Endothelial cell functions in vitro cultured on poly(l-lactic acid) membranes modified with different methods

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Abstract: We recently developed several methods to enhance the cell-polymer interactions. Optimal conditions for each method have been revealed separately by in vitro cell culture. As a practical consideration for construction of tissue-engineered organs, it is necessary to consider which is the most suitable and convenient in clinical applications. To compare the efficiency of these methods with respect to cell functions, poly-l-lactic acid (PLLA) was selected as matrix being modified by 1) aminolysis (PLLA-NH₂), 2) collagen immobilization with GA (PLLA-GA-Col), 3) chondroitin sulfate (CS)/collagen layer-by-layer (LBL) assembly (PLLA-CS/Col), 4) photo-induced grafting copolymerization of hydrophilic methacrylic acid (MAA) (PLLA-g-PMAA), and 5) further immobilization of collagen with 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDAC) (PLLA-g-PMAA-Col). The surface wettability of the modified PLLA was determined by water contact angle measurements. The cell response to the modified PLLA was quantitatively assessed and compared by using human umbilical endothelial cells (HUVECs) culture. Our results indicate that all the modifications can improve the cytocompatibility of PLLA (e.g., cells can attach with spreading morphology, proliferate and secret vWF and 6-keto-PGF₁α). All the collagen-modified PLLA showed more positive cell response than those purely aminolyzed or PMAA grafted. Among all the methods, collagen immobilization by LBL assembly or GA bridging after aminolysis is more acceptable for the convenience and applicability to scaffolds. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 69A: 436 – 443, 2004

Key words: poly(l-lactic acid); surface modifications; endothelial cells; cell culture

INTRODUCTION

Biodegradable polyesters such as poly(lactic acid) (PLA), polyglycol acid (PGA), poly(lactide-co-glycolide) (PLGA), and polycaprolactone (PCL) have been the most attractive biomaterials being used as scaffolds in tissue engineering. They have already been used for clinical purposes because they degrade to natural metabolites and may be tailored to particular applications by manipulation of shape, porosity, and degradation rate, and so forth. However, their hydrophobicity and poor cytocompatibility leads to the inefficiency of the scaffolds in constructing a friendly interface with living cells. It is the surface of a biomaterial that first comes into contact with living body. Hence, the initial response of cells to the biomaterial mostly depends on the surface properties. Therefore, surface modification of polyesters to improve their cytocompatibility is necessary. Many studies have been devoted to the surface modification of poly(lactic acid) (PLLA) toward improving its cytocompatibility, such as grafting polymerization, ozone oxidation, plasma modification, entrapment, and coating of natural polymers etc.1–6

As illustrated in Scheme 1, we have recently developed several methods to modify polymer surfaces aiming at enhancement of the cell-polymer interactions. All these methods can be applied onto polyester membranes, and some of them onto three-dimensional (3D) scaffolds. Reaction between ester groups of polyesters (exemplified here as PLLA) and amino groups of hexane diamine produces free amino groups on the PLLA surfaces through formation of amide bonds (PLLA-NH₂). By further reaction with glutaraldehyde, the amino groups were derivatized into aldehyde, by which macromolecules such as collagen are covalently attached (PLLA-GA-Col).7,8 The introduction of the amino groups also provides the feasibility to modify PLLA surface in a simpler manner [e.g., layer-by-layer (LBL) assembly technique] because the aminolyzed PLLA can be used as a polycationic substrate. LBL assembly of poly(styrene sulfonate, sodium

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salt) (PSS)/chitosan and chondroitin sulfate (CS)/collagen (type I) have been performed on PLLA-NH \(_2\) surfaces (for the latter pairs, naming PLLA-CS/Col).\(^9,10\) Modification of PLLA membranes or scaffolds has also been conducted through photooxidation and grafting copolymerization of methacrylic acid (MAA) initiated under UV light (PLLA-g-PMAA).\(^11,12\) By using the introduced carboxylic groups, the peptide bonds can be formed between PLLA-g-PMAA and collagen under the catalyzation of 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDAC), resulting in covalent immobilization of collagen (PLLA-g-PMAA-Col). The optimal conditions for each method with respect to cell functions have been elucidated by \textit{in vitro} cell culture of human endothelial cells or chondrocytes. These methods are applicable to modifying polyester scaffolds for different applications. However, for a specific application, such as endothelium formation on PLLA surfaces, one may need only the most effective and convenient way. Therefore, it is worth comparing the efficiency of these methods on improving cell-material interactions. Exemplified by poly-L-lactic acid (PLLA), herein the above-mentioned methods were used to modify the hydrophobic polyester, which initially causes poor endothelial cell compatibility. To avoid other influences, such as surface morphology of the substrata, cell lines, and culture conditions, and so forth, the same PLLA membranes and cell suspensions were used and the cells/materials were cultured simultaneously. In each method, the optimal conditions were used according to previous results.\(^7,9,10\) Cell response to the substrata was quantitatively assessed with respect to cell attachment, proliferation, and MTT viability as well as cell secretions such as von Willebrand factor (vWF) and 6-keto-prostaglandin \(F\)\(_{1\alpha}\) (a metabolite of prostacyclin).

