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Selective enhancement of human stem cell proliferation by mussel inspired surface coating

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In tissue engineering, promoted cell adhesion and proliferation on 3D scaffold is desired. Surface functionalization of the scaffoldis often utilized to realize this goal. Polydopamine (PDA) was extensively studied for promoted cell adhesion and proliferation. Norepinephrine, sharing a similar structure with dopamine, polymerizes slower and forms even and utltrasmooth surface coating on almost all substrates. However, the effects of polynorepinephrine (PNE) on stem cell adhesion and proliferation are barely studied. In this study, we compared the biocompatibility and cell adhesion properties of mussel inspired surface coating, the PDA and PNE, on poly(ε-caprolactone) (PCL) fiber for human mesenchymal stem cells (hMSC) and human induced pluripotent stem derived mesenchymal stem cells (hiPS-MSC). The surface modification of PDA or PNE on PCL fiber were characterized by environmental scanning electron microscope (ESME), X-ray photoelectron spectroscopy (XPS) and contact angle measurement. Biocompatibility of PDA and PNE coating to hMSC and hiPS-MSC were measured with lactase dehydrogenase (LDH) activity, live dead cell staining and cell counting kit 8 (CCK-8). Results showed that both PDA and PNE successfully formed a surface coatings on PCL fiber, which dramatically increase the hydrophilicity. PNE coating showed a much thinner and smoother surface while PDA coating form uneven aggregates among the fibers. Biocompatibility analysis and cell proliferation results suggest that the PNE coating is more biocompatible to both hiPS-MSC and hMSC cells than PDA coating. PNE coating preferentially promoted hiPS-MSC cell proliferation but not for hMSC, while PDA decreased cell proliferation of hiPS-MSC on PCL fiber. These results suggest that the effect of PDA and PNE surface coating on cell proliferation can be cell-dependent. The surface roughness of PDA coating can negatively affect the cell adhesion and proliferation. Different mechanisms of interactions between PDA or PNE coating might affect cell adhesion and proliferation, which need to be carefully investigated before their application in a specific cell type based tissue enginnering.

Introduction

Tissue engineering has been developed for around thirty years as a promising strategy to repair the damaged tissue or organ. Surface modification of scaffolds or implants with biocompatible adhesive molecules to promote cell adhesion and proliferation could benefit the success of tissue enginnering. PDA coating is inspired from marine organism mussel (mytiloida), which has a unique capacity of functionalizing virtually any material surface, including hydrophobic, synthetic polymers,[1] noble metal and metal oxides,[2, 3] carbon materials[4, 5] and so on. PDA, a catechol molecule, has been widely reported to increase the cell adhesion and proliferation, reduce inflammation and toxicity both *in vitro* and *in vivo*.[1, 3, 6] However, a few studies showed that PDA coating reduced the proliferation of smooth muscle cells, possibly due to the catechol oxidation induced ROS production.[7, 8] Polymerization of dopamine involves oxidation of catechol to quinine under alkaline condition (pH 8.5), which further react with other catechols or quinines to from an adherent polymer film.[2] The deposited polydopamine coating is chemically heterogeneous. Despite the advantage of its materials–independent functionalizability, the uncontrollable surface roughness after PDA polymerization has been an obstacle for its potential applications. To combat this challenge, a new surface coating derived from catecholamines, the polynorepinephrine (PNE), was introduced.[9] Unlike polydopamine coating, PNE have strong ability to activate surface-initiated, ring-opening polymerization in alkaline condition due to the presence of alkyl hydroxyl group in norepinephrine, forming nearly perfect smoothness at nanometer scale on the substrate surface.[9, 10]The residual quinine is stable under alkaline condition and reacts towards amine and thiol containing biomolecules, enabling facial conjugation of proteins with terminal amine possible.[2, 10, 11] Our previously study suggested that PNE surface functionalized PLCL fiber could facilitate PC12 cell differentiation.[12]

In tissue engineering, cell source is another critical factor for regenerate or restore damaged tissues and organs. Mesenchymal stem cells (MSCs) are commonly used for their multi-lineage differentiation capacities. However, the low population and limited proliferation capacity hampered their application in tissue engineering. Induced pluripotent stem (iPS) cell as a ground-breaking discovery by Takahashi and Yamanaka in 2006 enabled reprogramming somatic cells back to a pluripotent state with capacity to differentiate into cell types in three germ layers.[13] Based on this technique, MSCs derived from human iPS cells (hiPS-MSC) have been reported by several groups.[14, 15] hiPS-MSC shares similar in vitro and in vivo characteristics as MSC,[16, 17] and outperforms MSCs with greater cell proliferation capacity to proliferate for 120 population doublings without losing their renewal capacity and MSC characteristics,[18, 19] which make it an excellent cell source for tissue engineering.

Design and fabricate a suitable scaffold to mimic the extracellular matrix (ECM) to facilitate the cell adhesion and proliferation is critical for tissue engineering. Among many techniques developed for fabricating fibrous scaffolds, electrospinning attracts the most interests due to its straightforwardness, robustness and versatility capable of generating fiber with diameters ranging from 100 nm to 10 μm.[20] Electrospunfibers have larger surface area for cell interaction and communication than 2D cell culture plates. Poly(e-caprolactone) (PCL) is a synthetic, biocompatible and biodegradable polymer, which has been approved by FDA as implants, drug delivery devices, sutures. PCL and PCL composites have been used as scaffolds in tissue engineering for tissue regeneration of cartilage, bone, nerve and vascular tissue.[21] Our recent study used PCL and hydrogelcomposed 3D ECM mimicking scaffold to support hiPS-MSCsdifferentiation to fibroblasts.[22]

Based on mussel inspired surface coating, PDA has been extensively studied as a functional surface coating in tissue engineering for stem cell adhesion and proliferation.[8, 23-26] However, PNE, which share similar chemical structure with PDA but form more even and smoother surface coating, has not been well explored for stem cell-based tissue engineering.[10, 12, 27] Therefore, in the present work, we compared the adhesion and proliferation effect of PDA and PNE on hMSC and hiPS-MSC on both tissue culture plates (TCP) and PCL fiber. The surface functionalization with PDA or PNE on PCL fiber was characterized by ESEM, XPS and contact angle measurement. Cell adhesion and proliferation on surface functionalized substrate were further analyzed with cell viability assay and cell morphological characterization. By comparing stem cell proliferation on PDA or PNE surface coating, this research aimed at enriching the study of mussel chemistry on stem cells therapy fortissue engineering.

