PP82
Layer-by-layer functionalisation of polymeric blends to favor stent endothelialisation

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Introduction: Specific ligand immobilisation can enhance endothelial cells (ECs) adhesion on stent surface, avoiding migration of smooth muscle cells (SMCs) [1]. Wei et al. [2] demonstrated that the REDV peptide promote the EC growth respect to SMCs. In this work, polymeric substrates were coated through layer-by-layer technique to functionalize the final layer with the CRETTAWAC sequence able to favor ECs and inhibit platelet attachment [3].
Materials and methods: Aminolysed poly(L-lactide)/poly(DL-lactide-co-glycolide) (PLLA/PLGA) films were dipped into alternate aqueous solutions (0.5% w/v, pH: 4.5) of poly(sodium 4-styrene sulfonate) (PSS) and poly(diallyldimethylammonium chloride) (PDDA) for 15 minutes each, with intermediate washing steps in bidistilled water (pH: 4.5) for 5 minutes. Heparin (HE) was deposited as the last layer of the coating. Surface composition was evaluated through XPS analysis and via colorimetric method. The CRETTAWAC effect on re-endothelialization was evaluated using human umbilical vein endothelial cells (HUVECs). Cells were seeded onto three different surface: CRETTAWAC, bovine serum albumin (BSA) and fibronectin (FN).
Results: The deposition of multilayer coating was confirmed by XPS analysis.

Table 1. Atomic percentage of chemical elements of functionalised samples.

	C1s	O1s	N1s	S2p
Aminolyzed PLLA/PLGA	64.0	35.3	0.7	–
PLLA/PLGA + 14L	71.2	26.0	1.3	1.5
PLLA/PLGA + 15L	70.2	25.8	2.2	1.8

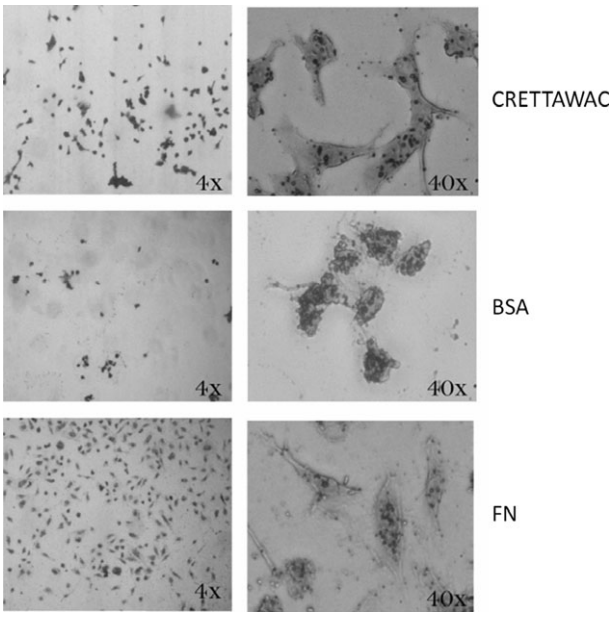

Figure 1. Microscopy images of adhered HUVECs on different functionalised surface after 4 hours.

After the deposition of 14 and 15 polyelectrolyte layers the percentage of N and S was higher respect to aminolysed PLLA/PLGA due to the deposition of PDDA, PSS and HE (Table 1). *In vitro* tests show that after 2 hours the number of adhered cells on CRETTAWAC functionalised substrate were less respect to FN. After 4 hours HUVECs seeded on CRETTAWAC showed a spread shape.
Discussion and conclusions: Through the LbL technique allows to obtain functional coatings without changing the bulk properties. CRETTAWAC sequence was shown to bind the α5β1 integrin that is present on the surface of ECs but lacking on platelets. LBL could be exploited for surface functionalisation with KKKKKKSGSSGKCRRETAWAC, able to electrostatically interact with HE.
Acknowledgments: NANOSTENT (Regional Project, Regione Piemonte).
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PP83
Realization of a soft-MI electrospun scaffold for tissue engineering applications

