Published in final edited form as:

J Biomed Mater Res A. 2014 May ; 102(5): 1408–1414. doi:10.1002/jbm.a.34802.

Plasma-assisted heparin conjugation of electrospun poly(L-lactide) fibrous scaffolds

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Abstract

Heparin conjugation of poly(L-lactide) fibrous scaffolds fabricated by electrospinning was accomplished by surface functionalization with amine ($-NH_2$) groups using a sequential treatment with Ar- NH_3 and H_2 plasmas. The density of the incorporated $-NH_2$ group was determined by combining a chemical derivatization method with X-ray photoelectron spectroscopy. The time of Ar- NH_3 plasma treatment significantly affected the N/C, $-NH_2/N$, and $-NH_2/C$ fractions, whereas the plasma power, Ar- NH_3 gas composition, and time of H_2 plasma treatment only influenced the $-NH_2/N$ and $-NH_2/C$ fractions. Scaffold surface functionalization by $-NH_2$ groups significantly increased the amount of covalently bonded heparin compared to a hydrolysis method. The function of immobilized heparin was confirmed by the decrease of platelet attachment during the exposure of the scaffolds to blood from Sprague-Dawley rats. *In vitro* experiments with bovine aorta endothelial cells demonstrated that heparin conjugation enhanced cell infiltration through the fibrous scaffolds, regardless of the amount of covalently immobilized heparin.

Keywords

amine groups; conjugation; endothelial cells; heparin; infiltration; platelets; plasma treatment; surface functionalization

INTRODUCTION

Polymer scaffolds with fibrous structures can mimic the structure of the external cell matrix, which makes them ideal for cell culture and tissue engineering. Additionally, fibrous scaffolds are good candidates for drug delivery¹ because of their high surface-to-volume ratio. Although polymer fibrous structures have been extensively studied for different applications, a surface treatment is usually required to enhance the hydrophilicity, biocompatibility, and surface density of functional groups for further biomolecule immobilization. Despite the extensive use of electrospun polymers consisting of various biomolecules (e.g., collagen, heparin, and laminin)^{2–4} and biomolecule physisorption onto surface-modified fibrous polymers,⁵ covalently immobilized biomolecules are critical for long-term functionality. For example, stable grafting of heparin molecules onto the inner

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wall surface of polymer vascular grafts is crucial to the prevention of thrombosis and the maintenance of normal blood circulation until the regeneration of new vascular tissue.

The effectiveness of surface functionalization strongly depends on the density of the covalently immobilized biomolecules. A traditional approach to enhance the density of functional groups on polymer surfaces is by a wet chemical treatment process. For example, to immobilize various bioactive molecules, NaOH solution hydrolysis or aminolysis (a chemical reaction in which a molecule is split into two parts by reacting with a molecule of NH_3 , for example) can be used to increase the surface density of $-\text{COOH}$ and $-\text{NH}_2$ groups, respectively.⁶⁻⁸ Grafting of an acrylic acid by exposure to ultraviolet (UV) light is another common method for increasing the $-\text{COOH}$ surface density.⁹ In addition to the former methods, plasma treatment or plasma polymerization have also been used to functionalize biomaterial surfaces for biomolecule immobilization.^{10,11} Optimization of the process conditions of these plasma methods is more straightforward than those of wet chemical methods and is also an environmentally friendly sterile process that can induce surface modification without affecting the bulk structure and properties.