Experimental Section

Electronspining of PCL fiber and surface modification

The polycaprolactone solution was prepared by dissolve PCL (Mw=70-90 KDa, Sigma, Germany) in 8 :2 chloroform/ethanol (v/v) into 12% solution (w/v). PCL solution were then electrospun at 20 kV through a 20 G blunt end needle with flow rate of 8 ml/h. Obtained fibers were lyophilized in a freezer dry overnight. The mesh was punched into circular shapes with 12 mm in diameter to fit in 48 well cell culture plates. Before use, the fibers were sterilized with 75% ethanol for 30 min and ultraviolet irradiation for 30 min. The surface coating with PDA or PNE was performed by simple immersion of electrospun PCL fiber or TCP into a dopamine hydrochloride(Sigma, Germany) solution or norepinephrine(Sigma, Germany) solution (2 mg/ml in 10 mM Tris-HCL, pH8.5) at 25 ℃ for 17 h. After coating, fibers or TCP were washed three times with PBS and lyophilized in a freezer dry, kept at 4 ℃ until use. From here,

TCP coated with PDA or PNE were refered as PDA-TCP, PNE-TCP, and PCL fiber coated with PDA or PNE were refered as PDA-PCL, PNE-PCL.

X-ray photoelectron spectroscopy

The atomic chemical composition of the PCL fiber before and after PDA or PNE coating was analyzed with X-ray photoelectron spectroscopy (XPS, ESCALAB250Xi,Thermo Fisher Scientific, US).

Contact angle

The contact angle of PCL fiber and PDA- or PNE-PCL fiber were measured with the DSA 100 (Germany) with the method depicted by the manufacturer at 25 °C and 60% relative humidity. In brief, 2 ul ddH_2O was added on the surface of the fibers and pictures were taken.

Environmental scanning electron microscope

Environmental scanning electron microscope (ESEM, Quanta 200 FEG, FEI, US) was applied to observe the surface morphology of PCL fiber before and after PDA or PNE coating and cell morphology of hMSC on these fibers. Cells were seeded on fibers for 9 days and then washed 3 times with PBS and fixed with 4% paraformaldehyde for 15min, After washed 3 times with PBS, PDA or PNE coated PCL fibers and fiber with cells were dehydrated with 30%, 50%, 70%, 85%, 95%, 100% ethanol, dried at room temperature. Fibers and cells were observed under ESEM with an acceleration voltage of 4 kV under low vacuum conditions.

Cell culture

Human bone marrow stem cell (hMSC) was purchased from Lonza (PT-2501) and cultured according to the instructions. hiPS-MSCs generated from human iPS cells as previously reported[28] was kindly provided by Dr Yonglun Luo from Department of Biomedicine, Aarhus University. Both cells were cultured in H-DMEM (Gibco, UK) plus 10% fetal bovin serum (FBS, Biowhittaker, Walkersvile, MD), 10% peneciline and streptomacin (Gibco, Grand Island, NY) in a humidified 37°C incubator with 5% CO_2. Cells were used between 7-8 passages.

LDH assay

LDH assay was applied to study the cytotoxicity of PDA- or PNE –TCP or PCL fiber. Briefly, cells were seeded on TCP or PCL fiber in 48 well plates.After 24 h, 200 µl cell culture medium was collected into 1.5 ml eppendorf tubes and

centrifuged at 2000 rpm, 5 min at 4 °C to discard the possible cell contamination. Then, 50 µl supernatant was transferred from eppendorf tube to 96 well plates and a 50 µl mixture of enzyme mix was added to each well and react for 30 min at RT, protected from light. The absorbance at 490 nm was measured with Victor X5 microplate reader. Cells cultured on TCP was set as normal control (0% cell death), while cells cultured on TCP treated with 1% TritonX-100 was set as positive control (100% cell death). The percentage of cytotoxicity was calculated using the equation:

$$\text{Cytotoxicity (\%)} = \frac{\text{exp.value} - \text{normal control}}{\text{positive control} - \text{normal control}} * 100$$

Live dead cell staining

Live dead cell staining was applied to study the biocompatibility of PDA- or PNE -TCP for hiPS-MSCs and hMSCs. Briefly, cells were cultured on PDA- or PNE -TCP for 1, 3 and 5 days. Then cells was washed with PBS and incubate with Calcein-AM (2 µM) and Ethidium (4 µM) at 37 ℃ for 30 min, cells was washed with PBS and observed under an inverted fluorescence microscope. The Ex/Em for Calcein-AM is 494/517 nm and for Ethiduim is 528/617 nm.

Cell viability assay

The cell viability of hiPS-MSCs and hMSCs cultured on TCP or PCL fiber was measured by cell counting kit 8 (CCK-8, Dojindo, Kumamoto, Japan) assay. In brief, cells were seeded and cultured for 1, 3 and 5 days on TCP or 1, 5 and 9 days on PCL fiber in 48 well plates, respectively. Cell culture medium was changed every other day. After that, PCL fiber was transfered into a new 48 well plates to measure cells on fiber and cells fall on TCP. The cell culture medium was discarded and 20% CCK-8/medium (300 ul/well) was added into 48 well plates and incubate at 37ºC for 2 h, 100 µl of aliquots from each sample were pipetted into a 96 well plate and the absorbance at 450 nm was measured with a Victor X5 microplate reader.

