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PAPER

Facile and universal immobilization of L-lysine inspired by mussels

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A novel functional molecule lysine-dopamine (LDA) was successfully synthesized and explored as a universal modifier for different types of surfaces, inspired by mussel adhesive moiety dopamine and bio-functional moiety L-lysine. The universal, robust, and efficient surface modification based on LDA was achieved through a facile and cost-effective dip-coating process, confirmed by Fourier transform infrared spectroscopy (FTIR), contact angle, X-ray photoelectron spectroscopy (XPS) and scanning electron microscope-energy dispersive spectroscopy (SEM-EDS) measurements. Meanwhile, LDA modification improved cell adhesion, promoted cell growth, and accelerated endothelialization on the substrate surface and provided plasma clot lysis activity. The results indicated that the surface biocompatibility was obviously improved by the one-step modification method for the immobilized L-lysine.

Introduction

Surface functionality plays a critical role in chemistry, materials science, physics, biology and many other sciences and technologies. In biomedical yield, surface properties strongly influence the performance of implanted biomaterials. A variety of strategies have been pursued to create biocompatible surfaces. For blood contacting materials, anti-thrombogenic surfaces can be created by functionalizing materials with bio-inert molecules (*e.g.* anti-platelet PEG),[1,2] immobilizing bio-active molecules (*e.g.* anticoagulant heparin or fibrinolytic lysine),[3,4] or seeding with endothelial cells.[1,5] For cell culture and tissue engineering, cell-adhesion surfaces can be achieved by immobilization of extracellular matrix (ECM) molecules (*e.g.* fibronectin),[4,6] cell recognition peptides (*e.g.* RGD),[4,5,7,8] polylysine (PLL, cationic adhesion mechanism),[8–10] and mussel proteins (Cell-Tak, DOPA-mediated adhesion).[8,11]

Lysine and PLL are widely used as biocompatible materials and as a platform for bioactive molecule conjugation to create biocompatible surfaces.[9,12–15] The immobilization of these functional moieties on a surface is crucial for their use in biomedical applications. Yavin, *et al.*[13] immobilized PLL on substrates by physical adsorption to improve biocompatibility and used it as a platform for conjugation with bioactive molecules.[14] However, the binding strength of adsorbed PLL is relatively weak, so the PLL can detach from the surface during the course of use. Young, *et al.*[15] reported that lysine immobilized through the acid group of grafted PAA onto a membrane served as a bioactive molecule and mimics the behavior of the very expensive PLL.

Many available surface modification techniques have limitations such as substrate specificity (self-assembled monolayer and silylation), requirements for complex instrumentation and expensive equipment (Langmuir–Blodgett and plasma), complicated processes (layer-by-layer assembly and grafting polymerization), or inherent instability (physical absorption). Therefore, development of facile and versatile strategies for surface modification of widely different materials has proven challenging. Inert and hydrophobic surfaces such as polytetrafluoroethylene (PTFE), polyethylene (PE), polypropylene (PP), and poly-(vinylidene fluoride) (PVDF) often suffer from poor cell adhesion due to low surface energy, lack of functional groups, and the absence of cell recognition sites. The immobilization of functional molecules on PTFE surfaces through simple methods to promote endothelial cell growth can be very challenging.

Mussels can adhere tightly to all types of inorganic and organic surfaces in a wet and turbulent environment by excreting adhesive proteins that are incredibly strong and durable.[16] Waite, *et al.*[17] discovered that the strong adhesion relies on the repeated DOPA-lysine motif in mussel adhesive proteins. Deming, *et al.*[18] identified that the catechol group in DOPA plays a key role in the universal adhesion property due to its chemical versatility and diversity of affinity. Messersmith, *et al.*[19] showed that low molecular weight dopamine containing catechol moiety can also mimic the powerful adhesive ability of mussel proteins and can be used as a universal coating for different surfaces. For cell adhesion promotion, cell-tak™ (polyphenolic proteins extracted from mussels)[8,11] is used to attach cells to many types of different surfaces. DOPA-lysine co-polypeptides[20] have also been reported to promote cell adhesion and growth on EVA. However, the cell-tak and DOPA-lysine co-polypeptides are very expensive.

