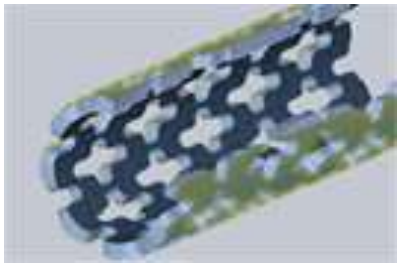

Deliverable 4.2

Development of optimised coating technology of stent prototypes

Editor:	Rocío Ortiz, Iban Quintana, TEKNIKER
Deliverable nature:	O
Dissemination level: (Confidentiality)	PU
Contractual delivery date:	M18 (30-06-2015)
Actual delivery date:	M18 (30-06-2015)
Suggested readers:	
Version:	1.0
Total number of pages:	11
Keywords:	Surface functionalisation, endothelialisation

Abstract

The ReBioStent project was inspired by the need to replace the metallic stent systems and produce a more reliable biodegradable stent with superior properties over the currently available biodegradable polymeric alternatives. In this context, surface functionalisation of the biopolymers is carried out to target two crucial aspects in the stent functionality: to improve the endothelialisation ability (healing) of the stent inner surface, and to reduce the occurrence of thrombosis as a consequence of platelet and protein adhesion. To this purpose the partners involved in WP4 developed different strategies, which were employed together in order to generate antithrombogenic surface promoting endothelialisation of the inner stent luminal surface. This deliverable is linked to the project milestone associated to the development of the ideal stent surface functionalisation for an optimal biocompatibility and long term stability of these implants.

[End of abstract]

Disclaimer

This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement n° 604251

Impressum

[Full project title]

Reinforced Bioresorbable Biomaterials for Therapeutic Drug Eluting Stents

[Short project title] ReBioStent

[Number and title of work-package] WP 4: Surface Functionalisation of Biomaterials to improve biocompatibility and long term stability of stents

[Number and title of task] Task 4.1: Antithrombotic surface promoting endothelialisation of the inner stent luminal surface

[Document title] Development of optimised coating technology of stent prototypes

[Editor: Rocío Ortiz, TEKNIKER]

[Work-package leader: Iban Quintana, TEKNIKER]

[Estimation of PM spent on the Deliverable] 12

Copyright notice

List of authors

Company	Author	Contribution
Politecnico di Torino	Irene Carmagnola	Results on biochemical functionalisation
Politecnico di Torino	Valeria Chiono	Results on biochemical functionalisation
Politecnico di Torino	Gianluca Ciardelii	Results on biochemical functionalisation
TEKNIKER	Rocío Ortiz	Results on physical functionalisation
TEKNIKER	Iban Quintana	Results on physical functionalisation
TEKNIKER	Adriana Serras	Results on physical functionalisation

Table of Contents

List of figures and/or list of tables

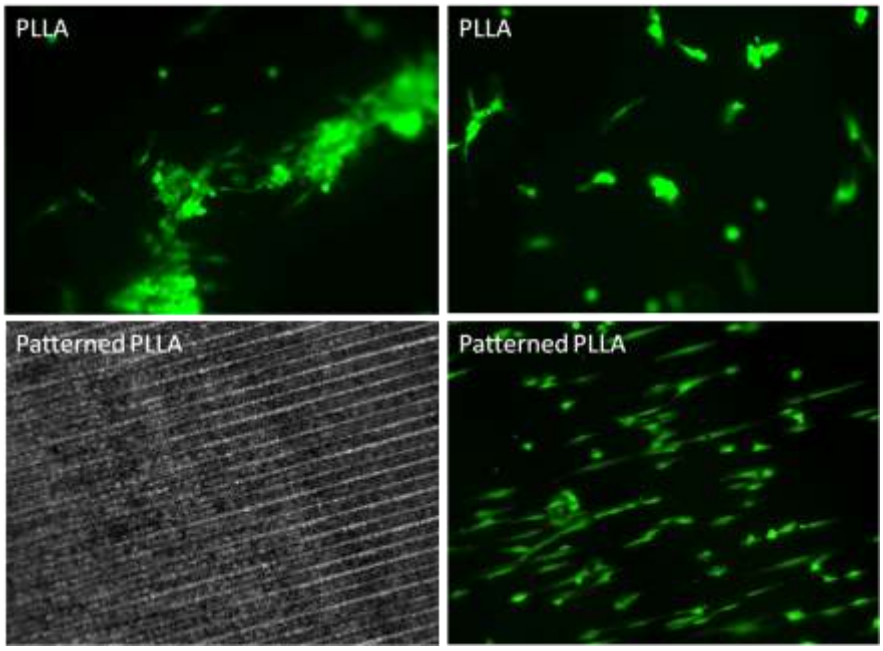

Fig.1. Effect of groove-patterns on endothelial cell adhesion (*in vitro* tests carried out by Research Center/Ruhr University Bochum, partner of the project).