Cell morphology characterization

The cell morphology of hiPS-MSC and hMSC cultured on PDA- or PNE-PCL fiber were observed under a Zeiss LSM 700 laser confocal microscope (Carl Zeiss Micro-Imaging GmbH,Germany). Briefly, cells were seeded on PDA- or PNE-PCL fiber and culture for 1, 5 and 9 days. Cell culture medium was changed every other day. Fibers were washed 3 times with PBS and fixed with 4% paraformaldehyde for 15 min at RT, permeabilized with 0.05% Triton for 5 min and then stained with phalloidion for 30 min, after washed 3 times, 10min for each wash with PBS, fibers were placed on a slide with a drop of mounting

medium with DAPI. Samples were then observed and taken pictures under confocol laser scanning microscope.

Statistical analysis. Data are expressed as mean ± standard deviation(SD). The statistical analysis was performed using Student's *t*-test to compare the significance in multiple data groups. Valus of $p < 0.05$ was considered as statistical significant.

Results and Discussions

Electrospinning and surface morphology of PDA or PNE coated PCL fiber

Electrospinning technology has been used for the fabrication of tissue-engineered scaffolds because of its ability to mimic the extracellular matrix (ECM) structures.[29, 30] PCL was chosen as the scaffold material due to its good biocompatibility and mechanical properties. The diameter of the fibers can be controlled by adjusting the electronspun parameters, including voltage, speed, needles, etc. The PCL fibers we electrospun are randomly arranged with an average diameter of 1.13±0.5 μm (Figure 1). PDA and PNE coating was prepared by dissolving dopamine or norepinephrine in 10 mM Tris buffer (pH 8.5) to make a 2 mg/ml solution. After 17 h of incubation, ESEM was applied to observe the surface morphology of PCL fibers and the surface coating PDA or PNE. As is shown in Figure 1 and Figure S1, PDA-PCL fibers revealed uneven surfaces with significant aggregates on the fibers. The average diameter of PDA-PCL fiber is 1.25±0.58 μm. Compared with PDA-PCL fiber, the PNE-PCL has much smaller aggregates and smoother surface. The average diameter of PNE-PCL fiber is 1.18±0.45 μm.This is in consistence with previous study that PNE coating form thin and ultra-smooth surface while PDA coating formed uncontrolled aggregates.[9] The emergence of the uncontrollable roughness during PDA polymeration is proposed due to the PDA particles in solution simultaneously attach and grow directly from surface.[9] Studies at molecular level found that it is the intermediate, 3,4-dihyfroxybenzaldehyde (DHBA) formed during polymerization of norepinephrine, resulted in the remarkable difference in surface morphology.

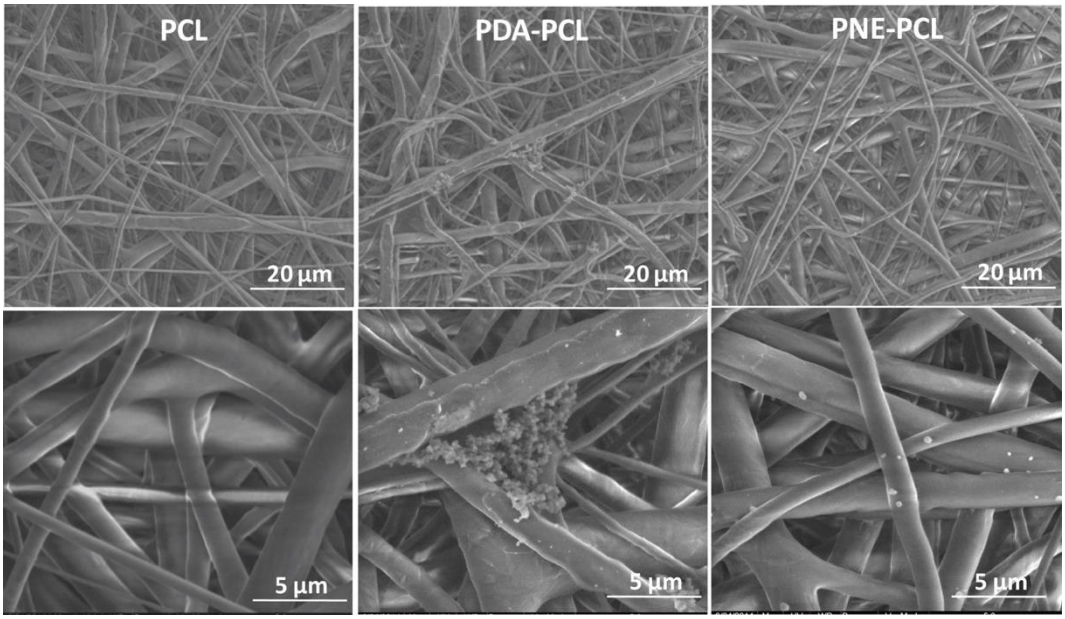

Figure 1, Surface morphology characterization of PCL fiber, PDA-PCL fiber and PNE -PCL fiber.

XPS was applied to quantify the surface elemental species of PCL fiber and PDA- or PNE-PCL fiber. As is shown in Figure 2, the uncoated PCL fiber consists of only carbon (C 1s) and oxygen (O 1s), while after PDA or PNE coating, a nitrogen (N 1s) peak was observed. The chemical compositions of three fibers were quantified and listed as Table S1 in supporting information. Along with coating time extend from 0.5 h to 17 h， the nitrogen composition increased in both PDA-PCL and PNE-PCL fiber. Interestingly, under the same incubation time, PDA-PCL fiber showed stronger nitrogen signal compared with PNE-PCL fiber. This is due to the fast polymerization and large aggregation formed during PDA coating on PCL fiber.