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Herein, we report a newly designed small molecule, lysine-dopamine (LDA), as a universal, robust, and efficient modifier for different surfaces. LDA can be applied by a simple, convenient, and cost-effective method on various substrates, and obviously improve the biocompatibility of substrate surface. The modified surface shows good durability, improves cell adhesion, promotes cell growth, and accelerates endothelialization.

Experimental

Materials

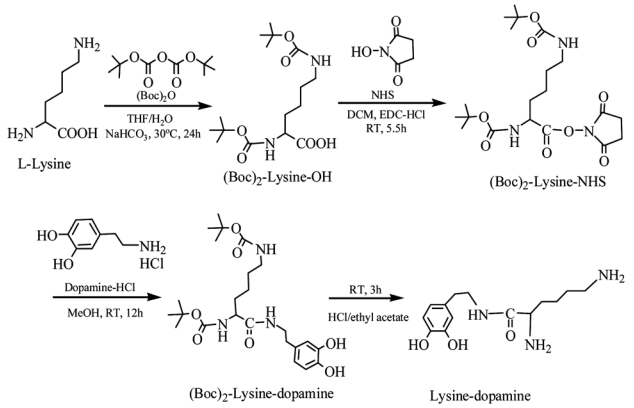
All reagents used were available from commercial sources. Lysine, *N*-hydroxysuccinimide (NHS), di-*t*-butyl dicarbonate ($(Boc)_2O$), dopamine hydrochloride (DA-HCl), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) were purchased from Aldrich and used without further purification. Tetrahydrofuran (THF), dichloromethane (DCM), ethyl acetate (EA), methanol, triethylamine (TEA), HCl, and $NaHCO_3$ were purchased from Sinopharm Chemical Reagent Co.

Titanium discs (grade 5, Ti-6Al-4V, Institute of Metal Research, Chinese Academy of Sciences), copper foil, monocrystalline silicon (Si), ceramic (Al_2O_3, TA Instruments), quartz (SiO_2), and glass discs were cleaned ultrasonically in 2-propanol for ten minutes before use. Microporous membranes such as polyethylene (PE, ET20-26, Entek), polypropylene (PP, Celgard 2500), polyvinylidene fluoride (PVDF, Dow), polytetrafluoroethylene (PTFE, Millipore), and dense films such as polyethylene terephthalate (PET, Toray), polyvinyl chloride (PVC), LDPE (Sinopec Corp.), PVDF (Shanghai 3F New Material Co., Ltd), and PTFE (Shanghai 3F New Material Co., Ltd) were used without treatment.

Synthesis of lysine-dopamine

Lysine-dopamine (LDA) was synthesized from lysine and dopamine. The detailed conditions are shown in Scheme 1.

(1) Synthesis of $(Boc)_2$-lysine-OH (BL). L-Lysine (10 mmol) was dissolved in the mixed solvent (150 mL deionized water and 50 mL tetrahydrofuran) in a flask and 12.6 g $NaHCO_3$ were added. Then di-*t*-butyl dicarbonate (Boc anhydride) (150 mmol) was added drop-wise into the flask. The reaction was carried out at 30 °C for 24 h under a nitrogen atmosphere. A rotary evaporator was used to remove the solvent and by-product. 1 mol L^{-1} HCl aqueous solution was added drop-wise into the flask until the pH was approximately 3. Ethyl acetate was applied to extract the product, then evaporated to isolate the product. Finally, the product was dried in an oven (60 °C) for 24 h. The yield of BL was 92%. ^{1}H-NMR (DMSO-*d*6, 300 MHz, δ in ppm): 12.42 (br. s, 1H, COO<u>H</u>), 6.98 (d, 1H, –OCON<u>H</u>–CH<), 6.78 (t, 1H, –OCON<u>H</u>–CH_2–), 3.78 (m, 1H, –OCONH–C<u>H</u><), 2.85 (m, 2H, –OCONH–C<u>H_2</u>–), 1.85–1.05 (m, 24H, –CH_2– and –CH_3).



Scheme 1 Synthesis of lysine-dopamine.