Plasma-assisted biomolecule immobilization via the formation of desirable surface functionalities (e.g., $-\text{COOH}$ and $-\text{NH}_2$ surface groups) has been the objective of several previous studies. For instance, plasma polymerization of allylamine¹²⁻¹⁴ and acrylic acid¹⁵⁻¹⁷ has been found to introduce a high density of $-\text{NH}_2$ and $-\text{COOH}$ surface groups, respectively. The most common treatment for functionalizing biopolymer surfaces with $-\text{NH}_2$ surface groups is NH_3 plasma treatment.¹⁸

Despite valuable insight into surface functionalization of biopolymers obtained from the previously mentioned studies, knowledge of plasma-assisted surface functionalization for biomolecule immobilization on porous fibrous structures is sparse. Although plasma polymerization is an effective method for coating various substrates by thin films of tailored physicochemical properties, most of the coating precursors are usually highly toxic and/or cannot be directly integrated into standard plasma processing systems. Therefore, the objective of this study was to examine the feasibility of a much less toxic gas such as NH_3 , which can easily be applied with most plasma systems, to impart biopolymer surfaces with the appropriate surface functionalities for biomolecule immobilization. Specifically, a two-step plasma treatment process involving a treatment with a mixture of Ar and NH_3 gas plasma followed by another treatment with H_2 plasma was used to surface functionalize electrospun fibrous scaffolds consisting of poly(L-lactide) (PLLA) with $-\text{NH}_2$ groups in order to increase the density of surface conjugation sites for heparin immobilization. The treatment process was optimized by varying the plasma power, treatment time, gas composition, and post-treatment conditions. Results from X-ray photoelectron spectroscopy (XPS), heparin conjugation measurements, and quantification of platelet attachment on heparin-conjugated PLLA scaffolds illustrate the potential of the present plasma process. *In vitro* experiments with bovine aorta endothelial cells (BAECs) reveal enhanced cell infiltration through the heparin-conjugated fibrous scaffolds.

MATERIALS AND METHODS

Scaffold fabrication

Fibrous scaffolds of biodegradable PLLA with an inherent viscosity of 1.09 dL/g (Lactel Absorbable Polymers, Pelham, AL) were fabricated by electrospinning as described previously.¹⁹ Briefly, after dissolving PLLA pellets in an ultrasonic bath with an aqueous solution of 19% w/v hexafluoroisopropanol, the solution was delivered by a programmable pump to a needle under a high voltage of 12 kV, and the electrostatically charged polymer fibers were ejected onto a grounded collector, resulting in the formation of a nonwoven fibrous scaffold. The fiber alignment and the scaffold thickness were controlled by adjusting the rotational speed of the collector and the deposition time, respectively. A low rotational speed of 150 rpm produced randomly oriented fibers. Scaffolds of thickness ~100 μm were used for surface chemistry characterization and platelet attachment, whereas scaffolds of thickness ~250 μm were used for heparin conjugation, toluidine blue detection, and *in vitro* cell infiltration. Scaffold thickness measurements were obtained with a micrometer gauge (Mitutoyo America, Aurora, IL).

Plasma-assisted surface modification

Scaffolds were surface treated in a radio-frequency capacitively coupled plasma reactor (Plasmalab 80plus, Oxford Instruments, UK) with a plate diameter of 20 cm and plate-to-plate distance fixed at 2 cm. Before each treatment, the chamber was cleaned with Ar plasma (300 W power, 100 sccm Ar gas flow rate, 0.9 Torr pressure) for 5 min. Control scaffold samples were only exposed to Ar plasma (30 W power, 100 sccm Ar gas flow rate, 0.5 Torr pressure, 2 min treatment time). Plasma-treated samples were produced by a two-step treatment process comprising a plasma mixture of Ar (10–50 vol%) and NH_3 (50–200 W power, 0.5 Torr pressure, 100 sccm gas flow rate, 2–10 min treatment time) and a subsequent H_2 plasma (10 W power, 50 sccm H_2 gas flow rate, 0.5 Torr pressure, 10–60 s treatment time) to increase the surface density of the primary $-\text{NH}_2$ groups onto the scaffold surfaces. Hereafter, this surface treatment will be denoted as the Ar- NH_3/H_2 plasma treatment.

