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Sticky Tubes and Magnetic Hydrogels Co-Assembled by a Short Peptide and Melanin-like Nanoparticles

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This paper describes the co-assembly of polydopamine spheres, either bare or coated with Fe_3O_4 magnetic nanoparticles, with the short aromatic peptide diphenylalanine. The combination of polydopamine particles and diphenylalanine generated tubular structures decorated with adhesive spherical particles, while the co-assembly of the polydopamine spheres coated with magnetic Fe_3O_4 nanoparticles resulted in the formation of a magnetic hydrogel. These new architectures may be useful as new vehicle for several applications including tissue regeneration and drug delivery.

Molecular self-assembly is a promising route for the formation of functional structures *in vitro*.[1] Biomolecules, specifically, are very appealing building blocks as they are versatile, biocompatible, and form spectacular architectures.[2] Peptides are a class of biomolecules that presents the highest diversity. Using self-assembled peptides, it is possible to form tubular structures, spheres, fibers, tapes and hydrogels.[3] The structural diversity that results from peptide self-assembly is however still limited. We have recently suggested that the co-assembly of different biomolecules would lead to a higher structural complexity.[4] We demonstrated this approach using short aromatic peptides that co-assembled into molecular necklaces or particles that resemble in their morphology to red or white blood cells.[4] Here, we report on the formation of new functional structures by the co-assembly of synthetic melanin-like particles and short peptides.

Melanins, typically defined as polyphenolic compounds derived from the oxidation of tyrosine or L-3,4-dihydroxyphenylalanine (DOPA).[5] They are ubiquitous in living organisms and serve a variety of purposes in pigments, antioxidants and photoprotectants.[6] Natural melanins are typically composed of catechol-amine monomers.[7] Catechol is a unique adhesive molecule, found in mussel proteins, [8] which can be used to form functional coatings.[9] This molecule can reduce metal ions while it is oxidized.[10] In addition it can self-polymerize into polydopamine (PDA) under oxidative and alkaline conditions.[11] The adhesive and cohesive properties of PDA are believed to be related to the reactivity of polyorthoquinoneindole by the formation of covalent bonds with various substrates via Schiff base type or Michael type reactions.[12] Moreover the catechol moiety of PDA can engage in hydrogen bonding, metal complexation, π-π interaction and quinhydrone charge transfer complexation.[9b, c, 13] Monodisperse PDA spheres with tunable diameter were synthesized by adapting a facile and low-cost methodology (ESI† Fig. S1).[5a, 14] These PDA spheres were used as an active template for the convenient synthesis of various nano composites such as PDA/Fe_3O_4 core/shell spheres.[14] This is due to the presence of versatile active surface functional groups (-OH, -NH_2), which are completely different from the previously reported PDA coated Fe_3O_4 nanoparticles (NPS).[15] This manuscript describes the use of these active particles for the decoration of peptide-based assemblies formed by the short aromatic peptide, diphenylalanine (DPA). DPA is the core recognition element of β– amyloid polypeptide which is involved in Alzheimer's disease.[3k] This peptide can self-assemble in aqueous solution into nanotubes.[3i, 3k, 16]

Table 1: The table lists the concentration of the building blocks and their resulted assemblies under different co-assembly conditions.

Condition	PDA (mg/mL)	DPA (mg/mL)	Fe_3O_4 coated PDA	Assemblies
1	1	2	--	No distinct structures
2	1	5	--	Sticky tubes
3	--	2	1	Hydrogel
4	--	5	1	Hydrogel

For the co-assembly experiments this peptide was dissolved in 1,1,1,3,3,3-hexafluoro -2- propanol (HFP) to a concentration of 100mg/ml. Then, we blended the peptide with either bare PDA spheres or PDA spheres coated with magnetic Fe_3O_4 nanoparticles (MNP) at several different proportions as indicated in table 1. Polar solvents (i.e. water) should allow the self-assembly of the peptides. To follow the process, we

analyzed a sample of the solution during different time intervals using High Resolution Scanning Electron Microscopy (HR-SEM). When the concentration of the peptide was 5mg/mL (condition 2), sparse tubular and spherical structures formed after 30 min of incubation (Fig. S2, ESI†). Then, after four hours some of the spheres attached to the tubular structures forming a coating on the tubular structures (Fig. S2, ESI†). We observed successive stacking of PDA spheres on the tubular surface as the time progress (Fig. S2, ESI†).Well-defined tubular structures decorated with PDA spheres were formed after 18h of incubation of the co-assemble mixture under condition 2 (Fig. 1a,c,d). When the concentration of the peptide was lower (2mg/mL, condition 1) no new distinct nanocomposites or structural arrangement were observed (Fig. 1b).

