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Tailoring of the titanium surface by preparing cardiovascular endothelial extracellular matrix layer on the hyaluronic acid micro-pattern for improving biocompatibility

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ABSTRACT

It has been proved that high molecular weight hyaluronic acid (HMW-HA, 1×10^6 Da) micro-strips on titanium (Ti) surface can elongate the human vascular endothelial cell (EC) morphology, subsequently enhance endothelial extracellular matrix (ECM) deposition in our previous work. The HMW-HA micro-strips were anticipated to possess good hemocompatibility and EC compatibility simultaneously. However, the single HMW-HA micro-strips on Ti substrate showed bad hemocompatibility. To solve this problem, a method combining HA micro-pattern and EC decellularization was developed, and the endothelial extracellular matrix layer on the HA micro-pattern (ECM/HAP) showed excellent hemocompatibility and endothelial progenitor cells (EPCs) compatibility (cell number: $14.3 \pm 0.5 \times 10^5$ cells/cm^2 > $2.2 \pm 0.7 \times 10^5$ cells/cm^2 on ECM/TiOH, $7.5 \pm 1.3 \times 10^5$ cells/cm^2 on TiOH, $3.4 \pm 0.9 \times 10^5$ cells/cm^2 on TiOH/HAP and $3.6 \pm 1.2 \times 10^5$ cells/cm^2 on Ti). We also found that the ECM/HAP coating could significantly inhibit the excessive proliferation of smooth muscle cells (SMCs) (cck-8 absorption: $0.25 \pm 0.06 < 1.18 \pm 0.09$ A.U. on ECM/TiOH, 0.87 ± 0.15 A.U. on TiOH and 1.55 ± 0.11 A.U. on Ti) and the attachment of macrophages (cell number: $1.3 \pm 0.1 \times 10^3 < 9.2 \pm 1.5 \times 10^3$ cells/cm^2 on ECM/TiOH, $8.8 \pm 0.3 \times 10^3$ cells/cm^2 on TiOH, $7.3 \pm 0.7 \times 10^3$ cells/cm^2 on TiOH/HAP and $9.6 \pm 0.9 \times 10^3$ cells/cm^2 on Ti in 12 h). These data suggest that the multifunctional ECM/HAP coating can be used to build the bionic human endothelial ECM on the biomaterials surface, which might provide a potential and effective method for surface modification of cardiovascular devices.

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1. Introduction

Cardiovascular disease is the number one cause of death in the world currently [1,2]. Stents implantation, which is the main method to treat coronary artery diseases, may provoke a series of cellular and biochemical events that induce pathological processes [3,4]. The sequence of events is: inflammation and smooth muscle hyperplasia that may produce thrombosis due to further inflammation or rupture of the plaque. The cycle does repeat itself if the thrombotic event does not occlude the vessel [3,5–8]. This thickening of the vessel wall may due to mechanical mismatch at the anastomosis between the stents and natural blood vessel [9,10] and poor re-endothelialization on the stents [3]. A monolayer of endothelial cells (ECs) cannot only prevent thrombosis but also mediate the phenotype and proliferation of the SMCs [3]. Thus, the available and effective techniques of re-endothelialization on the vascular implant are very important for clinical treatment to prevent the thrombosis and ISR.

Immobilizing biomolecules on biomaterials surface is an hot spot which has attracted a lot of attention and research in recent years [11,12]. Most biomolecules are components of the ECs extracellular matrix [1,3], and immobilizing specific extracellular matrix components can promote endothelial adhesion to biomaterials. Nevertheless, these extracellular matrix components also promote SMCs hyperplasia [3,13]. Meanwhile, the inflammatory response is another aspect that is often neglected [14]. In the stents

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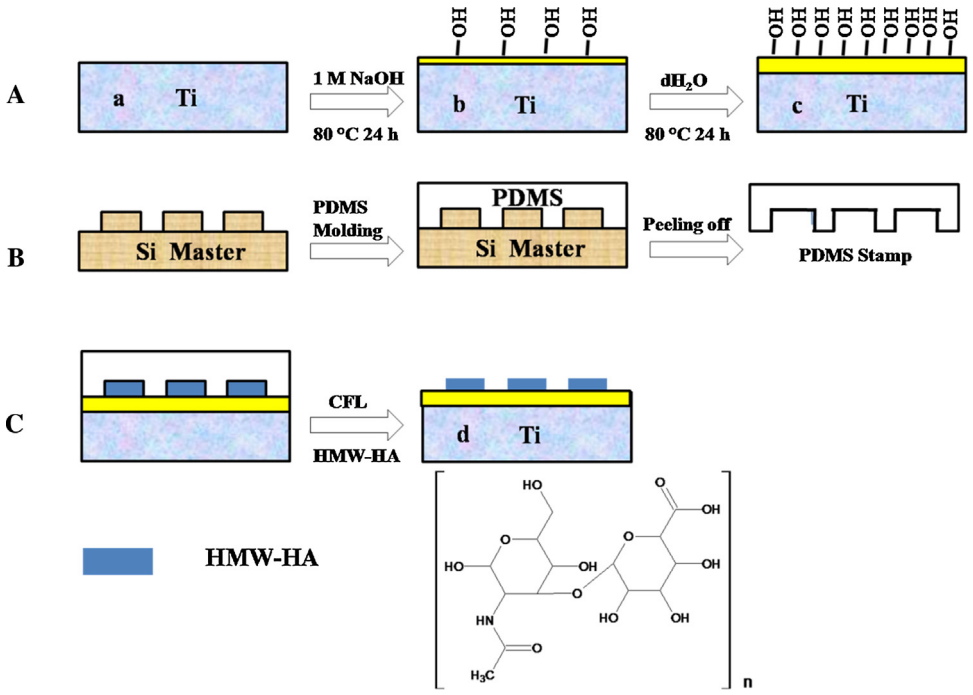