### MATERIALS AND METHODS

#### Preparation of PLLA membrane

The PLLA (\(M_n = 400,000; M_w = 800,000\))/1,4-dioxane solution with a concentration of 0.03g/mL was cast onto a stainless steel plate, evaporated the solvent at 35°C for 24 h and further dried under reduced pressure for another 24 h at 30°C to yield translucent PLLA membranes with a thickness of \(\sim 100 \mu m\). The membranes were immersed in alcohol/water (1/1, v/v) solution for 2–3 h to remove oily dirt, washed with large amount of deionized water, and dried under reduced pressure at 30°C to constant weight.

#### Aminolysis

To prepare NH\(_2\)-enriched PLLA (PLLA-NH\(_2\)), the PLLA membranes were immersed in distilled 1,6-hexanediame/p/316\(_2\)/propanol solution with a concentration of 0.06 g/mL for 3 min at 58°C. The membranes were rinsed with deionized water for 24 h at room temperature to remove free 1,6-hexanediame and dried under reduced pressure at 30°C to constant weight.

#### Covalent immobilization of collagen

PLLA-NH\(_2\) was immersed in 1.0 wt % glutaraldehyde (GA) solution for 3 h at room temperature, followed by rinsing with large amount of deionized water to transfer the amino to aldehyde groups. The GA-treated membranes were then incubated in 1 mg/mL collagen (type I; Sigma) solution (pH = 3.5) for 24 h at 2–4°C. The membranes immobilized with collagen (PLLA-GA-Col) were rinsed with 1.0% acetic acid solution and then rinsed with deionized water for 24 h to remove physically adsorbed collagen.

#### Layer-by-layer assembly on PLLA-NH\(_2\)

Chondroitin sulfate A (CS; Sigma) and collagen (type I; Sigma) were used as building blocks to modify the aminolyzed PLLA by LBL technique. The PLLA-NH\(_2\) was firstly treated in 0.012 M HCl solution for 15 min at room temperature to cationize the substrata. The membrane was subsequently incubated in CS solution (1.0 mg/mL; 0.5 M NaCl) for 20 min to adsorb a layer of CS and then rinsed with water containing 0.5 M NaCl for three times. In the next step, positively charged collagen was assembled by incubation in the CS-enriched PLLA membrane in collagen solution (1.0 mg/mL; 0.1 M Hac; 0.2 M NaCl) for 20 min, followed by rinsing with 0.1 M acetic solution (containing 0.2 M NaCl) at first and then rinsing with water. In this study, only one pair of CS/Col was assembled.
Photooxidation and grafting copolymerization

To modify the PLLA membranes by grafting copolymerization of hydrophilic methacrylic acid (MAA), the membranes were firstly put in a quartz tube containing excessive hydrogen peroxide solution (30%). Under rotation by a motor, the tube was exposed to UV light generated from 250 W high-pressure mercury lamp for 2 h at 25 °C. The photooxidized membranes were rinsed with water thoroughly to eliminate the free hydrogen peroxide and then were placed in a copolymerization tube containing 5% MAA aqueous solution. The oxygen in the tube was replaced by nitrogen. Grafting copolymerization was conducted under UV irradiation at a distance of 12.5 cm from the mercury lamp for 30 min. The grafting membranes (PLLA-g-PMAA) were rinsed with a large amount of water for at least 24 h at 37 °C to remove the adsorbed homopolymers.

Immobilization of collagen on PLLA-g-PMAA

To produce PLLA-g-PMAA-Col, the PMAA grafted membranes were further immersed in 10 mL of 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDAC; Aldrich) aqueous solution (10 mg/mL) for 24 h at 2–4 °C, followed by rinsing with a large amount of water. The membranes were then incubated in collagen solution (2 mg/mL in 0.6% acetic acid) for 24 h at 2–4 °C. After being rinsed with 0.6% acetic acid solution at first and then with water for at least 24 h to remove physically adsorbed collagen, the membranes were dried under vacuum at 30 °C to obtain collagen-immobilized PLLA. It is worth noting that EDAC is a reagent that can promote the condensation between —COOH and —NH₂ to form amide bonds.