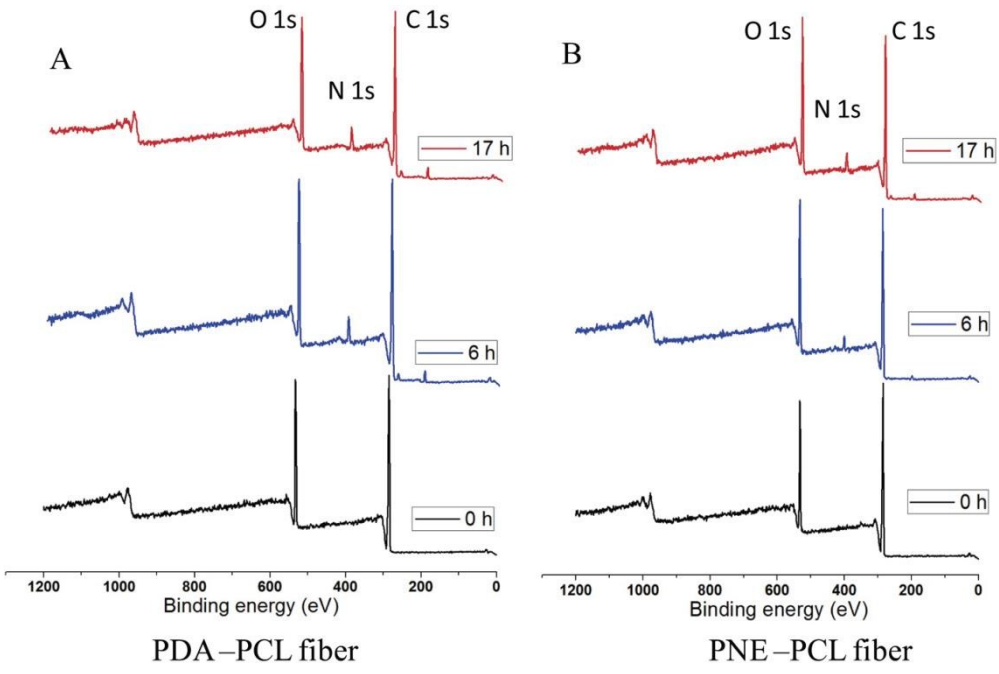

Figure 2, XPS spectra of PCL fiber before and after PDA or PNE coating. Chemical composition analysis of PCL fiber after 0, 6 h or 17 h coating in (A) PDA or (B) PNE solution. C 1s, O 1s and N 1s peaks are compared in parallel.

The water contact angle of the surface was measured in order to analyze the change of the surface wettability after surface modification with PDA or PNE. As is shown in supporting Figure S2, uncoated PCL fiber typically exhibited water contact angle of 125.5 º±1.1 º, while the contact angle for surface coated with PDA or PNE significantly decreased and was close to 0 º, which means the surface changed from hydrophobic to hydrophilic.

Cell viability of hiPS-MSCs and hMSC on PDA- or PNE-TCP

Before analyzing the cell proliferation on PCL fiber, we used TCP to evaluate the biocompatibility of PDA or PNE coating by LDH assay and live dead cell staining. LDH is a stable cytoplasmic enzyme present in all cells. It is rapidly released into the cell culture supernatant upon damage of the plasma membrane. Therefore it is a good biomarker for cytotoxicity assay. As is shown in Figure 3, compared with cells cultured on TCP, LDH release in hiPS-MSC cells cultured on PDA-TCP significantly increased, while in PNE-TCP group is as low as TCP group, suggesting PNE coating is more biocompatible than PDA coating for hiPS-MSC cells. Live dead cell staining results showed that both PDA and PNE coating did not induce significant cell death compared with cells on TCP. But the number of live cells and cell morphology is different on PNE-PCL fibers in comparison to those of PDA-PCL and the uncoated PCL fibers. More cells on PNE-TCP. This was further confirmed by the proliferation assay of hiPS-MSCs on PDA- or PNE-TCP for 1, 3 and 5 days as determined by

CCK-8 assay. As shown in Figure 3C, hiPS-MSC cell viability increased overtime and cell proliferation in PNE-TCP is higher than cells on TCP at the same incubation time, while PDA-TCP significantly decreased hiPS-MSC cell viability compared with hiPS-MSC on TCP. This is in accordance with the LDH results that the PDA-TCP increased the LDH release, which is a sign of cytotoxicity. These results suggest that hiPS-MSC adhere, spread and survive better on PNE-TCP than uncoated TCP. PDA-TCP decreased the cell viability of hiPS-MSC compared with uncoated TCP. While for hMSCs, as shown in Figure 4, PDA-TCP increased the LDH release of hMSC cells than uncoated TCP. Live dead cell staining suggested that both PDA and PNE did not induced significant cell death. Cell viability assay showed that PNE-TCP did not significantly affect their cell viability, while PDA coating decreased the hMSC viability on day 1 compared with hMSC cells on TCP and have no significant difference after 3 and 5 days incubation. Taken together, according to our results, PNE coating is more biocompatible to both hiPS-MSC and hMSC cells than PDA. PNE enhanced the cell adhesion and proliferation of hiPS-MSC, but have negligible effect on hMSCs. This is interesting because both dopamine and norepinephrine are catecholamine molecules, the only difference between these two molecules is that PNE have an hydroxyl on the side chain and formed a smoother surface than PDA while polymerized in pH 8.5 Tris buffer. As is reported before, PDA polymerization is rather fast and uncontrollable, during which large aggregates formed in solution and attached to functionalized surfaces, thus resulting insignificant variations in surface roughness.[9] Yang *et al.* and luo *et al.* have reported inhibitory effects of PDA-coated surface on smooth muscle cell (SMC) proliferation. They proposed that the phenolic/quininegroups present on the PDA coating played a key role in modulating vascular cell behaviour.[7, 8] Ding *et al.* further indicated that the quinone group on the PDA coating induces a substantially higher amount of protein adsorption, which plays a key role in promoting epithelial cell attachment and proliferation. Meanwhile, the reactive phenolic hydroxyl group on the PDA coating potently inhibits SMC proliferation.[31]