To examine what forces are involved in the interaction between the tubular structure and the spheres we performed XPS measurements to the samples of PDA (sample 1), DPA (sample 2) and the co-assembled mixture (sample 3; condition 2). The XPS spectra showed a significant difference in the content of oxygen (O1S) of sample 3 with respect to both sample 1 and 2 (Fig. S3, T1 ESI†). The O1s (I)/(II) fit with the characteristic regions in the XPS spectra assigned to O=C and O-C bonds. The co-assembly of DPA and PDA resulted in a significant variation of O1s (I). It changed from 29.2% for PDA (sample 1) or 75.6% for DPA (sample 2) to 70.0% for the co-assembled structures (sample 3). In addition, there was a decrease in O1s (II) from 58.3% (sample 1) and an increase from 24.4% (sample 2) to 30.2% (sample 3) indicating the successful attachment of the PDA spheres to the surface of the DPA tubes through the prominent interaction involving the catechol moieties.[17]

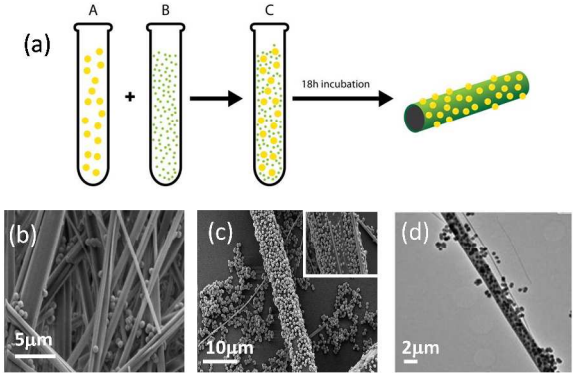

Fig. 1. (a) The scheme illustrates the co-assembly processes under condition 2 (Table 1); (A) PDA spheres in water (1mg/mL), (B) DPA in HFP (100mg/mL) and (C) PDA (1mg/ml): DPA (5mg/ml) in water (not to scale). (b) and (c) HR-SEM micrographs of the co-assembled structures under condition 1 and 2 respectively, (d) TEM micrograph of the co-assembled structures (condition 2) after 18h of incubation.

FT-IR analysis provided us with information on the chemical nature of the assemblies and their secondary structure. The tubular structures formed by the DPA peptide (5mg/mL) showed two distinctive peaks at 1621cm^{-1} and 1683 cm^{-1} indicating a β–sheet secondary structure as reported previously.[3k, 4b] The FT-IR spectra of the spherical PDA NPs had a single peak at the amide I region at 1614 cm^{-1} which can be ascribed to the aromatic nature of PDA.[15b] It did not have a distinct peak that can be related to a specific secondary structure. The amide bond (N-H) shearing band (1510cm^{-1}), the aromatic ring C-C vibration band (1442cm^{-1}) and the phenolic C-OH stretching band (1292 cm^{-1}) which are characteristic of PDA appeared in its FTIR spectrum (Fig. 2a).[15a] The amide I region of the co-assembled structures (condition 2) had two peaks (1684 cm^{-1}, 1620 cm^{-1}), which are similar to the peaks of the bare DPA and are probably related to their secondary structure. In addition, the bands correspond to the surface active functional groups of PDA (1510, 1442, and 1292 cm^{-1}), which may interact with the walls of the nanotube, could be clearly identified at the FT-IR spectrum of the co-assembled structures. This may indicate the interaction of the PDA spheres with the tubular wall.[8a, 8c, 18] Moreover, the appearance of broader signals with higher intensity around 3400 cm^{-1} and 3100 cm^{-1} might result from the surface active N-H / O-H stretching vibrations. This further confirms the interaction of poly-phenolic adhesive catechol moiety with the nanotubes (Fig. 2a).[18a] Furthermore, the intensity of the IR band at 1370 cm^{-1} of the PDA functionalized DPA nanotubes was clearly lower than that of bare DPA nanotubes. This effect can be associated with π-π stacking interaction and van der Waals interactions between the wall of the tubular structures and the aromatic structures of the PDA molecules.[18a] This indicates the interaction between the PDA sphere and DPA nanotubes. UV-vis spectral analysis showed that the DPA nanotubes had an absorption maximum at 258 nm, while the PDA spheres had an absorption maximum at 280 nm. The spectrum of the co-assembled sample contained both these peaks (Fig. 2b). However, the intensity of the characteristic absorption peak at 280 nm of the PDA spheres decreased and became broader suggesting that the PDA spheres successfully covered the DPA nanotubes.[15b] The nature of the absorbance spectra of DPA is mainly attributed to the intramolecular n–π* and π–π* transition involving the π electron cloud of the phenyl ring and the nonbonding electron of the N and O atoms. During the co-assembly state the π electron cloud of the phenyl ring might be involved in π-stacking interactions with the indole moiety of PDA and the non-bonding electron pair might be involved in the intermolecular hydrogen bonding with the catechol -OH moiety of the PDA. This could be the reason for the different absorbance spectra for the co-assembled structures. In this state the spectra is broader and the two peaks next to the absorbance maxima of 258 nm could not be clearly identified.