Wettability

The wettability of the control and the modified PLLA membranes was detected by measuring the water contact angles (Sessile drop) on a DSA10-MK2 contact angle measuring system from Krüss. All PLLA samples were dried under vacuum at 30 °C for 24 h. All the measurements were processed at room temperature at one time.

Human umbilical endothelial cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from the human umbilical cord veins with 1.0 mg/mL collagenase (type I; Sigma)/phosphate buffer solution (PBS; pH = 7.4) for 20–25 min at room temperature. The isolated HUVECs were incubated in a culture medium consisting of 20% (v/v) fetal bovine serum (FBS; Sijiqin Biotech. Co., China) and 80% RPMI1640 (Gibcobrl Co.) supplemented with 100 U/mL of penicillin and 100 μg/mL of streptomycin in a humidified air containing 5% CO₂ at 37°C. After incubation for 24 h, the culture medium was removed and then changed every 2 days. The HUVECs were fixed with 2.5% GA for 30 min for observation of cell morphology under scanning electron microscope (SEM) after being cultured for 2 days. The cell attachment and proliferation ratio were averaged from four parallel measurements at 12 h and 4 days, respectively, by trypsinization of the HUVECs and counting the cell number under a hemocytometer. The cell attachment and proliferation ratio are defined as N₁/N₀ × 100% and (N₂–N₁)/N₀ × 100%, respectively, where N₀, N₁, and N₂ represent the cell number per well of seeding at 12 h and 4 days, respectively.

The cell viability was measured by using the methylthiazolyldiazotetrazolium (MTT) method. Tetrazolium salts can be used to assess the activity of various dehydrogenase enzymes. MTT is a water-soluble tetrazolium salt yielding a yellowish solution when dissolved in PBS solution. Dissolved MTT can be converted into an insoluble purple formazan product by cleavage of the tetrazolium ring by dehydrogenase enzymes in mitochondria. Only viable cells can cause this conversion. This water-insoluble formazan can be dissolved by dimethylsulfoxide (DMSO) and can be quantitatively measured by absorbance spectroscopy. In the experimental assay, the MTT solution was prepared by dissolving 50 mg of MTT in 10 mL of PBS and filtered to sterilize and remove small amounts of insoluble residues, 20 μL of which was then added to each well in a 96-well TCPS plate. The samples were incubated at 37°C for 4 h. After incubation, the supernatants were removed and 100 μL of DMSO were added. The samples were shaken gently until the purple formazan crystals were thoroughly dissolved. The absorbance of the sample was recorded at 492 nm. Four parallel measurements were averaged for each sample.

Measurement of released vWF and 6-keto-PGF₁α in ELISA

Secretion of vWF and 6-keto-prostaglandin F₁α (6-keto-PGF₁α, a metabolite of prostacyclin) was measured in the supernatant of the cultured HUVECs by enzyme-linked immunosorbent assay (ELISA) kits obtained from Chinese Sun Diagnostics Co. After culture for 4 days, the cells were washed with the culture medium without FBS, followed by 5 h for vWF and 10 h for 6-keto-PGF₁α incubation in the culture medium supplemented with 20% FBS, respectively. For vWF measurements, the supernatant (six parallel for every sample) was collected and centrifuged (10 min; 400 rpm) to remove cell debris. For 6-keto-PGF₁α measurements, the supernatant was centrifuged for 2 min with 10,000 rpm to remove fibrin. The vWF or 6-keto-PGF₁α in the supernatant was bounded by peroxidase-conjugated anti-vWF antibody or anti-6-keto-PGF₁α antibody, which was detected quantitatively by using orthophenylenediamine (OPD) as the substrate. Optical density was determined at a wavelength of 492 nm and compared with standard curves constructed from human plasma.
RESULTS