Surface chemical analysis

The surface chemical characteristics of the plasma-treated scaffolds were examined with an XPS system (Perkin-Elmer PHI 5400 ESCA) without charge neutralization or monochromator, equipped with an Al-K α X-ray source of photon energy equal to 1486.6 eV. All XPS spectra were obtained with a take-off angle of 54.7° relative to the analyzer axis. During spectral acquisition, the pressure in the main chamber was maintained at $\sim 10^{-7}$ Torr. Survey spectra were acquired in the binding energy range of 0–1100 eV with pass energy of 178.95 eV. XPS results were calculated from at least three measurements derived from different areas of the scaffold surfaces. The presence of $-\text{NH}_2$ groups onto the surfaces of the PLLA scaffolds was detected by a chemical derivatization method.¹⁸ Briefly, before XPS analysis, both untreated and plasma-treated scaffold samples were exposed to trifluoromethyl benzaldehyde (TFBA) vapor (Fisher Scientific, Pittsburgh, PA) for 45 min and, subsequently, degassed with a mechanical pump at ~ 2 Torr for 1 h. Figure 1 shows a schematic of the chemical derivatization method and the formula used to determine the NH_2/C fraction.

Heparin conjugation

Heparin conjugation of the scaffold surfaces was accomplished with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) (Pierce Biotechnology, Rockford, IL). The heparin solution was prepared by dissolving first 20 mg/mL of EDC and 10 mg/mL of sulfo-NHS into 0.5 M 2-(N-morpholino)ethanesulfonic acid (pH = 5.5) and then adding 20 mg/mL of heparin. After incubating for 45 min in a shaker, the solution was first neutralized with 1 N NaOH (110 μ L of 1N NaOH per 1 mL of EDC/sulfo-NHS/heparin solution) and then incubated in a shaker together with the scaffold samples for 2 h to achieve full conjugation. Samples were also prepared by hydrolyzing PLLA scaffolds in 0.01 N NaOH for 10 min (to increase the surface density of the –COOH groups) and then conjugating with di-amino-poly(ethylene glycol) using the aforementioned EDC/sulfo-NHS method. Hereafter, these samples will be referred to as the PEG samples.

The presence of heparin on the scaffold surfaces was detected by toluidine blue staining. First, the scaffold samples with and without heparin conjugation were placed in 2 mL of 0.0005% (w/v) toluidine blue solution and vortexed for 10 min. Standard heparin solutions ranging from 0 to 250 μ g/mL were also prepared in 0.0005% (w/v) toluidine blue solution. After vortexing, 3 mL of n-hexane was added to all samples and standard heparin solutions to extract the remaining unbound toluidine blue. To determine the concentration of remaining toluidine blue, the absorbance was measured with a 631-nm spectrophotometer (BioRad, Model 550). The concentration of immobilized heparin on each scaffold was thus determined by comparing the absorbance values of unbound toluidine blue to those obtained from the standard heparin solutions.

Platelet attachment in vitro

To examine the effect of heparin conjugation on the platelet attachment, whole blood from Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) was used in *in vitro* platelet activation tests. Scaffold samples with and without heparin conjugation were incubated with rat whole blood at 37 °C for 2 h and then washed three times with phosphate buffered saline (PBS) fixed with 2% glutaraldehyde overnight. Subsequently, the samples with attached platelets were gradually dehydrated with ethanol of concentration 50% (5 min), 70% (5min), 80% (5 min), 90% (10 min), 100% (10 min), and 100% (10 min), and, finally, dried under a hood overnight. The density of the attached platelets was determined with a field-emission scanning electron microscope (SEM) (TM-1000, Hitachi, Pleasanton, CA) operated at 15 kv. Three sets of each group of samples were examined to ensure the reproducibility of the observed trend.