(2) Synthesis of $(Boc)_2$-lysine-NHS (BLN). The BL synthesized in the first step was dissolved in 125 mL dichloromethane, then *N*-hydroxysuccinimide (NHS) was added into the flask (NHS in excess). 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC-HCl) was dissolved in the dichloromethane at 0 °C (2 mol equivalents relative to NHS). After 30 min, the reaction was warmed to room temperature and allowed to proceed for another 5.5 h under a nitrogen atmosphere. Pure water was used to wash the solution 3 times; the solvent was removed, and the product dried in an oven (60 °C). The yield of BLN was about 88%. ^{1}H-NMR (DMSO-*d*6, 300 MHz, δ in ppm): 7.55 (d, 1H, –OCON<u>H</u>–CH<), 6.78 (t, 1H, –OCON<u>H</u>–CH_2–), 4.27 (m, 1H, –OCONH–C<u>H</u><), 2.96–2.65 (m, 6H, –N(CO–C<u>H_2</u>)$_2$ and –OCONH–C<u>H_2</u>–), 1.85–1.05 (m, 24H, –CH_2– and –CH_3).

(3) Synthesis of $(Boc)_2$-lysine-dopamine (BLDA). The BLN synthesized in the second step was dissolved in 125 mL methanol. Dopamine hydrochloride (dopamine-HCl) was added into the flask (dopamine-HCl in excess). After the dopamine-HCl dissolved, triethylamine (TEA) was added into the solution (the mole ratio of dopamine-HCl : TEA was 1.5 : 1.3). The reaction proceeded for 12 h at room temperature under a nitrogen atmosphere. The solvent and TEA were removed. Ethyl acetate was applied to dissolve the product, followed by washing with deionized water 3 times. The ethyl acetate was evaporated and the product was dried in an oven (45 °C) for 24 h. The yield of BLDA was about 71%. ^{1}H-NMR (DMSO-*d*6, 300 MHz, δ in ppm): 8.60–8.90 (d, 2H, –Ar(O<u>H</u>)$_2$), 7.76 (t, 1H, –CON<u>H</u>–CH_2–), 6.85–6.65 (m, 3H, –OCON<u>H</u>–CH< and –OCON<u>H</u>–CH_2–), 6.64–6.30 (m, 3H, –Ar), 3.78 (m, 1H, –OCONH–C<u>H</u><), 3.28–2.98 (m, 2H, –CONH–C<u>H_2</u>–), 2.85 (m, 2H, –OCONH–C<u>H_2</u>–), 2.45 (m, 2H, –CONH–CH_2–C<u>H_2</u>–), 1.85–1.05 (m, 24H, –CH_2– and –CH_3).

(4) Synthesis of lysine-dopamine (LDA). The BLDA synthesized above was dissolved in 100 mL ethyl acetate. 50 mL HCl/ethyl acetate solution was then added, and the reaction was carried out for 3 h at room temperature under a nitrogen atmosphere. After reaction, the solvent was removed and the product was oven-dried (45 °C) for 24 h. The final product is a bis-hydrochloride of LDA. The yield of LDA was about 99%. ^{1}H-NMR (DMSO-*d*6, 300 MHz, δ in ppm): 8.60–8.90 (d, 2H, –Ar(O<u>H</u>)$_2$), 8.46 (t, 1H, –CON<u>H</u>–CH_2), 8.13 (s, 2H, >CH–N<u>H_2</u>), 7.79 (s, 2H, –CH_2–N<u>H_2</u>), 6.64–6.30 (m, 3H, –Ar), 3.64 (m, 1H, >C<u>H</u>–NH_2), 3.42–3.10 (m, 2H, –CONH–C<u>H_2</u>–), 2.73 (m, 2H,

–C<u>H</u>$_2$–NH$_2$), 2.53 (m, 2H, –CONH–CH$_2$–C<u>H</u>$_2$–), 1.85–1.05 (m, 6H, –CH$_2$–). ES MS *m*/*z* 282.18 [M + H]$^+$.

Surface modification for different substrates by LDA

Many types of substrates (microporous membranes, films, *etc.*) were modified by LDA through a simple dip-coating process (Fig. 1). Substrates modified by dopamine (DA) were used for comparison. The substrates were immersed into 0.02 mol L^{-1} LDA or DA solution (pH = 8.5) and agitated at 30 °C for 40 h. The membrane was then removed and washed with deionized water. After drying to a constant weight at 40 °C in a vacuum oven, the resulting modified substrate was used for surface characterization and cell growth/adhesion experiments. In order to assess durability, the modified membranes were agitated with deionized water in a shaker (150 rpm) at 45 °C for 3 days. The membranes were then removed, washed with deionized water, and dried for contact angle characterization.

