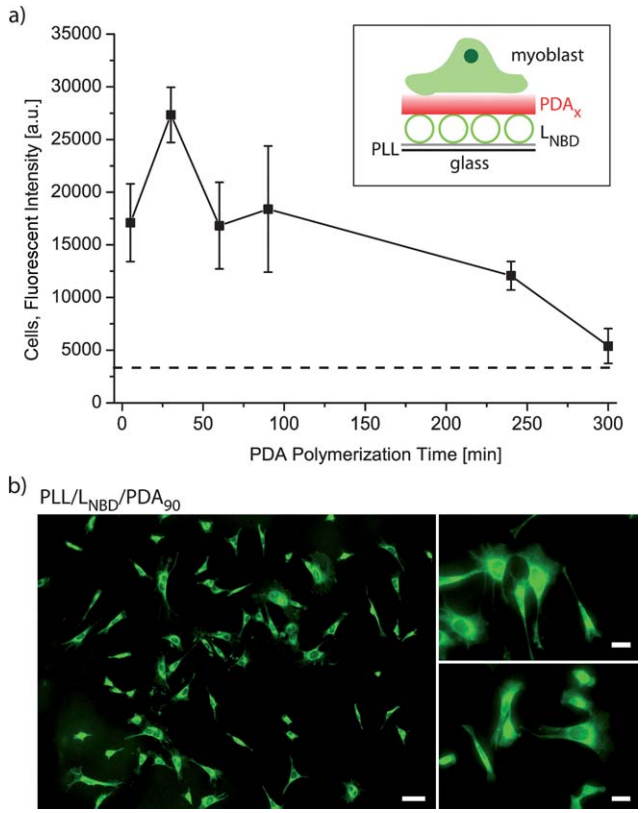


Fig. 3 (a) Myoblast cells were grown on a substrate pre-modified with poly(L-lysine), fluorescently labeled liposomes and PDA with different polymerisation times (inset). The fluorescent intensity of cells adhering for 4 h to such a coating was measured using flow cytometry, demonstrating that the fluorescent lipids are taken up by the cells from the surface. Further, increasing the PDA polymerisation times led to reduced fluorescence, suggesting that the access of the cell to the fluorescent lipids was hindered by the increased thickness of the PDA layer. (b) Representative fluorescent microscope image of myoblast cells exhibiting fluorescence due to the uptake of fluorescent lipids from the surface. Reprinted with permission from ref. 59. Copyright 2011 American Chemical Society.

Biosensing

The need to improve and newly develop sensitive and selective high throughput biosensing platforms for diagnostics and drug screening or drug discovery purposes remains tremendous. The approaches and target molecules are various, from DNA[107] and antibody-based[108,109] sensors to lipid-based platforms equipped with membrane proteins[110] often assembled in micro- or nano-arrays.[111] PDA coatings are expected to complete the tool box to design biosensors and they have already been employed in a few different ways in this context.

Molecular imprinting of proteins, recently critically reviewed by Verheyen *et al.*,[112] is expected to have important applications in biosensors for protein detection and sorting, but often involves complex and time consuming steps to produce the sensing platform. In 2006, Lui *et al.* were the first ones to consider PDA-imprinted films as recognition element.[113] They produced imprinted sensors *via* the electrochemical oxidation of dopamine in the presence of nicotine. The reproducible and selective detection of nicotine in PBS, and the recovery of over 98% of 5 μM nicotine in human serum were reported. In a different report, Ouyang *et al.* electrochemically copolymerized *o*-phenylenediamine and dopamine in the presence of L- or D-glutamic acid on an electrode and showed the chiral discrimination of glutamic acid measured as relative capacitance changes of the sensor.[114] In a later publication, they demonstrated the creation of protein-imprinted PDA nanowires (IPWs), prepared by the immersion of a protein-coupled alumina membrane in a solution of dopamine and ammonium persulfate using the self-polymerisation of dopamine in alkaline pH, followed by the removal of the membrane and the template proteins, bovine or human hemoglobin (Fig. 4a).[115] The IPWs showed good monodispersity, binding capacity and selectivity towards target proteins. The high quality of the latter two aspects was attributed to the PDA cavity structure which exhibits binding possibilities *via* amino- and hydroxyl-containing groups, π–π bonds and van der Waals forces. Further, Zhou *et al.* coated superparamagnetic nanoparticles (Fe_3O_4 NPs) with PDA in the presence of human hemoglobin, yielding imprinted particles with preserved magnetic properties for their separation after exposure to targeting proteins.[116] The versatility of the hemoglobin imprinted Fe_3O_4 NPs was tested in a competitive binding assay using five different non-templated proteins. In all assays, the relative binding of hemoglobin was over 80%, suggesting that these Fe_3O_4 NPs have the potential to serve as affinity materials for protein separation and detection.

