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Stent Surface Functionalisation via Plasma Treatment to Promote Fast Endothelialisation

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Abstract : Thrombosis and restenosis after stenting procedure can be prevented by promoting fast stent wall endothelialisation. It is well known that surface functionalisation with antifouling molecules combining with extracellular matrix proteins is a promising strategy to design biomimetic surfaces able to promote fast endothelialization. In particular, REDV has gained much attention for the ability to enhance rapid endothelialization due to its specific affinity with endothelial cells (ECs). In this work, a two-step plasma treatment was performed to polymerize a thin layer of acrylic acid, used to subsequently graft PEGylated-REDV and polyethylene glycol (PEG) at different molar ratio with the aim to selectively promote endothelial cell adhesion avoiding platelet activation. PEGylate-REDV was provided by Biomatik and it is formed by 6 PEG monomer repetitions (Chempep Inc.), with an NH2 terminal group. PEG polymers were purchased from Chempep Inc. with two different chain lengths: m-PEG6-NH2 (295.4 Da) with 6 monomer repetitions and m-PEG12-NH2 (559.7 Da) with 12 monomer repetitions. Plasma activation was obtained by operating at 50W power, 5 min of treatment and at an Ar flow rate of 20 sccm. Pure acrylic acid (99%, AAc) vapors were diluted in Ar (flow = 20 sccm) and polymerized by a pulsed plasma discharge applying a discharge RF power of 200 W, a duty cycle of 10% (on time = 10 ms, off time = 90 ms) for 10 min. After plasma treatment, samples were dipped into an 1-(3-dimethylaminopropyl)-3- ethylcarbodiimide (EDC)/N-hydroxysuccinimide (NHS) solution (ratio 4:1, pH 5.5) for 1 h at 4°C and subsequently dipped in PEGylate-REDV and PEGylate-REDV:PEG solutions at different molar ratio (100 µg/mL in PBS) for 20 h at room temperature. Surface modification was characterized through physico-chemical analyses and in vitro cell tests. PEGylated-REDV peptide and PEG were successfully bound to the carboxylic groups that are formed on the polymer surface after plasma reaction. FTIR-ATR spectroscopy, X -ray Photoelectron Spectroscopy (XPS) and contact angle measurement gave a clear indication of the presence of the grafted molecules. The use of PEG as a spacer allowed for an increase in wettability of the surface, and the effect was more evident by increasing the amount of PEG. Endothelial cells adhered and spread well on the surfaces functionalized with the REDV sequence. In conclusion, a selective coating able to promote a new endothelial cell layer on polymeric stent surface was developed. In particular, a thin AAc film was polymerised on the polymeric surface in order to expose –COOH groups, and PEGylate-REDV and PEG were successful grafted on the polymeric substrates. The REDV peptide demonstrated to encourage cell adhesion with a consequent, expected improvement of the hemocompatibility of these polymeric surfaces in vivo. Acknowledgements—This work was funded by the European Commission 7th Framework Programme under grant agreement number 604251-ReBioStent (Reinforced Bioresorbable Biomaterials for Therapeutic Drug Eluting Stents). The authors thank all the ReBioStent partners for their support in this work.

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