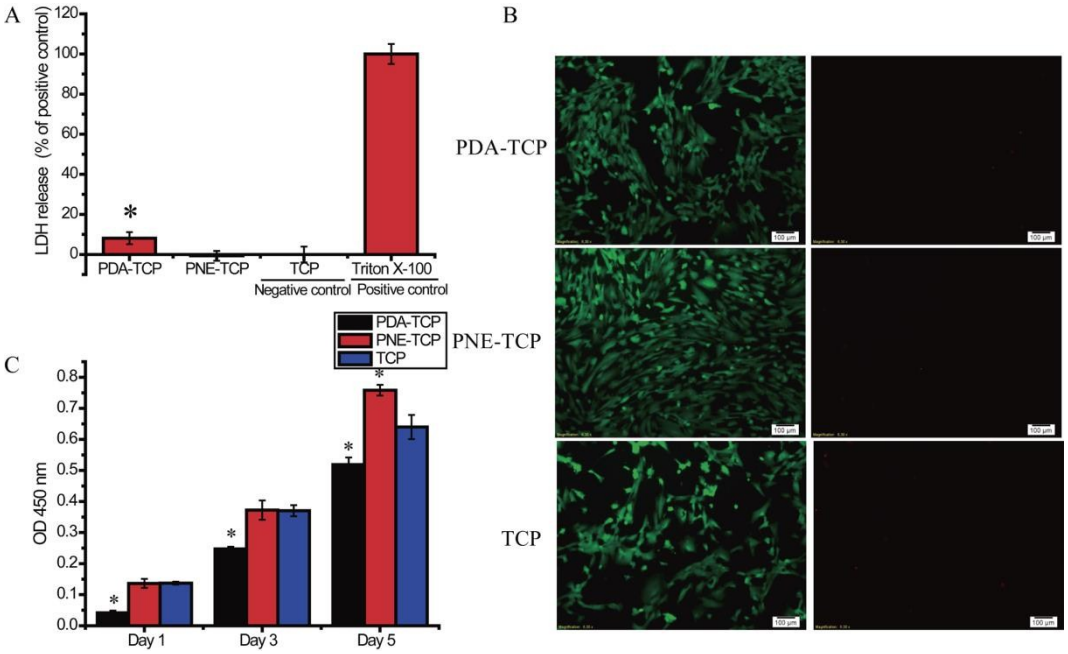

Figure 3, hiPS-MSCs cell viability on TCP and PDA- or PNE-TCP. (A) LDH assay of hiPS-MSCs cultured on TCP and PDA- or PNE-TCP. (B) Live dead cell staining of hiPS-MSCs cells cultured on TCP and PDA- or PNE-TCP (C) Cell viability of hiPS-MSCs cultured on TCP and PDA- or PNE-TCP for 1, 3 and 5 days. Data are presented as mean ±SD. n=3. The statistical significance is presented as $p < 0.05$.

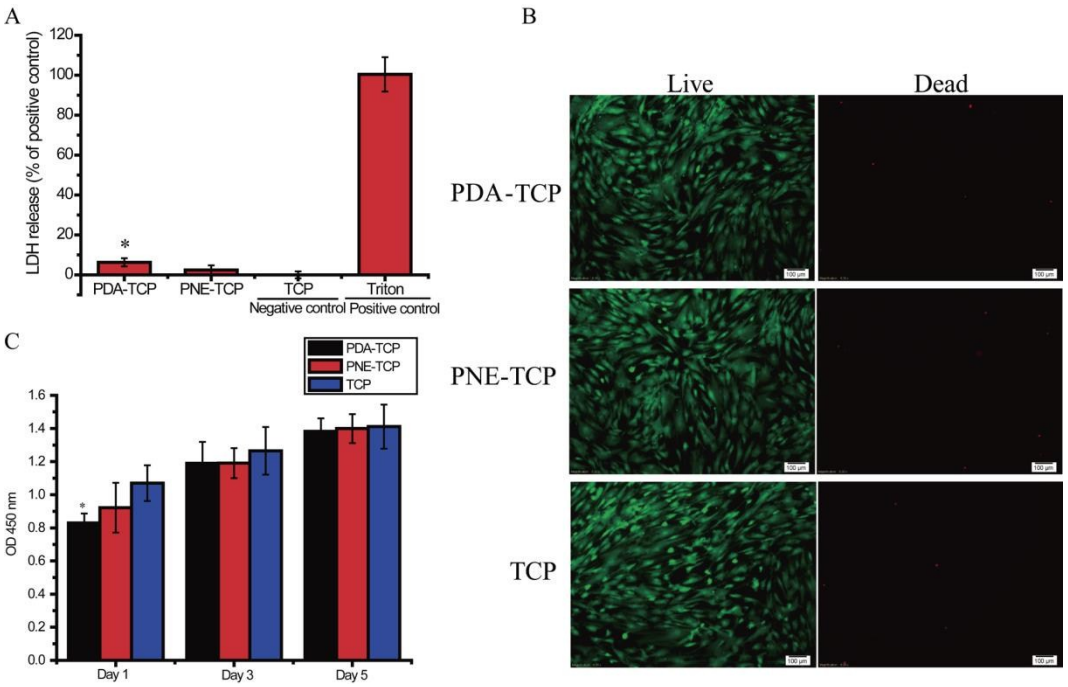

Figure 4, hMSCs cell viability on TCP and PDA- or PNE-TCP. (A) LDH assay of hMSCs cultured on TCP and PDA- or PNE-TCP. (B) Live dead cell staining of cells cultured on TCP and PDA- or PNE-TCP (C) Cell viability of hMSCs cultured on TCP

and PDA- or PNE-TCP for 1, 3 and 5 days. Data are presented as mean ±SD. n=3. The statistical significance is presented as $p<0.05$.