An important challenge in biosensing is the immobilization of enzymes on surfaces of *e.g.* electrodes while preserving their activity. To this end, a glucose biosensor entrapping glucose oxidase (GOx) and gold nanoparticles (Au NPs) in a PDA film deposited on a glassy carbon electrode was considered.[117] It was shown that the Au NPs accelerated the transfer of the electrons in the PDA film on the surface of the electrode. This biosensor exhibited improved performances in terms of sensitivity, lower detection limit, linearity, long term stability, and preserved enzyme's bioactivity in the determination of glucose compared to other designs where GOx was embedded in chitosan-Au NPs films. Moreover, this platform was used to detect glucose in diluted human serum, demonstrating the potential of this PDA-based platform for clinically use as an amperometric biosensor.

On a different note, there is still a need for novel labels with improved sensitivity in immunosensing.[118] Platinum nanoparticle (Pt-NP)-rich polymer nanocomposites, which can catalytically oxidize hydrogen peroxide, are considered for signal read-out. Fu *et al.* reported a first attempt in this direction using PDA/Pt-NPs/antibody bionanocomposites as labels in a sandwich-type electrochemical assay and proof-of-concept results using a human immunoglobulin G immunoassay.[119]

Encapsulation of living cells is of great importance in the area of cell-based sensors and bioreactors. PDA encapsulation of living yeast cells made it possible to protect the biological species against harsh environments *i.e.* lysis with lyticase, yet still allowed cell division and further modification of the PDA coating with streptavidin for immobilization onto biotin-presenting surfaces in this case (Fig. 4b).[120]