Characterization

FTIR and attenuated total reflection (ATR) spectra were measured with a Perkin-Elmer (Spectrum 1000) FTIR spectrometer at room temperature. Powdered samples were studied (KBr pellets) by FTIR. The film or membrane samples were studied by FTIR or ATR-FTIR.

^{1}H-NMR spectra were obtained with a Varian (Mercury plus-400) NMR spectrometer at room temperature in DMSO-*d*6, and chemical shifts were reported in ppm relative to tetramethylsilane (TMS).

The UV-vis spectrum of LDA in water was recorded on a Perkin Elmer (Lambda 750S) UV-vis spectrometer. All measurements were performed in quartz cuvettes. Water was used as a blank.

Contact angles were measured on a Contact Angle System OCA20 (Data Physics Instruments GmbH, Germany) using the sessile drop method with deionized water; the drop volume was 3.5 μl. All of the measurements were taken at ambient temperature and the values reported in this paper are the average of five measurements.

The surface chemical composition was analyzed using a Shimadzu-Kratos (AXIS Ultra) X-ray photoelectron spectroscopy (XPS). The XPS measurements were carried out at a base pressure of about 5 × 10^{-10} mbar using an Mg X-ray (1253.6 eV) source.

The surface elemental distribution was analyzed by scanning electron microscope-energy dispersive spectroscopy (SEM-EDS) on a Hitachi (S-2150) field-emission scanning electron microscope.

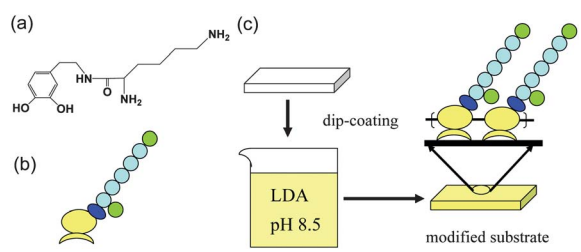


Fig. 1 Lysine-dopamine (LDA): chemical structure (a) and schematic representation (b); surface modification of LDA by dip-coating (c).

Cell adhesion and growth experiments

(1) Cell culture. Human umbilical vein endothelial cells (HUVEC) were obtained from Shanghai Veterinary Research Institute. Cells were sub-cultured twice a week and maintained in a 37 °C incubator containing a humidified 5% CO_2/95% air atmosphere. Dulbecco's modified eagles medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) was changed every 2 days.

(2) Cell seeding of the membrane. Membranes were cut into a circular shape (1.5 cm diameter) and sterilized with 75% alcohol and ultraviolet light. Then, membranes were transferred into a 24-well tissue culture polystyrene plate (TCPS, Corning), and secured by a silicon rubber ring. 500 μl cell suspension (about 30 000 cells per well) was used for culture seeding.

(3) Evaluation of cell viability and membrane cytotoxicity. The WST-1 (Beyondtime Bio-Tech, China) assay was used to measure cell viability. Briefly, after cells were cultured on PTFE surface or LDA modified PTFE surface for 24 h, the culture medium was changed and then 10% (v/v) WST-1 reagent was added to each well. The cells were incubated for another 1 h, and 150 μl of the cell suspension in each well was transferred into a 96-well flat plate (Corning). The absorbance was measured at 450 nm using a microplate reader.

(4) Evaluation of cell adhesion and cell proliferation. After 24 h of cell incubation, the medium was discarded. Poorly adhered cells were washed with 3 × PBS. The cells attached to the membranes were then stained with acridine orange/ethidium bromide (AO/EB) and observed with a fluorescence microscope.