Surface wettability

The occurrence of surface modifications by these methods has been verified by X-ray photoelectron spectroscopy (XPS), attenuated total reflectance infrared spectroscopy (ATR-IR), ultraviolet-visible (UV-Vis) spectroscopy, fluorescence spectroscopy, scanning force microscopy, and so forth. The introduction of these hydrophilic \( \text{NH}_2 \), \(-\text{COOH}\) groups and collagen, the main component of extracellular matrix, shall alter the surface properties such as wettability and biorecognition with cells, which in turn modulate cell-material interactions. To follow the surface wettability alteration, water contact angles were measured and summarized in Table I. The data show that all the water contact angles were decreased after modifications compared with the control PLLA, meaning that the surface wettability was improved although the extent was different. Only a slight decrease of the contact angle was found for PLLA-NH\(_2\). This is reasonable because the introduced NH\(_2\) groups could not cover all the top surface of PLLA. After PMAA grafting, however, the contact angle decreased apparently, attributed to the nature of PMAA (i.e., one COOH group in each repeating unit). Lower contact angles were also found for both PLLA-GA-Col and PLLA-g-PMAA-Col, but higher for PLLA-CS/Col. This may be attributed to the difference of the collagen conformation (the dominance of the hydrophilic-hydrophobic parts of collagen molecules) or due to the collagen amount on the PLLA surface, or both. For example, a much thinner layer of collagen was obtained by LBL immobilization, resulting in partial exposure of the hydrophobic PLLA.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Water Contact Angle/Degree (sessile drop)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control PLLA</td>
<td>85.3 ± 0.1</td>
</tr>
<tr>
<td>PLLA-NH(_2)</td>
<td>81.7 ± 3.3</td>
</tr>
<tr>
<td>PLLA-GA-Col</td>
<td>71.8 ± 1.7</td>
</tr>
<tr>
<td>PLLA-CS/Col</td>
<td>80.3 ± 0.8</td>
</tr>
<tr>
<td>PLLA-g-PMAA</td>
<td>75.9 ± 3.0</td>
</tr>
<tr>
<td>PLLA-g-PMAA-Col</td>
<td>74.5 ± 1.5</td>
</tr>
</tbody>
</table>

Cell response to the modified PLLA

Figures 1–4 present the growth behavior, viability, and secretion ability of HUVECs cultured on PLLA membrane surfaces of PLLA-NH\(_2\), PLLA-GA-Col, PLLA-CS/Col, PLLA-g-PMAA, and PLLA-g-PMAA-Col. On the control PLLA, although cells could adhere [Fig. 1(a)] and retain some extent of MTT viability [Fig. 2(a)], the negative proliferation ratio [Fig. 1(a)] means that cell death occurred during the culture process, showing that HUVECs cannot grow and divide properly. Cell morphology observation under SEM confirmed no cell division or cell spreading after culture for 4 days [Fig. 3(a)]. On the contrary, cell clusters could be occasionally found [Figure 3(a1)]. All these results indicate that the control PLLA was unfavorable for endothelial cell growth.

All the modified PLLA improved endothelial cell compatibility. After aminolyzed with diamine, more cells were attached with a positive cell proliferation [Fig. 1(b) and improved MTT viability [Fig. 2(b)], although these values are still lower than those on other modified PLLA surfaces. A typical spreading and polygonal morphology for endothelial cells [Fig. 3(b,c)].
3(b)] were observed after culture for 4 days. However, the absolute cell number was not high enough to cover all the surfaces, leaving some bare areas not occupied by the cells [Fig. 3(b2)]. Nevertheless, this morphology generally indicates that the materials are favorable for cell growth, spreading, and differentiation. ELISA detection confirmed that endothelial cells cultured on PLLA-NH2 surface could secrete vWF and 6-keto-PGF1α, as those cultured on TCPS, although the absolute value was lower [Fig. 4(a)].

When the aminolyzed PLLA was further immobilized with collagen using GA as a coupling reagent (PLLA-GA-Col), the cell attachment behavior was significantly enhanced [Fig. 1(c)]. Figure 1(c) shows that the cell attachment ratio was even higher than that of TCPS. MTT viability assessment was consistent with the cell attachment [i.e., a highest value was recorded in all the specimens [Fig. 2(c)]. Although the cell proliferation rate was not the fastest after a 4-day culture, it was still higher than that of TCPS [Fig. 1(c)]. The cells spread very well and interacted strongly with the substrate through their pseudopods [Fig. 3(c1)]. The entire surface was covered by the cells to form a uniform and continuous endothelium monolayer [Fig. 3(c2)]. However, the ability to secrete vWF and 6-keto-PGF1α was poor compared with other samples [Fig. 4(b)]. When PLLA membrane was modified by LBL assembly of CS and collagen (PLLA-CS/Col), HUVECs displayed high attachment, proliferation, and viability properties [Figs. 1(d) and 2(d)]. SEM observations also found spreading endothelium morphology and high cell density [Fig. 3(d)]. It was remarkable that the secretion of both vWF and 6-keto-PGF1α was the highest among all the detected samples, including TCPS [Fig. 4(c)].