Cell attachment and infiltration in vitro

To examine the effect of heparin conjugation on cell infiltration, BAECs were seeded on both untreated and plasma-treated scaffold samples with or without heparin conjugation. Untreated (control) samples were sterilized in 70% ethanol under UV light for 30 min and, subsequently, washed five times with sterile PBS. Then, three samples from each group were attached to non-tissue-culture-treated polystyrene dishes using sterile double-sided tape. Cells were seeded at 100% confluency onto scaffold samples with a culture medium

consisting of Dulbecco's Modified Eagle's Medium, 10% fetal bovine serum, and 1% penicillin streptomycin, and kept in a humidified incubator (37 °C, 5% CO₂) for 5 days to allow cell infiltration into the scaffolds. Sufficient medium was used in each dish in order to avoid the need to change the medium. After incubation for 5 days, the samples were fixed, stained with 4',6-diamidino-2-phenylindole (DAPI), and placed in optimal cutting temperature compound (TissueTek, Elkhart, IN) on dry ice. Cross-sections of 20 µm thickness were obtained with a cryosectioner at -20 °C. Fluorescent signals from the cells within these cryosections were viewed with an upright fluorescence microscope (Zeiss HAL 100, Carl Zeiss MicroImaging, Thornwood, NY). Cell number was counted based on the DAPI staining of nuclei. At least 30 cryosections of each group were examined to confirm the consistency of the results among different cryosections.

Statistical analysis

Results are presented in the form of mean and standard deviation (error bar) data, calculated under the assumption that the data followed normal distributions. All statistical data were calculated from three repeated measurements. Student t-test was used to determine statistical differences in results obtained with different treatment.

RESULTS AND DISCUSSION

Surface chemistry of plasma-treated scaffolds

Experimental results from a parametric study of the effect of controlling plasma parameters on the surface chemical modification of the scaffold surfaces are presented in this section. Only one of the following parameters was varied in each experiment: (a) Ar-NH₃ plasma treatment (power = 50–200 W, treatment time = 2–10 min, Ar gas = 10–50 vol%) and (b) H₂ plasma post-treatment (treatment time = 10–60 s). The effects of the resulting plasma conditions on the surface chemical characteristics of the PLLA fibrous scaffolds were examined in the context of results of the N/C, NH₂/N, and NH₂/C fractions obtained with the XPS and chemical derivatization techniques. These fractions can be interpreted as indicators of the incorporation of N into the surface (N/C), the selective incorporation of -NH₂ groups over other N-containing chemical groups (NH₂/N), and the overall effectiveness of surface functionalization by -NH₂ groups (NH₂/C).

Figure 2 shows the effect of the Ar-NH₃ plasma power and treatment time on the surface chemistry of the fibrous scaffolds. Although a higher plasma power produced a higher N/C fraction [Fig. 2(a)], it reduced the fraction of -NH₂ groups at the surface [Figs. 2(b–c)]. This trend may be attributed to the increased fragmentation of the NH₃ molecules under the relatively high-power plasma conditions. Therefore, a relatively low-power (i.e., 50 W) Ar-NH₃ plasma treatment yields a higher density of -NH₂ surface functionalities. The effect of the Ar-NH₃ plasma treatment time on the N/C fraction was secondary [Fig. 2(d)]. However, the increase of the treatment time decreased the NH₂/N fraction [Fig. 2(e)], while it increased the NH₂/C fraction [Fig. 2(f)]. In view of the trends shown in Fig. 2(d–f), a relatively short treatment (i.e., 2 min) with the Ar-NH₃ plasma is preferred to minimize possible structural changes and/or damage in the scaffold material.