Plasma clot lysis assay

The plasma clot lysis assay was carried out according to the literature reported by Li *et al.*[4] Briefly, samples were incubated in pooled normal human plasma (PNP) for 2 h at room temperature. The samples were rinsed in tris-buffered saline (TBS, pH 7.4) for three times, and placed in clean wells. 0.1 mg ml^{-1} of tissue plasminogen activator (t-PA) in TBS was added and incubated for 30 min. The samples were rinsed extensively with buffer. Then the clot-lysing potential was assessed using a modified plasma recalcification assay. A 100 μl PNP was added to the wells containing the surfaces. After a 5 min equilibration period at 37 °C, 100 μl $CaCl_2$ (0.025 M) was injected into the wells. Absorbance at 405 nm was measured at 30 s or 60 s intervals over a 80 min period.

Results and discussion

Characterization of LDA

The functional bio-molecule LDA is a newly synthesized small molecule containing a mussel-derived adhesive moiety (catechol) and a biofunctional moiety (lysine). It was synthesized from

lysine and dopamine, both of which are non-toxic and biocompatible. The structures of LDA and intermediate products including BL, BLN, and BLDA were confirmed by FTIR, ^{1}H-NMR and UV-vis spectroscopy.

In the FTIR spectra (Fig. 2), for BL, the characteristic N–H stretching vibration appear at 3348 cm^{-1}; the 1710 cm^{-1} band is associated with the stretching vibration of the carbonyl C=O. Bands at 2975, 2933, 2869, 1456, 1392 and 1365 cm^{-1} are due to the alkyl groups, the latter two bands are Boc C–$(CH_3)_3$ group overtone bands. The characteristic absorption of C–O–C appears at 1170 cm^{-1}. Compared to BL, there are some new absorption bands at 1816, 1784, 1212 and 1070 cm^{-1} in BLN, which can be assigned to the *N*-hydroxysuccinimide (NHS) group. Furthermore, compared to BL and BLN, the shift of the carbonyl from 1710 cm^{-1} to 1673 cm^{-1} in BLDA and LDA, and the appearance of a band at 1603 cm^{-1} corresponding to the phenyl ring implies the successful amidation and grafting of dopamine. For LDA, the amine absorption peak broadens and the alkyl absorption weakens, indicating the removal of the amine-protecting groups.

In the ^{1}H-NMR spectra (Fig. 3), for BL, the chemical shifts at 12.42 ppm, 6.98 ppm, 6.78 ppm, 3.78 ppm, 2.85 ppm and 1.36 ppm correspond to protons on the –COOH, –CH(COOH)–NH-Boc, –CH_2–NH-Boc, –CH(COOH)–NH-Boc, –CH_2–NH-Boc, and –O–C(CH_3)$_3$ moieties. Compared with BL, new chemical shifts in BLDA at 8.60–8.90 ppm, 7.76 ppm, and 6.64–6.30 ppm correspond to protons on –Ar(–OH)$_2$, –CO–NH–CH_2–, and the Ar of dopamine, due to the successful amidation reaction. Compared with BLDA, there was no peak at 1.36 ppm in LDA, indicating successful deprotection.

In the UV-vis spectrum of LDA (Fig. 4), there is an absorbance peak for the catechol group at 280 nm, which confirms the conjugation of dopamine with lysine.

The FTIR spectra, ^{1}H-NMR spectra and UV-vis spectrum confirm the successful synthesis of LDA.

Surface modification for different substrates by LDA

It is well known that the universal adhesion ability of mussels relies on the repeated DOPA-lysine motif in mussel foot adhesive proteins. In the adhesive protein, the pendant catechol from DOPA and the amine from lysine are responsible for the remarkable adhesion. The newly designed small molecule LDA can mimic the universal and strong adhesion of the mussel adhesive protein (Fig. 1), and it can be used as a biocompatible modification to membranes since lysine is a bio-functional molecule and dopamine is an excellent anchoring moiety.

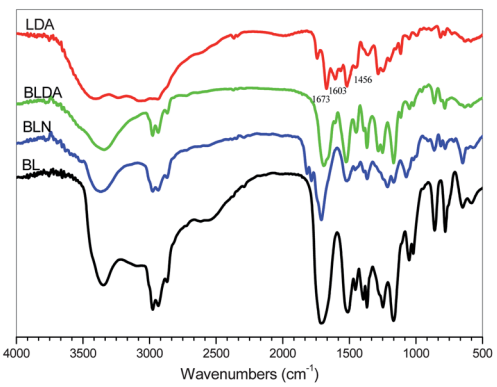


Fig. 2 FTIR spectra of Boc$_2$-lysine-OH (BL), Boc$_2$-lysine-NHS (BLN), Boc$_2$-lysine-DA (BLDA) and lysine-dopamine (LDA).