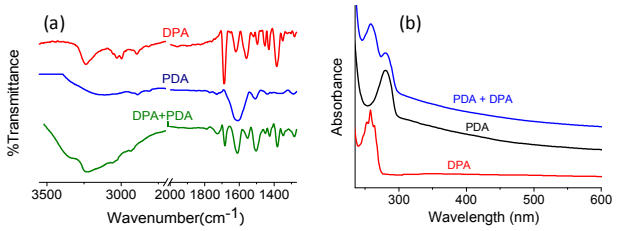

Fig. 2. (a) FT-IR transmittance spectra and (b) UV-Vis absorbance spectra for DPA, PDA and PDA co-assembled with DPA (condition 2).

To examine if the tubular structures covered by the PDA spheres have new adhesive properties, we deposited decorated and bare tubular structures on a titanium surface and tried to remove them from the substrate by washing with water (See experimental section ESI†). HR-SEM analysis showed that while the bare tubular structures detached from the titanium surface by simple washing, the decorated tubular structure remained on the surface unless the sample was treated by sonication or dipped in strong acid/alkali (10M) solutions for five minutes (Fig. 3). These results indicate the adhesive nature

of the co-assembled structures, which are probably due to the surface active functional groups of the decorated PDA.

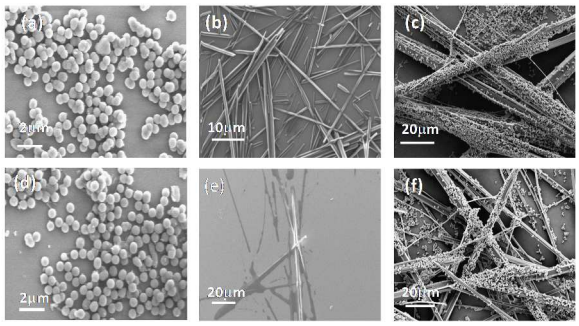

Fig. 3. Representative HR-SEM micrographs of the PDA spheres, DPA nanotubes and the co-assembled structures (condition 2) on a titanium surface before (a,b,c) and after (d,e,f) washing with water.

The PDA spheres can also be decorated with magnetic Fe_3O_4 NPs and form core shell spherical particles (Figure S4-6, ESI†).[14] To examine the co-assembly of these core shell particles with the DPA structures, we blended the two at different concentrations of DPA (condition 3 and 4, Table 1, Fig 4b). Both conditions led to the formation of a hydrogel which resisted flow upon inversion of the screw scraped vials (Fig. 4a).[19] It is important to note that when the preformed tubular structures were mixed with the PDA/Fe_3O_4 core/shell MNPs a gel did not form (Fig. S7, ESI†). To evaluate the viscoelastic properties and mechanical strength of the hydrogel we performed rheological measurements. The changes in storage modulus (G') and loss modulus (G'') under shear strain (σ) at a constant frequency of 1 Hz. were recorded (Fig S11a ESI†). These measurements show that at low strain values, the G' values are one order of magnitude higher than those of G''. This result indicates the dominant elastic character of the hydrogel. In addition, both G' and G'' were roughly constant below the critical strain value. When the strain value was above the critical strain both moduli gradually decreased to a very low value, demonstrating a partial breakup of the gel. This critical stress value is referred to as yield stress (σ_y). In this case the calculated σ_y value is 637Pa. A dynamic oscillatory frequency sweep experiment was performed at room temperature (298K) to examine the mechanical strength of the hydrogel (Fig. S11b). Frequency sweep rheometry measurement indicated that at any given frequency G' values were much higher than G'' (one order of magnitude). Moreover, both G' and G'' are frequency independent in the linear viscoelastic regime. This linear viscoelastic behavior further supports the formation of a hydrogel.[20]