Figure 3 shows the effect of the Ar-NH₃ plasma gas composition (left column) and the post-treatment time with H₂ plasma (right column) on the scaffold surface chemistry. Although the variation of the gas composition did not affect the N/C fraction [Fig. 3(a)], the increase of the Ar content to 50 vol% decreased the NH₂/N fraction [Fig. 3(b)] and, in turn, the NH₂/C fraction [Fig. 3(c)]. This trend may be attributed to the intensification of the Ar⁺/scaffold interactions and the more pronounced fragmentation of the NH₃ molecules by energetic Ar⁺ ions. Similar to the Ar-NH₃ gas composition, although the effect of the post-treatment time with H₂ plasma on the N/C fraction was insignificant [Fig. 3(d)], it significantly affected the formation of -NH₂ surface groups. In fact, the NH₂/N and NH₂/C fractions reached their peak values for a post-treatment time of 30 s [Figs. 3(e-f)]. The data shown in Fig. 3 indicate that the H₂ plasma post-treatment effectively converted other N-containing chemical groups into -NH₂ groups; however, the effect was saturated after 30 s under the given H₂ plasma conditions. Although discernible damage or structural changes of the fibrous scaffold surfaces were not observed even with high-resolution SEM, the results shown in Figs. 2 and 3 indicate that for the range of plasma conditions examined in this study, a high density of -NH₂ surface functionalities is achieved after a 5 min Ar-NH₃ plasma treatment at a power of 50 W with 30 vol% Ar, followed by a 30 s H₂ plasma post-treatment. Thus, the results for heparin conjugation, platelet attachment, and cell infiltration presented below are for the following plasma treatment conditions: (a) Ar-NH₃ plasma (50 W power, 30 vol% Ar, 0.5 Torr pressure, 100 sccm gas flow rate, 5 min treatment time) and (b) H₂ plasma (10 W power, 50 sccm H₂ gas flow rate, 0.5 Torr pressure, 0.5 min treatment time).

Heparin conjugation

The amount of covalently immobilized heparin on the untreated, plasma treated with Ar or Ar-NH₃/H₂ plasma, and PEG scaffold samples is shown in Fig. 4. As expected, very small amounts of heparin conjugation were detected on the untreated and Ar plasma-treated samples, because of the limited number of available conjugation sites on these surfaces. This finding indicates that the amount of heparin conjugation on the Ar-NH₃/H₂ plasma-treated samples cannot be attributed to nonspecific adsorption of heparin on surface sites activated during plasma treatment. The significantly higher amount of heparin measured on the Ar-NH₃/H₂ plasma-treated samples compared to the PEG samples shows that this plasma treatment was more effective than the method of NaOH hydrolysis and aminolysis to incorporate -NH₂ groups onto the fibrous scaffold surfaces for subsequent biomolecule immobilization.

Platelet attachment

Additional evidence of the efficacy of Ar-NH₃/H₂ plasma treatment to enhance heparin conjugation on the scaffold surfaces was obtained from *in vitro* experiments with blood from Sprague-Dawley rats. Figure 5 shows high-resolution SEM images of scaffold surfaces obtained from these tests. Significant platelet attachment was observed on the untreated [Fig. 5(a)] and treated with Ar plasma [Fig. 5(b)] or Ar-NH₃/H₂ plasma [Fig. 5(c)] scaffold surfaces without heparin conjugation. Platelet attachment on the heparin-conjugated untreated [Fig. 5(d)] and Ar plasma-treated [Fig. 5(e)] scaffold surfaces was slightly reduced compared to non-conjugated scaffolds; however, the effect was most pronounced with the

heparin-conjugated Ar-NH₃/H₂ plasma-treated scaffold surfaces [Fig. 5(f)]. The significant decrease of platelet attachment on the latter surfaces illustrates the effectiveness of the Ar-NH₃/H₂ plasma treatment process to induce extensive heparin immobilization on the PLLA fibrous surfaces and is consistent with the toluidine blue staining results shown in Fig. 4. Further studies need to be performed with platelets isolated from healthy human donor to confirm this finding for clinically relevant applications.

Cell infiltration

The effect of heparin conjugation on BAEC infiltration through the PLLA fibrous scaffolds is demonstrated in Fig. 6. While cell infiltration was enhanced by the Ar and Ar-NH₃/H₂ plasma treatments [Fig. 6(b) and (c), respectively] compared to untreated scaffold samples [Fig. 6(a)], heparin conjugation increased cell attachment and infiltration through all samples [Fig. 6(d–f)], independent on the amount of conjugated heparin.