Cell proliferation and cell morphology of hiPS-MSCs and hMSC on PDA- or PNE-PCL fiber

Electrospun fiber, with a three-dimensional architecture, high porosity, interconnected pore structure, and a high surface-to-volume ratio provides great advantages in *in vitro* cell culture for tissue engineering. PCL as a FDA approved synthetic polymer has been widely used due to its good biocompatibility and suitable mechanical property for tissue engineering. The biocompatibility of uncoated PCL fiber and PDA- or PNE-PCL fiber for hiPS-MSCs and hMSC was evaluated by LDH assay and CCK-8. As is shown in Figure 5A, for hiPS-MSC, PDA-fiber induced significant increase in LDH release compared to uncoated PCL fiber, while PNE-PCL fiber significantly decreased the LDH release. LDH release in PDA-PCL possibly originated from cells failed to adhere on the fiber, which was further verified by cell viability assay. Cell proliferation data (Figure 5B) suggest that PDA-PCL significantly decreased cell viability compared with uncoated fiber, while PNE-PCL increased cell viability especially on day 9. This is in accordance to the cell proliferation results on PDA- and PNE-TCP. However for hMSC cells, as shown in Figure 6, both PDA and PNE did not induce significant increase in LDH release, the cell viability of hMSC cells in PDA-or PNE-PCL fiber is significant lower than uncoated PCL fiber. But if you only compare the cell viability of cells grown on the fiber, PNE have a similar cell viability with uncoated PCL fiber, but PDA still have a lower cell viability compared with uncoated PCL fiber.

In general, mammalian cells are undergo a cell adhesion process of substrate attachment, spreading and cytoskeleton development.[32, 33] We further investigated the cell morphology of hiPS-MSCs and hMSC on PDA- or PNE-PCL fiber by staining cytoskeleton actin filament with FITC-phalloidin. As is shown in Figure 5C, on day 1, hiPS-MSCs adhered more on PNE-PCL than PDA-PCL and uncoated PCL fiber. The cytoskeleton of hiPS-MSCs on uncoated and PDA-PCL is not as spread and stretched as cells on PNE-PCL. After 5 and 9 days culture, the difference in cell morphology and cell density on three fibers became more evident, PNE coating facilitate the hiPS-MSCs cell adhesion and proliferation on PCL fiber. However, as shown in Figure 6C, Hmsc cell morphology on PDA and PNE-PCL fiber did not show much difference from cells on uncoated PCL fiber. PDA coating has been reported to selectively reduced cell proliferation of human umbilical artery smooth muscle cell but increase human umbilical vein endothelial cell proliferation due to the reactive phenolic hydroxyl group on the PDA.[8] Study indicated that dopamine polymerized in air is rough due to the agglomerate deposition and inhomogeneous stacking of the deposited molecules.[34] The decreased cell adhesion and proliferation is possibly due to the surface roughness of PDA aggregates. The PNE formed smooth surface and

react with thiol and amine containing molecules, therefore the enhanced cell proliferation could be attributed to the immobilization of serum proteins from cell culture medium on PNE layer.

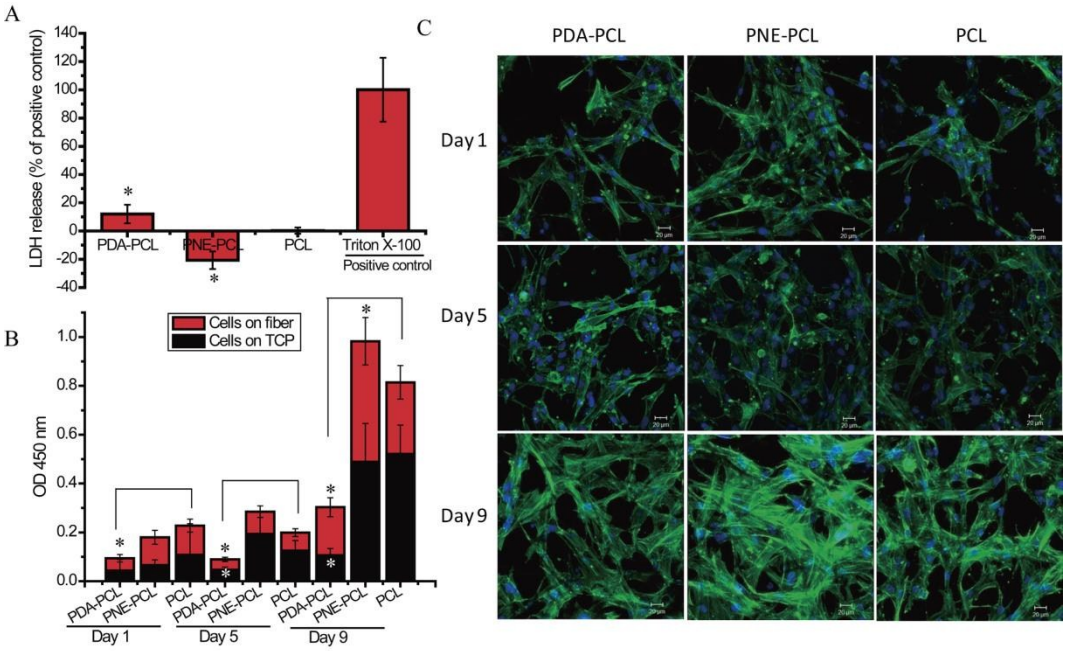

Figure 5, Biocompatibility of PCL fibers and PDA- or PNE-PCL fibers to hips-MSCs. (A) LDH release of hiPS-MSCs on PCL fibers and PDA- or PNE-PCL fibers. (B) Cell viability of hiPS-MSCs after 1, 5, and 9 days incubation on PCL fibers and PDA- or PNE-PCL fibers. (C) Cell attachement and morphology of hiPS-MSCs after 1, 5, and 9 days incubation on PCL fibers and PDA- or PNE-PCL fibers. The data represented mean ± SD. n=3.The statistics significance compared with PCL fiber was presented as $p < 0.05$.