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Introduction: The fabrication of bioactive scaffolds able to mimic the *in vivo* cellular microenvironment represents a challenge for tissue engineering (TE). In the present work, we propose the combination of electrospinning (ESP) and soft-molecular imprinting (soft-MI) [1] to create a soft-molecular imprinted fibrous scaffold for TE applications. By combining these techniques, we fabricated poly-L-lactide acid (PLLA), PolyActive (PA) and poly lactic-co-glycolic acid (PLGA) based scaffolds functionalized using different protein arranged onto the surface.
Materials and methods: A functionalized PDMS mold was used as a target for the ESP jet. The PDMS mold was prepared using a commercial product (Sylgard 184 kit, Dow Corning, MI) with a 10:1 (w/w) ratio. The mixture was casted onto a silica wafer comprising different custom-made geometries, degassed, and finally cured in an oven for 4 hours at 70°C. To reduce the hydrophobicity of its surface, the PDMS mold was treated with Piranha Solution (3:1). After the derivatization of its surface with a 1% solution of (3-Aminopropyl)-trimethoxysilane in Toluene, the PDMS mold was activated with an EDC-NHS solution. Finally, the mold was immersed in a protein solution for 24 hours. The proteins used as templates were FITC-albumin and TRITC-lectin. Three different polymer solutions were prepared: PLGA 4% (w/v) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), PLDLA 5% (w/v) in HFIP and PA300 20% (w/v) in $CHCl_3$/HFIP 70:30 (v/v). The functionalized PDMS mold was positioned under the ESP jet and the ESP fibers fabricated at a flow rate of 1 mL/h and a voltage of 20 kV. The imprinted scaffolds were tested for their ability to rebind the template protein and observed with a fluorescence microscope. To evaluate the selectivity for a particular template molecule, the imprinted scaffolds were immersed in a solution of both FITC-albumin and TRITC-lectin. The obtained scaffolds were analyzed by fluorescence microscopy to ensure that no weakly bound protein on the PDMS was transferred to them.

Results: The imprinted scaffolds demonstrated affinity and selectivity for their template molecule over the other fluorescent protein competitor. Specifically, the FITC-albumin imprinted scaffolds showed protein absorption in a solution of FITC-albumin (Fig. 1a) while no protein adsorption was seen in a solution of TRITC-lectin. Similar results were obtained with the TRITC-lectin imprinted scaffolds (Fig. 1b).

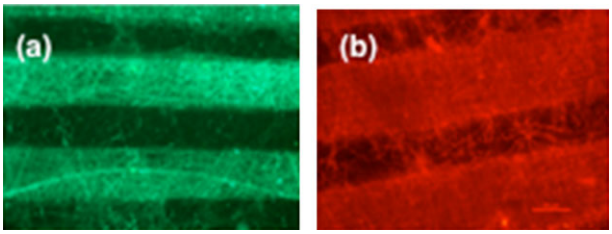

Figure 1. (a) FITC-albumin imprinted PLGA scaffold; (b) TRITC-lectin imprinted PLGA scaffold.

Discussion and conclusions: The integration of ESP and soft-MI represents a promising technique for the realization of scaffolds for TE applications, because it allow us to mimic the biological environments through the selective bindings of specific endogenous proteins.

Reference

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PP84
Electrospun microyarns for vascularization in tissue engineering

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Introduction: As an interdisciplinary field Tissue Engineering is aiming at the reproducibility of damaged tissue or organs. Although there are constantly achieved successes in the design of tissue engineered constructions, there is still the difficulty to provide those with a sufficient vascularization. Successful applications in Tissue Engineering involve thin avascular tissue like cartilage and skin without a vasculature.

Aim of the present research work is the production of electrospun microyarns to support angiogenesis in fibrin gel scaffolds.