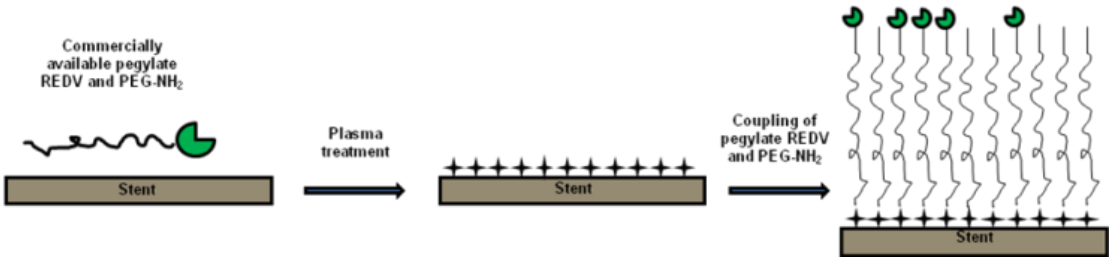

Fig.2 Schematic representation of the surface functionalisation with pegylated REDV peptide.

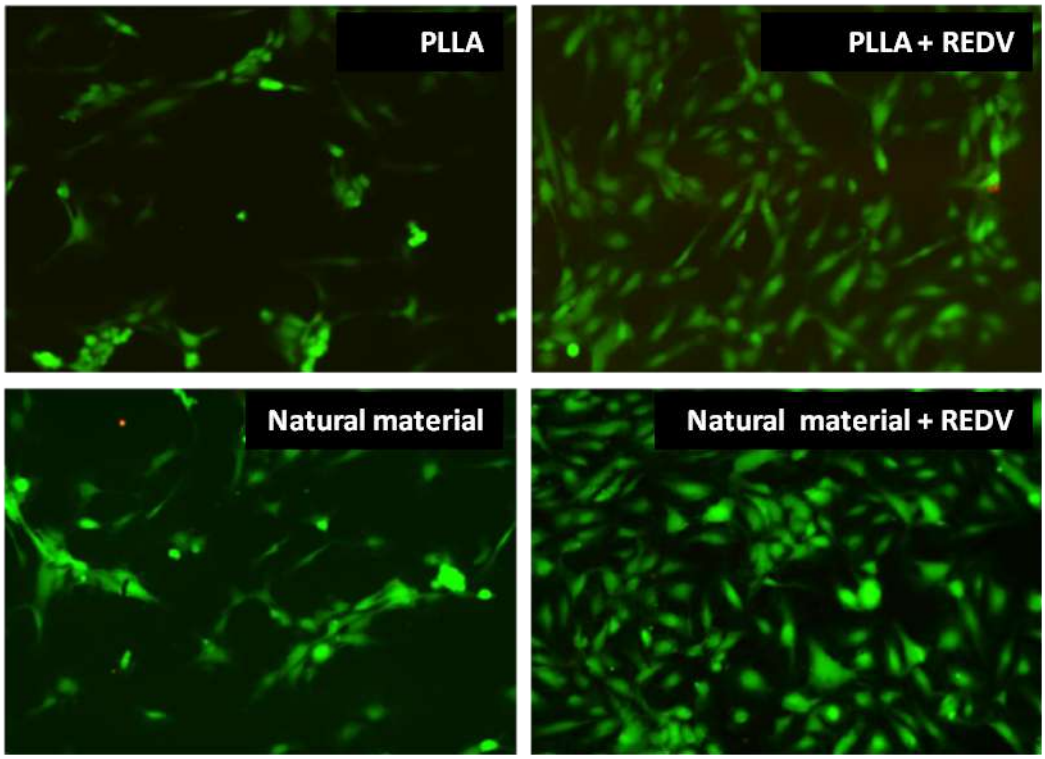

Fig.3 Endothelial cell behavior on PLLA and natural material (PHAs) films functionalised with REDV in comparison to the non-functionalised films: fluorescence microscopy images of adhered endothelial cells.