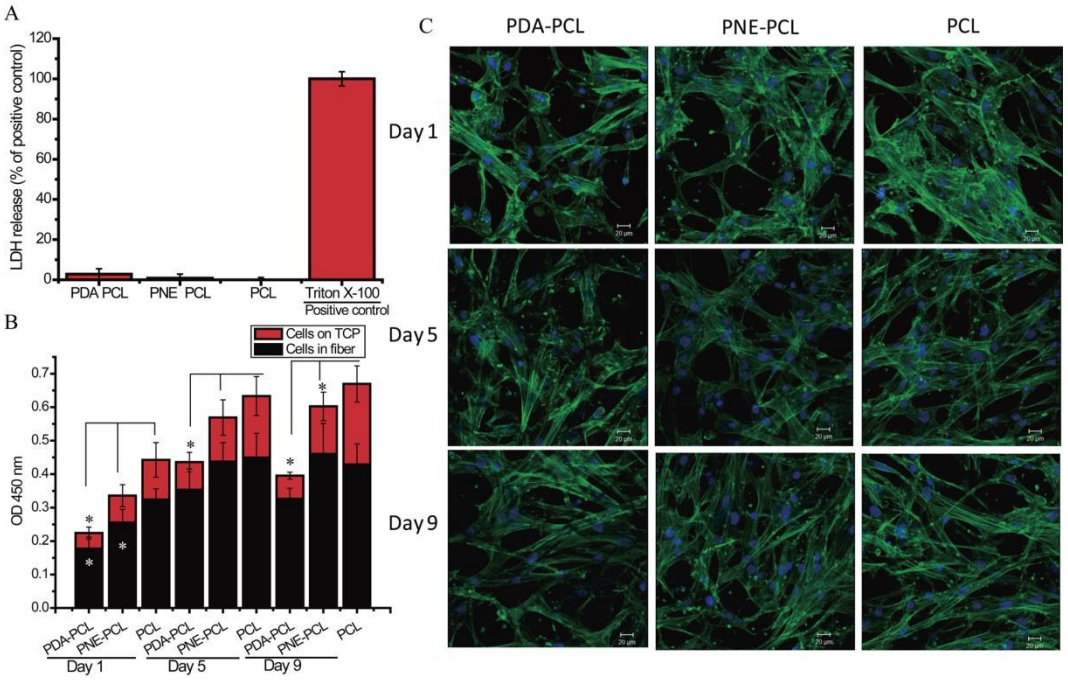

Figure 6, Biocompatibility of PCL fibers and PDA- or PNE-PCL fibers to hMSCs. (A) LDH release of hMSCs on PCL fibers and PDA- or PNE-PCL fibers. (B) Cell viability of hMSCs after 1, 5, and 9 days incubation on PCL fibers and PDA- or PNE-PCL fibers. (C) Cell adhesion and morphology of hMSCs after 1, 5, and 9 days incubation on PCL fibers and PDA- or PNE-PCL fibers. The data represented mean ± SD. n=3. The statistics significance compared with PCL fiber was presented as $p < 0.05$.

Cell morphology of hMSCs on PDA -or PNE- PCL fiber

To investigate the interaction between the fibers and hMSC cells, we applied ESEM to observe the cell morphology and localization of hMSC on PCL fiber. As is shown in Figure 7, most of the cells are located on the surface of the fiber, there are also some cells migrated into the fibers. The uncoated PCL fiber had more cells adhered on the fiber surface, while PDA-PCL and PNE-PCL had less cell number but more spread cell adhesion. PDA and PNE were reported to promote the cell proliferation and differentiation by immobilizing the thiol and amine containing molecules or growth factors.[35] But in our study, it is true for hiPS-MSC but not for hMSC. The underlying mechanisms need further investigations.