Materials and methods: Microyarns with different morphological properties (polymer concentration, polymer blend, diameter, torsion angle) were produced by electrospinning[1]. Polylactide (PLA) and polyethylene glycol (PEG) were used as polymers. The electrospun microyarns were UV- sterilized for 30 minutes under the laminar flow hood. After sterilization the microyarns were seeded with 1.4×10^6 human umbilical vein endothelial cells (HUVECs) and incubated (37°C, 5%CO_2) in 24-well plates for 24 hours. Subsequently the microyarns were stained with 4′,6-Diamidin-2-phenylindol (DAPI) and analyzed under the fluorescence microsope[2]. Those of the microyarns that showed the best proliferations were used for further experiments. To proof their capability of developing capillary-like structures, the microyarns were cultivated in 3D. The micoyarns were first seeded in 24 well plates with HUVECs; then embedded in fibrin gel containing both HUVECs and human dermal foreskin fibroblasts (HDFFs). After 14 days of co-cultivation the fibrin gels were fixed and immunostained (CD31). Verification and quantification of the capillary-like structures were implemented by two-photon laser scanning microscopy (TPLSM) and ImagePro Analyzer software. All experiments were replicated three times with different donors.

Results: After 14 days of co-cultivation, it could be observed that there is formation of capillary-like structures within the HUVEC/HDFF co-culture system. There were also significant distinctions in formation between the different morphological properties of the microyarns.

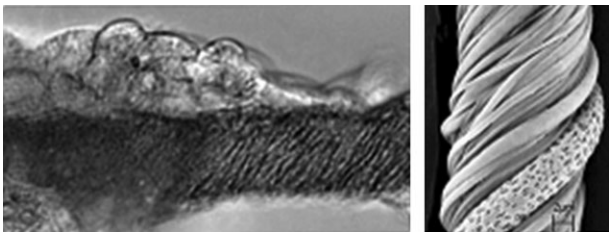

Figure 1. Cell adhesion on PLA/PEG microyarn (l), SEM image of PLA/PEG microyarn (r).

Discussion and conclusions: The present work shows, that microyarns can be applied as textile scaffolds for vascularization. Depending on their morphological property, they are also capable to influence the orientation of cell adhesion.

References

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PP85
Fabrication of a functionally layered membrane designed for periodontal tissue regeneration

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Introduction: Guided bone regeneration (GBR) is employed in dental implantology and the treatment of periodontal bone loss. It is based on the surgical placement of a barrier membrane that excludes soft tissue growth that would otherwise prevent bone healing [1-2].Unfortunately, non-resorbable membranes require a second operation for their removal while resorbable collagen membranes have an associated risk of disease transmission, and also existing commercial systems do not promote tissue regeneration beyond their barrier function. The aim was therefore to develop a functionalised, bilayered resorbable membrane with a dense layer to contact soft tissues, and a porous layer to promote bone healing.

Materials and methods: Compact films of poly(D,L-lactide-co-glycolide) (PLGA, 75:25) were prepared by solvent casting from acetone. Porous membranes were prepared by dissolving PLGA (22% w/v) and nano-hydroxyapatite powder (20% w/w) [3] in acetone prior to electrospinning. Both layers were subjected to plasma polymerisation for acrylic acid grafting, and were modified by layer-by-layer (LbL) technique to generate functional polyelectrolyte (poly (styrene sulfonate) /poly(allyl amine) (PSS/ PAH); Sigma) to obtain 20 nanolayers to incorporate (1) an antibiotic drug (Metronidazole; Sigma) to provide antibacterial activity for the compact layer, and (2) specific bone matrix peptides (KRSR and FHRRIKA; GenScript) to promote bone healing at the porous surface. The layers were assembled using fibrin glue. Drug release from the compact layer was evaluated by UV-Vis, while *in vitro* biocompatibility was investigated using L292 mouse fibroblasts (compact layer) and rat mesenchymal stromal cells (porous layer).

Results and discussion: FTIR-ATR showed that the typical absorption bands of PAH and PSS increased with layer number (i.e. SO^{3-} stretching vibrations at 1130/cm for PSS, and NH scissoring vibrations at 1580/cm for PAH). The contact angle values had an alternate behaviour from the 9th and from 11th nanolayer for the compact and porous layer respectively. XPS spectra showed a N_{1s} peak at 399.5 eV and S_{2p} peak at 168 eV, indicating PAH and PSS had been introduced (Fig. 1).

DOI: 10.1002/term.1932