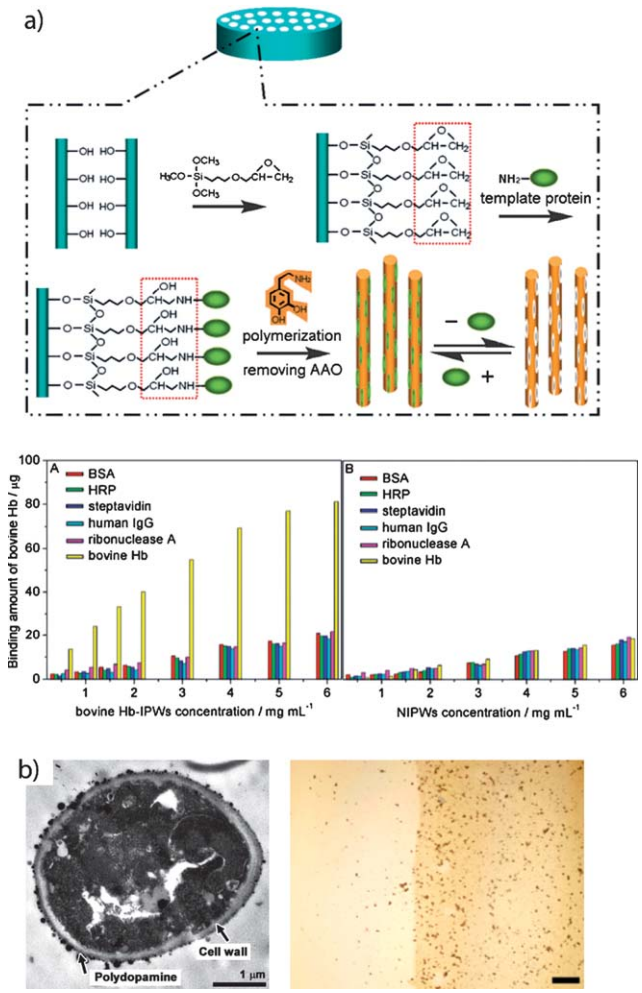


Fig. 4 (a) Top: schematic illustration of surface protein-imprinted nanowires. An anodic alumina oxide (AAO) membrane was modified with 3,4-dihydroxyphenylalanine, followed by the creation of the protein-imprinted PDA nanowires. Bottom: bovine haemoglobin and other reference protein binding haemoglobin (left) and non-imprinted (right) PDA nanowires. Reprinted with permission from ref. 115. (b) Left: image of a negative-stained PDA coated yeast cell. Right: the binding of avidin-linked PDA coated yeast cells to a biotin presenting (left) and a PEGylated (right) surface shown in an optical micrograph. Reprinted with permission from ref. 120. Copyright 2011 American Chemical Society.

Conclusions and future perspectives

This feature article gives an overview over the current state of the art for PDA coatings towards biomedical applications. This approach, only a few years old, has already yielded a variety of different successful examples where the unique properties of PDA have proven to be highly beneficial. The simple deposition protocol applicable to virtually any shape and substrate material, reproducibility, the straightforward possibility for bio-conjugation, low toxicity, *etc.* make this polymer uniquely and widely relevant, also demonstrated by the broad spectra of applications reported to date. As the knowledge of the polymerisation of dopamine and related materials increases, new rationally designed materials with high functionality can be synthesised that address the need for specific materials for biomedical applications, and also for other areas *e.g.* for sensors or photoactive/protective materials. PDA formation is expected to be similar to melanins. Their creation starts with oligomer formation followed by assembly into larger aggregates *via* π–π stacking. Although their detailed mechanism and origin remain largely ambiguous, these films exhibit plenty of interesting properties from photoprotection to superparamagnetism. Further, other monomers can be co-polymerized with PDA or the deposited PDA films can be conjugated *via* thiol and amine chemistry. Due to this variety of basic properties, a large number of applications for PDA coatings have been reported. The PDA coating of CNTs and the creation of PDA capsules are giving access to biologically active micro/nano-assemblies which have the potential to find applications in biosensing or drug delivery. Further, PDA turned out to be particularly promising for coating a variety of surfaces to control and guide cell/bacteria/fungus–surface interactions. In particular, PDA coatings can be post-functionalized with biomolecules and/or PEG, hydrophobic substrates can be rendered (bio-)adhesive, and initial potential to use these coatings in surface-mediated drug delivery has been shown. From a different perspective, PDA has shown promise in biosensing *i.e.* as a matrix for molecular imprinting, as a coating in an amperometric biosensor, as a novel label or it can be used to encapsulate entire cells.

Due to its novelty and potential, the applications are scattered across the field of biomedicine, leaving more questions unanswered than providing in-depth insight. In the future, fundamental aspects such as understanding the PDA polymerisation mechanism in detail and how the post-functionalization could be better controlled and optimized will have to be addressed. Understanding why and how PDA is deposited on virtually any surface would be another interesting aspect to study together with many fundamental properties such as pH, temperature or salt effect in order to provide a more coherent image of the PDA films *per se*. Getting thorough understanding as to what side reactions and side products are formed, and how to systematically control and guide the chemical reactions which occur during the surface modification is also important, should these coatings be translated into clinical applications and get FDA approval. Further, determining if PDA is (bio)degradable and by which mechanism is a crucial aspect which will have to be tackled to ensure the success of PDA for biomedical applications *i.e.* in drug delivery. To this end, Simons *et al.* mentioned in their review the observation that drugs *i.e.* lipophilic β-blockers can bind to melanosomes.[13] Although it is not understood yet in detail how the drug is binding to the melanosomes that could be a potential possibility for drug storage and time-dependent release. Further, a more specific consideration of melanin-like materials for implants is likely to be a successful path due to its inherent properties. Last but not least, although so far only few, the proposed biosensing approaches are holding promise, especially since PDA seems to gently interact with fragile biomolecules. This allows for improving current or developing new sensing platforms, taking the biggest challenges such as high sensitivity and selectivity, low cost, multiplexing or low sample volume into account.

Taken together, PDA is fast on the way to becoming one of those popular polymers which are being investigated and considered by a large number of scientists with different expertise

and applications in mind due to its ease of use combined with its fascinating properties.

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