Fig. 1. The scheme of preparing HA micro-pattern on Ti surface. (A) Preparation of the functional layers of hydroxyl groups: (a) polished Ti substrate; (b) preparation of the functional layers of hydroxyl groups by 1 M NaOH at 80 °C for 24 h; (c) further enriching the hydroxyl groups by immersing b in the dH_2O at 80 °C for 24 h; (B) fabrication of the PDMS stamp from a silicon mold; (C) fabrication of the HA micro-pattern on the hydroxyl groups enriched Ti substrate by the PDMS stamp: (d) HA micro-patterned TiOH substrate.

implantation, response of SMCs and macrophages to vascular intimal damage and endothelial cell death play a great part in the EC dyfunctions, thrombosis and inflammation [14,15]. Therefore, it is also an urgent need to develop a multifunctional surface which possesses the functions of anticoagulation, promoting endothelialization, inhibiting SMCs hyperplasia and anti-inflammation simultaneously.

It is well known that the monolayer of endothelial cells from the body's own blood vessels is the best natural barrier and functional organization [16], and the autologous ECs are elongated by the blood flow shear stress (BFSS) and grow along the blood flow *in vivo* [17]. It has been generally accepted that simulating the biological microenvironment *in vivo* of the ECs on the biomaterials surfaces *in vitro* may be a effective method to build the autologous EC monolayer [18–21]. The micro-strips of biomolecules fabricated on biomaterials by soft lithography using a polydimethylsiloxane (PDMS) stamp can elongate the cells without the shear stress [20]. Several micro-patterns of biomolecules have been prepared to be applied for the biomaterials, including hyaluronic acid (HA) [20], phosphatidyl choline [22], collagen I [23] and the mixtures of fibronectin and heparin [24], and all these micro-strips can elongate the EC morphology. However, the extracellular matrix covered surface fabricated by the method of the elongated EC detachment and its biocompatibility have not been reported.

HA is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine linked by β(1, 4) and β(1, 3) glucosidic bonds [25]. This biomolecules have high viscosity and absorbent [25], thus can be easily fixed on a hydrophilic and rough surface by physical adsorption [20]. The presence of —COOH group leads to the weak acidity of HA, which can cause an acid–base reaction between the HA and metal surfaces, and the both can be easily combined with hydrogen bonds. In addition, HA is a component of the ECM, owning good cell compatibility and no cytotoxicity [26]. High molecular weight HA (HMW-HA, $\geq 1 \times 10^6$ Da, 5 mg/ml in PBS) has been widely used for the regulation of cell behavior [20,27]. Therefore, HA is chosen as an excellent material of biomolecules for preparing micro-patterns on biomaterials surface for the purpose of mimicking BFSS effect.

In this work, HMW-HA micro-patterns were fabricated on the titanium (Ti) surface for the excellent biocompatibility [11] and mechanical performance [28]. The ECM of elongated ECs was prepared on the HMW-HA micro-patterned titanium (Ti) surface by the method of cell detachment. This bionic surface was demonstrated not only having good blood compatibility and EC compatibility, but also obtaining excellent ability in inhibiting the proliferation of SMCs and adhesion of macrophages. We expect this bionic ECM surface will provide potential application for blood contact implanted devices.

2. Materials and methods

2.1. Materials and reagents

Ti substrates (diameter = 10 mm) were prepared using 99.5% pure Ti foils (Baoji, China). Ti substrates were polished and ultrasonically cleaned four times successively in acetone, ethanol and deionized water (dH_2O) for 5 min each and then dried at room temperature. HMW-HA (Sangon Biological Engineering Co. Ltd., China) was diluted to a concentration of 5 mg/ml in dH_2O. Polydimethylsiloxane (PDMS) prepolymer and catalysts (Sylgard 184) were obtained from Dow Corning (Midland, MI, USA). Medium 199 (M199), medium DMEM-F12 (F12), fetal calf serum (FBS), trypsin and type II collagenase were supplied by Gibco BRL. Cell counting kit-8 (CCK-8), mouse anti human SM-myosin antibody (bs-2178R), FITC conjugated rabbit anti-human type IV collagen antibody (bs-0806R-FITC) and TMB (3,3′,5,5′-etetramethylbenzidene) for the immunochemistry tests were provided by BD Biosciences (China). The actin staining reagent kit (SABC-FITC, SA2004), 4,6-diamino-2-phenyl indole (DAPI), horseradish peroxidase (HRP) conjugated goat antimouse IgG antibody (BA1050) and mouse anti human α-SMA antibody (BM0002) were stocked by Boshide (China). Mouse monoclonal antihuman Collagen IV antibody (ab6311),

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