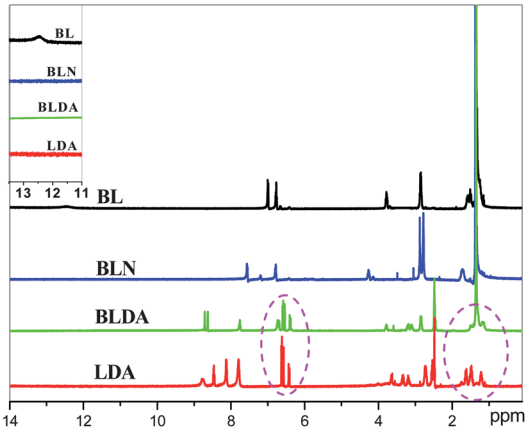


Fig. 3 ^{1}H-NMR spectra of BL, BLN, BLDA and LDA.

As shown in Fig. 5, the brown color indicated that surfaces had been successfully modified by LDA. L-Lysine immobilization can be achieved through simple dip coating in a LDA/PBS solution for all different kinds of substrates including metal (Ti alloy, Cu), ceramic (Al_2O_3, glass), inorganic (silicon, quartz), and polymer (PTFE, PVDF, PE, PP, and PET). The modified surfaces all show similar water contact angles near 60°, though the different original surfaces show quite different water contact angles (Fig. 6).

The successful surface modification on the surfaces by LDA can be also confirmed by FTIR, XPS, and SEM-EDS measurements. The FTIR spectra of a PP microporous membrane (PP-pore) before and after modification by LDA show the different surface chemical structures. Compared with the PP-pore membrane, new bands at 1660 cm^{-1}, 1560 cm^{-1} (amide I, II stretching vibration) and 1520 cm^{-1} (NH_3^+ bending vibration) are observed in LDA modified PP porous membrane (PP-pore-LDA) (Fig. 7).

Compared with the unmodified membrane, a LDA modified PTFE porous membrane (PTFE-pore-LDA) shows an obvious increase in surface oxygen content, the appearance of surface nitrogen, and a sharp decrease in surface fluorine content (Fig. 8) by XPS. These results indicate that LDA was indeed introduced onto the PTFE membrane surface.

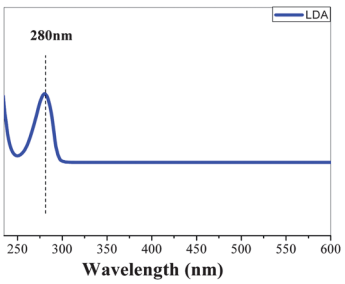


Fig. 4 UV-vis spectrum of lysine-dopamine (LDA).

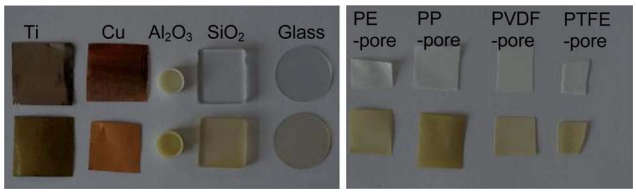


Fig. 5 Facile and universal surface modification by LDA: photograph of substrates before (upper row) and after immersion in LDA (lower row).

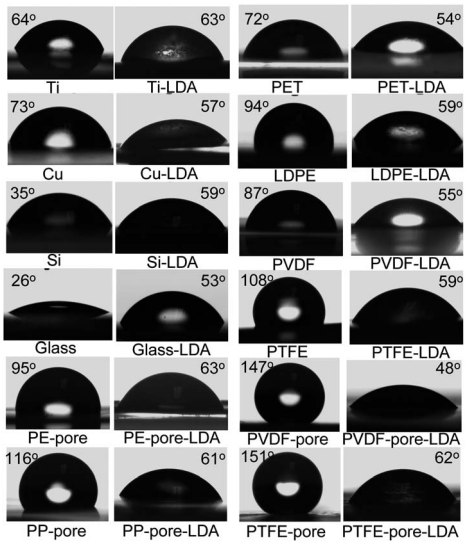


Fig. 6 Water contact angle of original and LDA modified substrates.