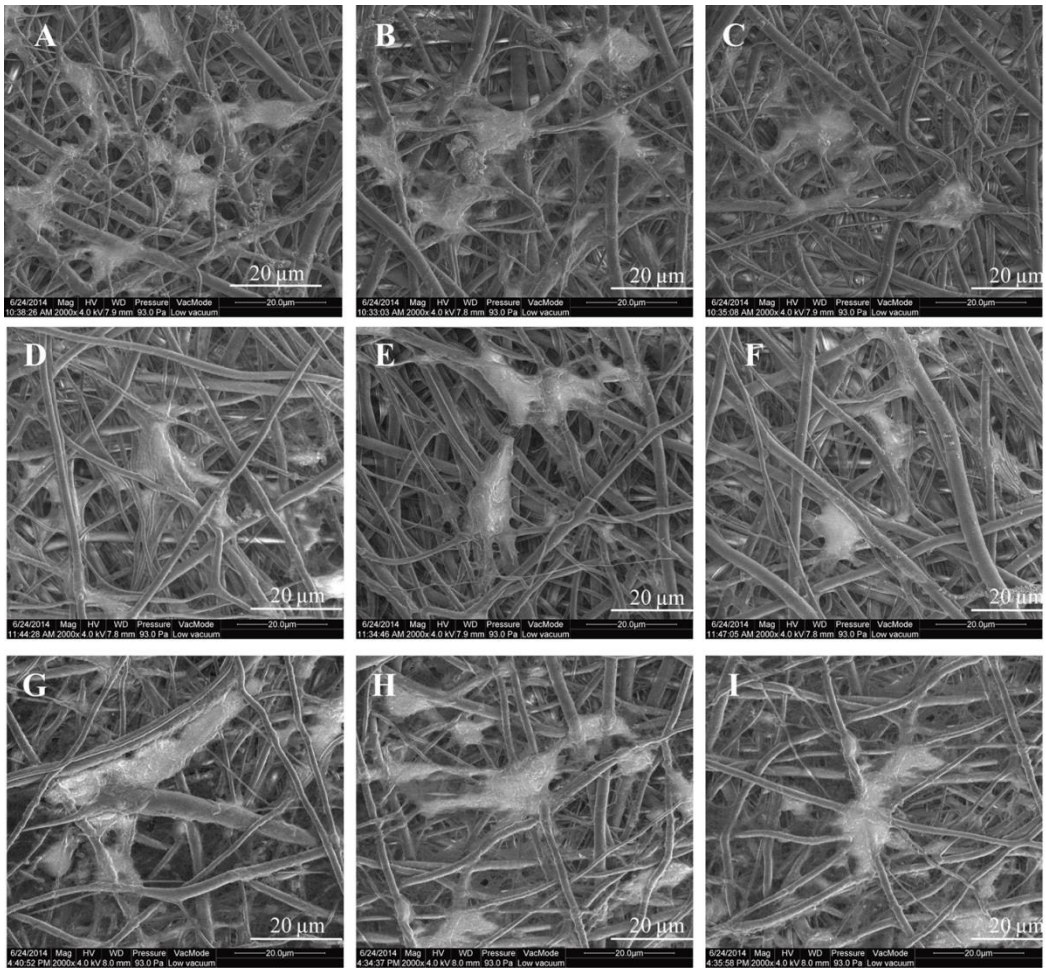

Figure 7, ESEM images of hMSCs on PCL fiber with or without PDA or PNE coating. hMSC cells on PCL fiber (A, B, C), PDA-PCL fiber (D, E, F) and PNE-PCL fiber (H, I, G).

Conclusions

Enhanced cell adhesion and proliferation are desired in tissue engineering. Surface modification with biofunctional molecules is a good way to make it possible. Mussel inspired surface coating was introduced into tissue engineering as a functional surface coating for promoted cell adhesion and proliferation. Dopamine has been extensively studied for cell proliferation on different substrate. However, norepinephrine, which shares a similar molecular structure with dopamine but form superior homogenous coating, was not as well studied as dopamine for cell adhesion and proliferation. Therefore, we here performed the study by comparing the biocompatibility and cell adhesion property of PDA and PNE on two kinds of stem cells, the human mesenchymal stem cells and human pluripotent stem cells derived mesenchymal stem cells. Our results suggest that both PDA and PNE successfully formed a surface coating on PCL fiber and dramatically increased the hydrophilicity as characterized by ESEM, XPS and contact angle measurement. PNE coating showed a much thinner and smoother surface in comparison with PDA coating. Biocompatibility analysis and cell proliferation results showed that PNE coating is more biocompatible to both hiPS-MSC and hMSC cells than PDA coating. PNE coating preferentially promoted hiPS-MSC cell proliferation, while PDA decreased cell proliferation of hiPS-MSC on PCL fiber. These results suggest that the effect of PDA and PNE surface coating on cell proliferation can be cell-dependent. The surface roughness of PDA coating can negatively affect the cell adhesion and proliferation. A careful investigation should be taken before using PDA or PNE on scaffold surface coating for stem cell therapy and tissue engineering. Further study is needed to illustrate the different mechanisms of PDA and PNE coating on different cells.

Acknowledgements

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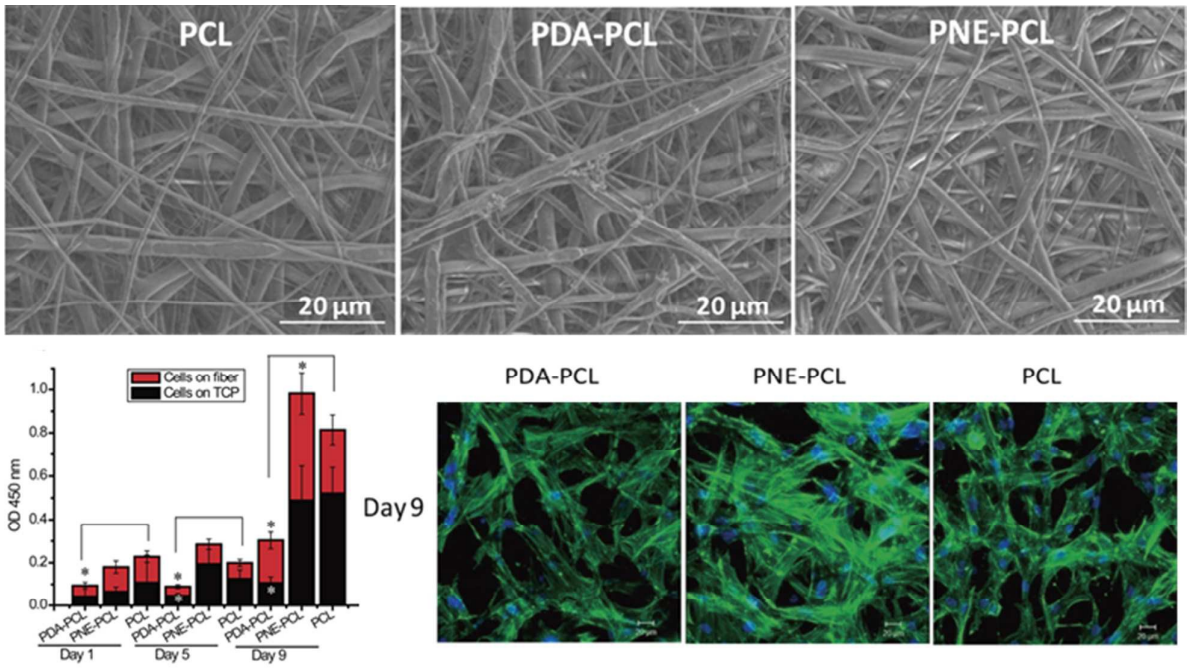

Biocompatibility and cell adhesion properties of mussel inspired surface coating, Polydopamine and polynorepinephrine, on PCL fiber for human mesenchymal stem cells and human induced pluripotent stem derived mesenchymal stem cells were investigated.