The enhancement of cell infiltration in the presence of heparin is consistent with previous findings,²⁰ which may be attributed to the negative charge of heparin and its capability of attracting and retaining water, resulting in scaffold swelling and a cell-friendly hydrophilic microenvironment. Alternatively, heparin may allow the binding of growth factors from serum, enhancing the scaffold bioactivity. The use of immobilized heparin as an adaptor for growth factor binding and delivery for broad applications in tissue regeneration will be explored in future studies. Co-axial electrospinning and hydrogel coating have been previously used for drug loading in electrospun scaffolds.^{21,22}

It is believed that for cell culture *in vitro*, both physical adsorption and chemical immobilization of heparin on the scaffold surfaces contributed to the cell attachment and subsequent infiltration, and when the total amount heparin exceeds a threshold, the effect on cell attachment and infiltration is saturated, as observed in this study. Therefore, the increased cell attachment on the heparin-conjugated untreated scaffolds is attributed to heparin adsorption, whereas cell attachment on the heparin-conjugated plasma-treated scaffolds is attributed to plasma-induced surface chemistry modification, resulting in both heparin adsorption and conjugation.

Considering that surface –NH₂ groups are conjugation sites for various biomolecules, the present plasma method for incorporating –NH₂ groups onto PLLA scaffold surfaces is not only useful for heparin conjugation but can also be used for the conjugation of other biomolecules. In addition, the current plasma treatment method uses common nontoxic gases, such as NH₃, Ar, and H₂, and produces a density of conjugations sites significantly higher than those obtained with wet chemical methods. Thus, the results of this study indicate that the present plasma method is an effective method for increasing the density of desirable surface functionalities on various biopolymers.

CONCLUSIONS

Electrospun fibrous scaffolds consisting of PLLA were subjected to a two-step treatment process with Ar-NH₃/H₂ plasma to incorporate –NH₂ surface groups to enhance heparin conjugation. Results of a parametric study demonstrated that the power, time, and gas

composition of the Ar-NH₃ plasma treatment and the time of the H₂ plasma post-treatment affected the surface chemical characteristics of the scaffold surfaces, in particular the density of -NH₂ surface functionalities. A 10-min plasma treatment with Ar(30 vol%)-NH₃ at a power of 50 W followed by a 0.5-min post-treatment with H₂ plasma were found to be optimum for maximizing the surface density of -NH₂ groups for all other plasma parameters fixed. *In vitro* experiments demonstrated that scaffolds with a high density of -NH₂ surface functionalities exhibited a high amount of covalently immobilized heparin and minimal platelet attachment. Scaffold treatment with Ar or Ar-NH₃/H₂ plasmas increased the infiltration of BAECs *in vitro*, while heparin conjugation further increased BAEC infiltration *in vitro*, independent of the amount of covalently immobilized heparin on plasma-treated scaffolds with a high density of -NH₂ surface functionalities. The improved heparin conjugation can increase the amount and the stability of heparin on electrospun scaffolds, which can enhance the anti-thrombogenic property and improve the long-term performance of electrospun vascular grafts. In addition, heparin-coated electrospun scaffolds can be used for the effective loading and delivery of growth factors, which has broad applications in tissue engineering.

Acknowledgments

The authors thank J. Henry and A. Wang for assistance with the toluidine blue test and mouse blood sample, respectively. The XPS measurements were carried out at the Molecular Foundry at the Lawrence Berkeley National Laboratory. This research is partially supported by the National Science Foundation (Grant CMS-0528506) and the National Institute of Health (Grant HL083900 and EB012240).

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$$\frac{[NH_2]}{[C]} = \frac{[F]/3}{[C] - 8[F]/3}$$

Fig. 1.