After grafting of PMAA (PLLA-g-PMAA), there were lower cell attachment, viability, and secretion of vWF and 6-keto-PGF1α [Figs. 1(e), 2(e), and 4(d)]. The attachment ratio was even lower than the control PLLA. However, the attached cells had a higher proliferation rate, resulting in spreading cell layers on the modified PLLA surface [Fig. 3(e)]. A further immobilization of collagen by EDAC (PLLA-g-PMAA-Col)
could effectively improve cell attachment, proliferation, viability, and vWF and 6-keto-PGF\(_{1\alpha}\) secretion [Figs. 1(f), 2(f), and 4(e)]. A large number of cells could also be observed under SEM [Fig. 3(f)].

In summary, the cell attachment can be apparently enhanced by these modifications, especially for those with collagen, because the outermost layers (PLLA-GA-Col, PLLA-CS/Col, and PLLA-g-PMAA-Col), except for PLLA-g-PMAA whose cell attachment was even poorer than the control PLLA. Regarding proliferation, the cells on PLLA-g-PMAA divided fastest, and other modified PLLA were also favorable for cell division similar to or better than TCPS. In addition, consistent alteration tendencies of MTT viability with cell attachment were shown (i.e., PLLA membranes with collagen as the outermost layer showed higher viability). Except for the PLLA-NH\(_2\), confluent cell lines such as endothelial cells, chondrocytes, and osteoblasts.4,10,12 Cells can adhere on PLLA surface, but viability may be preserved. These phenomena have been confirmed in our study. It has been proposed that cells communicate with the substratum through an adsorbed protein layer.29 This adsorbed protein layer plays a crucial role in regulating the subsequent cell attachment, proliferation, viability, and functionality. For a beneficial cell-substratum interaction, the cells mostly reorganize the stereo confirmation of the adsorbed proteins, so that proper specific recognition and binding can be formed between the cell membranes and the adsorbed proteins.29 Subsequently, the attached cells can move, proliferate, and spread. The strong hydrophobicity of a substrate will cause the denaturation or tight adhesion of the adsorbed proteins, leading to the difficulty or even inability of the cells to reorganize the proteins’ confirmation. As a result, the attached cells may be tightly anchored and cannot move, proliferate, spread, and finally die. After introducing hydrophilic groups such as —NH\(_2\) or —COOH as described here, the surface wettability of PLLA is enhanced (e.g., decrease of water contact angle). Moreover, these groups may also interact with proteins, glycoproteins, and proteoglycans both in the culture medium and the cell membranes through electrostatic force and hydrogen bonds. All these interactions are advantageous for accelerating cell response to the matrix, resulting in improved cytocompatibility (e.g., cells can adhere and proliferate properly) although they are not as better as on collagen-existed PLLA.

It should be noted that suitable wettability with regard to different substrate materials and cell lines has been found (e.g., a balance between the hydrophobicity and hydrophilicity is required for a specific cell line grown on a definite material, not too hydrophobic nor too hydrophilic).25,26,30 In the latter case, the over-existing hydrophilic groups may retard the adhesion of both proteins and cells by thermodynamic and dynamic mechanisms, which is often reported in hydrogel studies such as polyethylene oxide (PEO). This may cause the attachment difficulty of the cells, which is the first step for these anchorage-dependent cells to proliferate and spread. For PLLA-g-PMAA, the best compatibility for chondrocytes or endothelial cells was found when the —COOH density was ~3.5 \(\times\) 10\(^{-7}\) mol/cm\(^2\) on the surface,12 as used in the present study. The lower cell attachment ratio might be caused by the negative charged property of the PMAA (pKa = 4.2) chains in the culture conditions (pH = 7.4), which repulse the cell anchorage through charge interaction because the cell membranes are generally negatively charged.31 Hence, the surface wettability is only one of the controlling factors that influence cytocompatibility.

The above conclusion is further verified when one compares the overall cell behavior on collagen-existed PLLA surfaces with different immobilization methods. All these collagen-existed PLLA present pronounced enhancement of cell proliferation, viability, spreading, and functionalities, although different contact angles have been measured. In this case, the decisive factor is apparently the collagen, which is the main component in extracellular matrix. Collagen is one type of adhesion proteins that can accelerate cell attachment and proliferation through specific interactions between the domains such as RGD in collagen molecules and the integrin in cell membranes.32 Their specific bindings result in focal contact of cells on the substratum, which benefit cell attachment and movement. From our experimental results, we conclude that the specific interactions are more powerful to accelerate cell anchorage, proliferation, spreading, and secretion of vWF and 6-keto-PGF\(_{1\alpha}\). However, one can still find some differences between these immobilization methods with respect to cell viability and func-
tionalities, the most important aspects to assess cytocompatibility.