Introduction

Rapid re-endothelialisation of the arterial wall and the inner surface of the stent are critical for the prevention of the main causes of in-stent restenosis or thrombosis[1]. Drug eluting is recognized as the method of choice for addressing undesirable cell and protein adhesion that leads to inflammatory reaction and restenosis after stent implantation[2]. However, issues related to the drug elution lifespan and the risk of restenosis as a consequence of a fail on wound healing remain unresolved[3]. Topographical modification of the inner surface of the stent by different techniques has gained prominence as a method to encourage the formation of a healthy endothelium[4]. Among all the surface properties that influence cell behaviour, topography has emerged as a major tool to control and influence different cellular cues, such as cell size, cell shape, proliferation, adhesion or migration [5,6]. Literature collects the effect of surface topography in a wide variety of cell types including endothelial cells (ECs) [7,8,9]. In combination with biochemical modifications, physical modifications can be applied to recruit endothelial cells, to support their adhesion, proliferation and migration.

Experimental methods

Surface patterning of poly(lactic acid) (PLLA) sheets was performed by using picosecond laser micromachining technology (PLM). Laser ablation was applied on different biopolymers (that are being used for producing BVSs) to generate grooves of variable dimensions and evaluate the effect of topography on endothelial cells response. Biochemical strategies were used in combination with micropatterning to functionalize the surface: Plasma technology was employed to polymerize acrylic acid (AAc) on the PLLA substrate surface with the aim to covalently graft a pegylated REDV peptide with specific endothelialisation ability. Additionally, this biochemical functionalisation promotes adhesion of ECs against platelet and SMC attachment

Results

Micropatterning and chemical modification were optimised using PLLA films produced by the company Vornia Biomaterials, a partner in the project. Surface topography has been optimized to induce and increase the alignment of the endothelial cells on PLLA patterned surfaces compared to the non-patterned PLLA surfaces (Fig. 1).

AAc deposition via plasma treatment was successfully performed on the surface of PLLA, as demonstrated by physicochemical characterisation, allowing the grafting of pegylated-REDV on the surface (Fig. 2).

The presence of the pegylate-REDV peptide was demonstrated through physico-chemical characterisation. The adhesion of the endothelial cells, shown in Fig. 3, was increased on polymeric substrates functionalised with pegylate-REDV, confirming the efficacy of the coating.

General discussion and conclusions

The *in vitro* cell culture tests performed on the functionalised PLLA surfaces showed that EC alignment and adhesion was improved on the laser-patterned surface, and the EC proliferation and attachment was promoted on the peptide coated surface. These observations disclosed that combined physical and biochemical surface functionalisation may favour the formation of a healthy endothelium, first, promoting the adhesion of the ECs to the surface, and then, increasing the proliferation and migration of the ECs along the blood flow, leading to the improvement of the vessel healing process after stent implantation. In addition, the encouragement of the EC adhesion may reduce the attachment of platelets and proteins to the vessel wall, decreasing the risk of thrombosis. The ultra-short pulsed laser applied to obtain the physical pattern is a very versatile technology in terms of materials, geometries, areas and features that can be processed. Moreover, the processing time is significantly reduced compared to the clean room techniques usually applied in this field (lithography based technologies) showing a higher manufacturing capacity. This technology allows obtaining a wide range of groove dimensions on the biopolymers, enabling the identification of the optimum groove topography to get the desired EC alignment. The biochemical functionalisation applied via peptide grafting to stimulate local endothelial cell adhesion allowed the preparation of stable coatings, highly demanded in the case of the stents to resist the shear stresses caused by the blood flow. This coating was also provided with an antifouling functionality to improve the stent performance. These benefits will be confirmed by *in vitro* culture tests in functionalised stent prototypes under blood flow conditions to verify the effects of the multifunctional surface on the overall healing of the stent inner surface.

References

1. Kipshidze N, *et al*. J Am Coll Cardiol 44(4):733-739, 2004.
2. Nazneen f, *et al*. J Biomed Mater Res Part B 100B:1989-2014, 2012.
3. Martinez, C. Wiley Interdiscip Rev Nanomed Nanobiotechnol 3(3):256–268, 2011.
4. Mel A, *et al*. Biomacrom 9(11):2969-79, 2008.
5. Bettinger C.J. *et al*. Angew. Chem. Int. Ed. 48:5406 – 15, 2009.
6. Ross A.M. *et al*. Small 8(3):336-55, 2012.
7. Kilian K.A. *et al*. Proc Nat l Acad Sci USA 107:4872-77, 2010.
8. Ortiz R. *et al*. Mat Sci Eng C 37:241-250, 2014.
9. Liliensiek S.J. *et al*. J Biomed Mater Res A 79:185-192, 2006.