Schematic of the chemical derivatization process of the $-NH_2$ group with TFBA on PLLA fibrous scaffolds subjected to $Ar-NH_3/H_2$ plasma treatment and formula for computing the NH_2/C fraction.

Fig. 2.

Effect of plasma power (left column) and plasma treatment time (right column) on the surface chemical characteristics of PLLA fibrous scaffolds subjected to Ar-NH₃/H₂ plasma treatment: (a,d) N/C, (b,e) NH₂/N, and (c,f) NH₂/C fraction. (Plasma parameters: (a)–(c) Ar-NH₃ plasma (50–200 W power, 30 vol% Ar, 0.5 Torr pressure, 100 sccm gas flow rate, 5 min treatment time); H₂ plasma (10 W power, 50 sccm H₂ gas flow rate, 0.5 Torr pressure, 0.5 min treatment time) and (d)–(f) Ar-NH₃ plasma (100 W power, 30 vol% Ar, 0.5 Torr pressure, 100 sccm gas flow rate, 2–10 min treatment time); H₂ plasma (10 W power, 50 sccm H₂ gas flow rate, 0.5 Torr pressure, 0.5 min treatment time)).

Fig. 3.

Effect of Ar-NH₃ plasma gas composition (left column) and H₂ plasma post-treatment time (right column) on the surface chemical characteristics of PLLA fibrous scaffolds subjected to Ar-NH₃/H₂ plasma treatment: (a,d) N/C, (b,e) NH₂/N, and (c,f) NH₂/C fraction. Plasma parameters are: (a)–(c) Ar-NH₃ plasma (50 W power, 10–50 vol% Ar, 0.5 Torr pressure, 100 sccm gas flow rate, 5 min treatment time); H₂ plasma (10 W power, 50 sccm H₂ gas flow rate, 0.5 Torr pressure, 0.5 min treatment time) and (d)–(f) Ar-NH₃ plasma (50 W power, 30 vol% Ar, 0.5 Torr pressure, 100 sccm gas flow rate, 5 min treatment time); H₂ plasma (10 W power, 50 sccm H₂ gas flow rate, 0.5 Torr pressure, 10–60 s treatment time).

Fig. 4. Heparin conjugation density on untreated, plasma-treated (Ar or Ar-NH₃/H₂), and PEG-conjugated (by the NaOH hydrolysis method) PLLA fibrous scaffolds. Plasma parameters are: Ar-NH₃ plasma (50 W power, 30 vol% Ar, 0.5 Torr pressure, 100 sccm gas flow rate, 5 min treatment time); H₂ plasma (10 W power, 50 sccm H₂ gas flow rate, 0.5 Torr pressure, 30 s treatment time).

Fig. 5.

SEM images showing the attachment of platelets from whole blood of Sprague-Dawley rats on PLLA fibrous scaffolds without (first row) and with (second row) heparin conjugation (by the NaOH hydrolysis method): (a,d) untreated (using the NaOH hydrolysis method for heparin conjugation in d), (b,e) Ar plasma-treated (controls), and (c,f) Ar-NH₃/H₂ plasma-treated scaffolds. (UT = untreated, Ar = Ar plasma treated, Ar-NH₃ = Ar-NH₃/H₂ plasma treated, Hep = heparin conjugated).

Fig. 6.

Cross-sectional fluorescence images showing through-thickness infiltration of BAECs into PLLA fibrous scaffolds without (left column) and with (right column) heparin conjugation: (a,d) untreated (using the NaOH hydrolysis method for heparin conjugation in d), (b,c) Ar plasma-treated (controls), and (c,f) Ar-NH₃/H₂ plasma-treated scaffolds. Cells were seeded at 100% confluency at the top scaffold surface with serum medium for 5 days. Top and bottom scaffold surfaces are distinguished by dashed lines. DAPI staining for nuclei is shown in blue color. (UT = untreated, Ar = Ar plasma treated, Ar-NH₃ = Ar-NH₃/H₂ plasma treated, Hep = heparin conjugated).