Normal vascular endothelium plays an important role in regulating blood vessel tone and platelet function by releasing vaso- and platelet-active substances. The biomaterials used in tissue-engineered vascular prostheses shall not only have good qualitative properties such as cell attachment ratio, proliferation ratio, viability, and spreading morphology but also contain endothelial function. The latter is usually more important because the differentiation should be preserved for cells cultured in vitro. In the present study, the endothelial functions were assessed through measurement of the secretion of vWF and 6-keto-PGF$_{1α}$. vWF is an adhesive glycoprotein synthesized exclusively in endothelial cells and megakaryocytes. Endothelial vWF is stored in rod-shaped organelles called Weibel-Palade bodies and accelerates the platelet adhesion. Prostacyclin (PGI$_2$), a prostanoid, is an inhibitor of platelet activation and a powerful vasodilator. Both affect platelet aggregation and disaggregation, blood coagulation, and circulation. 6-keto-PGF$_{1α}$ is a stable hydrolysis product of PGI$_2$. Therefore, the HUVECs functions can be revealed through the measurements of the vWF and the PGI$_2$-product secreted by the cells.

Taking into account the viability and secretion of vWF and 6-keto-PGF$_{1α}$, we find that the best substrata are PLLA-CS/Col and PLLA-g-PMAA-Col. The level of viability and secretion of cells on these materials is quite close to that on TCPS surface, showing that a normal cell function is preserved when cultured in vitro. This finding, as discussed above, is quite important for construction of tissue-engineered organs because only the differentiated cells can secrete these factors. It is worth noting that the chondroitin sulfate/collagen pairs are better for improvement of endothelial cell compatibility than the former polystyrene sulfate/chitosan pairs with the same layer numbers. The difference between these collagen-existed PLLA (i.e., PLLA-GA-Col, PLLA-CS/Col, and PLLA-g-PMAA-Col) might be caused by the different states of the collagen molecules, for instance, a higher degree of denaturation by GA bridging can be expected. However, it is not clear what the intrinsic mechanism is at present. Nevertheless, we have shown that biocompatible components can be successfully introduced onto the surfaces of tissue-engineering scaffolds, resulting in cytocompatible materials with preserved differentiation properties. These methods are not restricted to collagen and can be extended to other biomacromolecules such as cell growth factors, cell morphology proteins, polysaccharides, and so forth.

Considering the applicability and convenience of the above methods, collagen immobilization by LBL assembly or GA bridging after aminolysis maybe more acceptable, because they are suitable for both plane membrane and scaffolds with irregular shape and inner structure, where traditional methods are generally unavailable. Moreover, the modification process is rather easy to control and a timesaver. Hence, these two strategies are more practical and applicable for polyester scaffold modifications.

CONCLUSIONS

With respect to endothelial cell attachment, proliferation, viability, morphology, and secretion of vWF and 6-keto-PGF$_{1α}$, herein we have compared the efficiency of five modification methods for PLLA: 1) aminolysis (PLLA-NH$_2$), 2) collagen immobilization with GA (PLLA-GA-Col), 3) chondroitin sulfate (CS)/collagen layer-by-layer (LBL) assembly (PLLA-CS/Col), 4) photo-induced grafting copolymerization of hydrophilic methacrylic acid (MAA) (PLLA-g-PMAA), and 5) further immobilization of collagen with EDAC (PLLA-g-PMAA-Col). In general, all the modifications can apparently improve cell response to the substrate PLLA, resulting in an improved cytocompatibility. Taking into account the viability and secretion of vWF and 6-keto-PGF$_{1α}$, the most important aspects for assessment of endothelial cell functions, the best modification methods are PLLA-CS/Col and PLLA-g-PMAA-Col. Considering the applicability and convenience of the above methods, collagen immobilization by LBL assembly or GA bridging after aminolysis is more acceptable. These two methods are suitable for both plane membrane and scaffolds with irregular shape and inner structure, where traditional methods are generally unavailable. These methods are not restricted to collagen and can be extended to other biomacromolecules such as cell growth factors, cell morphology generation proteins, polysaccharides, and so forth.

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