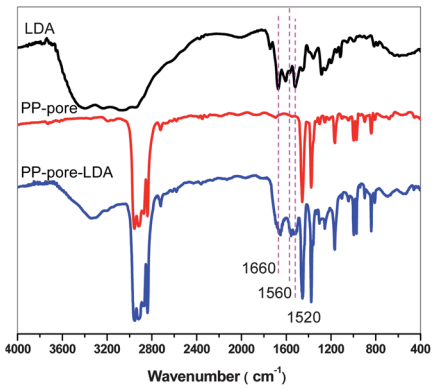


Fig. 7 FTIR spectra of original and LDA modified PP porous membranes.

Thus, classically adhesion-resistant materials, such as PTFE, were successfully modified by LDA through a simple dip-coating process. The LDA modified PTFE surface shows an even distribution of N and O as shown in Fig. 9.

Stability of surface modification

In order to confirm the stability of surface modification by LDA, durability testing on PTFE-pore-LDA was carried out. As shown in Fig. 10, no obvious change of water contact angle (near 60°) was observed for PTFE-pore-LDA membrane before or after testing, which suggests that the LDA modification of the PTFE-pore membrane is durable.

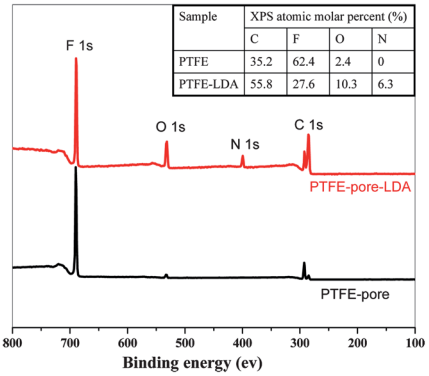


Fig. 8 XPS and elemental analysis of PTFE-pore and PTPE-pore-LDA.

Cell viability, cell adhesion and cell proliferation

Since lysine is a bio-functional molecule and dopamine is an excellent anchoring moiety, a biocompatible surface can be created by the one-step modification method for the immobilization of lysine *via* the mussel adhesive moiety. Surfaces modified by LDA show potential for cell culture and cell immobilization.

As shown in Fig. 11, the PTFE-pore-LDA substrate has remarkable cell affinity compared to the corresponding control PTFE membrane (PTFE-pore). LDA modified PTFE membrane shows a much higher WST reduction activity (+300%) than that of PTFE membrane. The PTFE-pore-LDA substrate is favorable for the culture of HUVEC. Therefore, a LDA modified surface improves the cell viability and shows potential in promoting cell proliferation.

LDA also improves adhesion to HUVEC and accelerates endothelialization. As shown in Fig. 12, few cells were observed on the surface PTFE-pore membrane after poorly adhered cells were removed by washing with PBS, while lots of live cells (in green color) can adhere to and grow on the surface of the PTFE-pore-LDA membrane. As is well known, HUVEC can barely adhere to an inert PTFE surface. The good cell adhesion and cell proliferation on the PTFE-pore-LDA surface are attributed to the biocompatibility of LDA through two

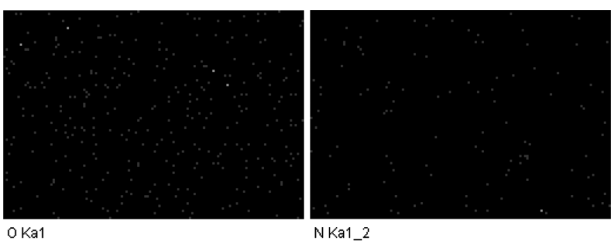
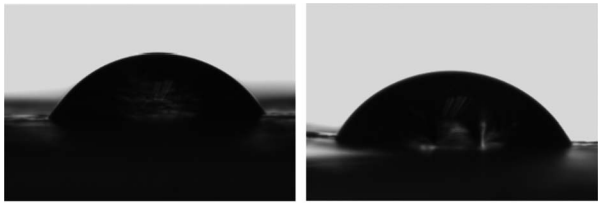


Fig. 9 Elemental distribution on PTFE-pore-LDA surfaces by SEM-EDS.