We further analyzed the morphology of these gels using HR-SEM. Both the air-dried and frozen-dried gels exhibited a three-dimensional (3D) network of tubular structures with embedded MNPs (Fig. 4d, e and Fig. S8, ESI†). We assumed that the magnetic interactions among the PDA/Fe_3O_4 core shell particles promoted the aggregation of the tubular structure in such a fashion that water molecules were trapped between the aggregates voids to form a hydrogel. We investigated the thermal stability of the hydrogels formed under condition 3 and 4 (Table 1) by measuring the gel to sol transition temperature (T_{gs}), using the inverted tube method.[20a] The T_{gs} values of the hydrogels were 321K and 326K for condition 3 and condition 4 respectively (Fig. 4f). The higher thermal stability of the gel formed under condition 4 might be attributed to the higher concentration of DPA which allowed tighter packing of the gel. These temperatures are comparable with other peptide-based hydrogels.[20b, 21] In addition, the hydrogels were stable for at least one month when preserved at room temperature in a sealed tube. XRD analysis of the hydrogels showed the characteristic peaks of the magnetic Fe_3O_4 NPs in the 2θ range of 20° to 70° similar to that of PDA/Fe_3O_4 Core/Shell MNPs (Fig. S9, ESI†).[15b, c] Furthermore, the FT-IR spectra of the PDA/Fe_3O_4 Core/Shell MNPs and the hydrogels formed by co-assembly showed a characteristic absorption peak at ~580cm^{-1} which is assigned to the Fe-O stretching modes of magnetite (Fig. S10, ESI).[15a, 15c, 22] This peak slightly shifted (from 580 to 569 cm^{-1}) for the hydrogels probably due to the incorporation of the PDA/Fe_3O_4 Core/Shell MNPs in the aggregated tubular network. Overall, these results demonstrate that the Fe_3O_4 MNPs retained their magnetic properties after functionalization with PDA and further functionalization with DPA through co-assembly.

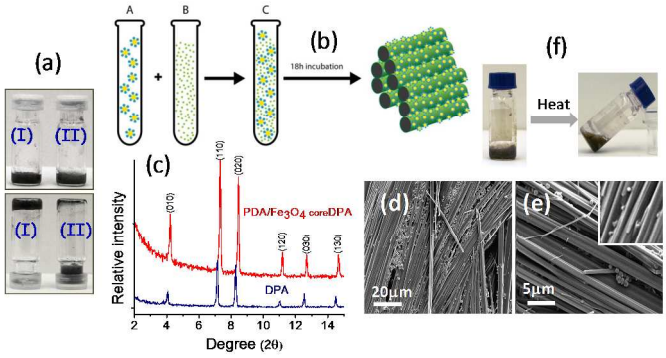

Fig. 4. (a) Photographs of straight and upside down vials containing the material formed under condition 3 or 4 (I) and condition1 or 2 (II). (b) The scheme illustrates the co-assembly processes under condition 3 or 4 (Table 1); (A) PDA spheres in water (1mg/mL), (B) DPA in HFP (100mg/mL) and (C) PDA/Fe_3O_4 Core/Shell spheres(1mg/ml): DPA (2 or 5mg/ml) in water (the illustration is not to scale). (c) XRD pattern of the hydrogel formed by co-assembly under (condition 4, blue) and DPA tubes (red). (d, e) HR-SEM micrographs of the co-assembled structures under condition 4 (after freeze drying) (the inset shows the incorporation of MNPs). (f) Gel to sol transition of the hydrogel with increasing temperature from X to Y.

We further investigated the nature of the gelated mass by X-ray diffraction pattern (XRD) of an air dried gel and compared it to the self-assembled bare DPA tubes. The spectra of bare tubular structures of DPA and the hydrogels had periodic peaks in the range of 2θ = 2° to 15° (Fig. 4c). These results demonstrate the hexagonal unit cell of the DPA tubular structure and the hydrogel. The intensity of these reflection peaks increased and sharpened in the case of the hydrogels. This indicates an increase in the amount of crystalline phase with a more close packing and suggests the formation of ordered self-assembled network.[23] In addition, the crystallite size decreased for the hydrogel network compared to bare DPA nanotubes from 3298Å to 1659Å.

Peptide based gelators are of great importance since they are biocompatible and therefore can serve as soft materials for tissue engineering and cell/drug delivery.[21] Magnetic gels are a new class of soft polymer materials consist of swollen polymeric networks with incorporated magnetic particles.[24] These magnetic hydrogels have been studied as a potential treatment of cancer.[22] Various methods have been developed to fabricate magnetic hydrogels such as blending,[25] *in situ* preparation [26] and grafting onto methods.[27] The method that

we describe here is an efficient strategy for the preparation of magnetic hydrogel. This approach involves the spontaneous self-assembly of monomeric DPA in the presence of Fe_3O_4/PDA MNPs by a simple mixing of the two in aqueous solution. We propose that the incorporation of MNPs during the growth period of the tubular structure is based on the chelation capacity of the active amino group of the DPA with Fe_3O_4 MNPs.[28]

In summary, we presented a simple method to generate sticky tubular structures or magnetic hydrogels by the co-assembly of melanin-like particles and peptide monomers. These assemblies are formed spontaneously under mild conditions. These results are another demonstration for the complexity that one can achieve by co-assembly.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Peptide synthesis, experimental details and additional figures

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