(a) (b)

Fig. 10 Water contact angle of PTFE-pore-LDA before (a) and after (b) agitation with deionized water in a shaker (150 rpm) at 45 °C for 3 days.

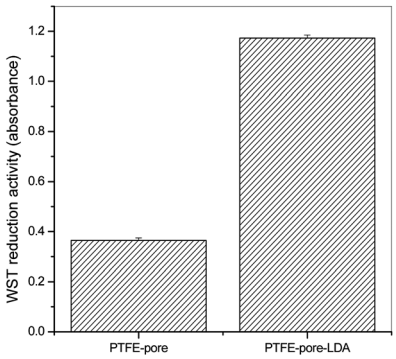
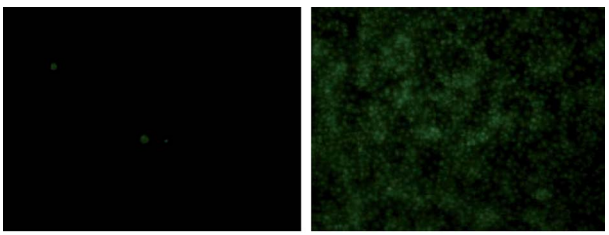


Fig. 11 Cell viability of human umbilical vein endothelial cells (HUVEC) after culturing for 24 h.



(a) (b)

Fig. 12 Photomicrograph of HUVEC on membranes after culturing for 24 h (AO/EB staining): (a) PTFE-pore, (b) PTFE-pore-LDA.

adhesion mechanisms (cationic adhesion and DOPA-mediated adhesion).[8]

Plasma clot lysis

For the fibrinolytic ability of lysine,[4] the substrate modified by LDA shows potential for creating a clot-lysing surface. In the plasma clot lysis assay, clot formation was expressed as absorbance at 405 nm *vs.* time. The onset of coagulation is indicated by a steep rise in the absorbance *vs.* time curve following recalcification of the plasma. As shown in Fig. 13, the PP-pore and PP-pore-DA surface showed a typical clot formation curve: a plateau in absorbance was reached and maintained indicating a fully formed, stable clot. In contrast, for the PP-pore-LDA surface, the absorbance returned to baseline, indicating that the clot formed and then was lysed. This result indicates that the LDA modified surface is bioactive and shows specific binding capacity of plasminogen, based on the immobilized fibrinolytic lysine through a simple coating process.

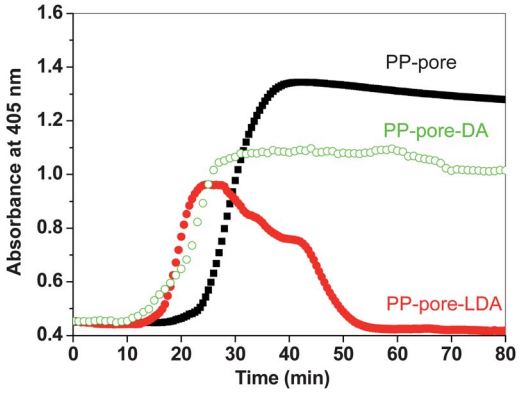


Fig. 13 Clot formation in plasma expressed as absorbance at 405 nm *versus* time for PP-pore, PP-pore-DA and PP-pore-LDA.

Conclusions

Inspired by mussel adhesive moiety dopamine and bio-functional moiety lysine, a novel molecule LDA was successfully synthesized and explored as a universal and robust surface modifier for different materials including metal, ceramic, inorganic, and polymer. Impressively, inert surfaces (even PTFE) can be also well modified by LDA through a facile and cost-effective dip-coating process. More importantly, lysine was immobilized on the surface *via* mussel adhesive moiety dopamine during the approach. Furthermore, the PTFE membrane modified by LDA showed excellent durability, improved cell adhesion, promoted cell growth, and accelerated endothelialization. Also, for the immobilization of fibrinolytic lysine, LDA modified surface shows plasma clot lysis activity. For the unique properties, functional molecule LDA shows potential in surface chemistry, materials science, bio-technology, tissue engineering and many other applications.

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