Applied Surface Science 393 (2017) 82–92

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full Length Article

Functionalization of CoCr surfaces with cell adhesive peptides to promote HUVECs adhesion and proliferation

Maria Isabel Castellanos[a,b], Carlos Mas-Moruno[a,b], Anna Grau[a,b], Xavier Serra-Picamal[d,e,f], Xavier Trepat[d,e,f], Fernando Albericio[g], Michael Joner[h,i], Francisco Javier Gil[a,b], Maria Pau Ginebra[a,b,d], Jose María Manero[a,b], Marta Pegueroles[a,b,*]

[a] Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgical Engineering, Technical University of Catalonia (UPC), ETSEIB, 08028 Barcelona, Spain
[b] Centre for Research in Nanoengineering (CRNE), UPC, 08028 Barcelona, Spain
[d] Institute for Bioengineering of Catalonia (IBEC), 08028 Barcelona, Spain
[e] University of Barcelona and CIBER-BBN, 08036 Barcelona, Spain
[f] Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain
[g] Department of Chemistry, University of Barcelona, CIBER-BBN, 08028 Barcelona, Spain
[h] Department of Cardiology, Deutsches Herzzentrum München, 80636 Munich, Germany
[i] CVPath Institute, Gaithersburg, MD 20878, USA

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form
19 September 2016
Accepted 21 September 2016
Available online 22 September 2016

Keywords:
CoCr alloy
Surface functionalization
Cell adhesive peptides
HUVEC proliferation
Endothelialization
SMCs adhesion

ABSTRACT

Biomimetic surface modification with peptides that have specific cell-binding moieties is a promising approach to improve endothelialization of metal-based stents. In this study, we functionalized CoCr surfaces with RGDS, REDV, YIGSR peptides and their combinations to promote endothelial cells (ECs) adhesion and proliferation. An extensive characterization of the functionalized surfaces was performed by XPS analysis, surface charge and quartz crystal microbalance with dissipation monitoring (QCM-D), which demonstrated the successful immobilization of the peptides to the surface. Cell studies demonstrated that the covalent functionalization of CoCr surfaces with an equimolar combination of RGDS and YIGSR represents the most powerful strategy to enhance the early stages of ECs adhesion and proliferation, indicating a positive synergistic effect between the two peptide motifs. Although these peptide sequences slightly increased smooth muscle cells (SMCs) adhesion, these values were ten times lower than those observed for ECs. The combination of RGDS with the REDV sequence did not show synergistic effects in promoting the adhesion or proliferation of ECs. The strategy presented in this study holds great potential to overcome clinical limitations of current metal stents by enhancing their capacity to support surface endothelialization.

1. Introduction

Intracoronary stenting is a common practice in interventional cardiology to treat blood vessels with reduced flow due to atherosclerosis. The stent is a metallic mesh tube, which is expanded in the narrowed artery recovering blood flow. Restenosis due to excessive proliferation of smooth muscle cells (SMCs), neointimal hyperplasia, has been a relatively common complication associated with bare metal stents (BMS) after implantation [1–3]. Currently, drug-eluting stents (DES) are polymer-coated stents, which release anti-proliferative drugs that successfully minimize restenosis. However, the drugs not only limit SMCs proliferation but also delay endothelialization of the device after implantation [4]. This may result in higher risk of late in-stent thrombosis, requiring long-term anti-platelet aggregation therapies after implantation. Thus, new surface functionalization should combine the benefit of decreasing acute restenosis, while keeping low levels of thrombogenicity and recovering artery's function.

Biomimetic surface modification with proteins or peptides that have specific cell-binding moieties is a promising approach to

* Corresponding author at: Department of Materials Science and Metallurgical Engineering, Av. Diagonal 647, Barcelona, 08028, Spain.
E-mail addresses: maria.isabel.castellanos@upc.edu (M.I. Castellanos), carles.mas.moruno@upc.edu (C. Mas-Moruno), agraugar@gmail.com (A. Grau), xserrapicamal@gmail.com (X. Serra-Picamal), xtrepat@ub.edu (X. Trepat), fernando.albericio@irbbarcelona.org (F. Albericio), michaeljoner@me.com (M. Joner), francesc.xavier.gil@upc.edu (F.J. Gil), maria.pau.ginebra@upc.edu (M.P. Ginebra), jose.maria.manero@upc.edu (J.M. Manero), marta.pegueroles@upc.edu (M. Pegueroles).

http://dx.doi.org/10.1016/j.apsusc.2016.09.107
0169-4332/

improve endothelialization [5,6]. In this regard, stent functionalization with proteins [7], biopolymers [8,9], or with biologically relevant peptide sequences [4,10,11] has been shown to promote endothelial cells (ECs) adhesion. Proteins purified from the extracellular matrix (ECM) have a strong biological efficiency because their native structure and synergistic sequences are preserved. However, this strategy may present problems of immunogenicity, denaturation after sterilization and poor control of functionalization. An alternative to such shortcomings is the use of short synthetic peptides, which are non-immunogenic, easy to purify and can be immobilized on surfaces in a controlled manner [12].

Surfaces modified with bioactive cell-adhesive peptides have shown to mediate anchorage-dependent cell functions, including adhesion, migration and proliferation [13–16]. A prominent example is illustrated by the well-known adhesive sequence RGDS (Arg-Gly-Asp-Ser), present in fibronectin and other ECM proteins, which is recognized as the minimal amino acid sequence necessary to promote cell adhesion [16]. This sequence has been applied to different surfaces including poly(ethylene glycol) (PEG), polyethylene terephthalate (PET) and polytetrafluoroethylene [17,18], poly(L-lactic acid) scaffold [19] and titanium surfaces and its alloys [20,21], in order to improve cell attachment and the bioactivity of the surfaces. Besides the RGDS motif, other adhesive ligands such as the laminin derived YIGSR (Tyr-Ile-Gly-Ser-Arg) sequence [22,23] have been shown to promote EC adhesion and migration without enhancing platelet adhesion [24]. For instance, this peptide has enhanced the adhesion of ECs in hydrogels [13,25], PET [26], polyurethane [27,28], poly(2-hydroxyethyl methacrylate) [29] and decellularized scaffolds [30]. Finally, another cell adhesive sequence found in fibronectin, the REDV (Arg-Glu-Asp-Val) peptide, which targets ECs via the integrin $\alpha4\beta1$, has been reported to selectively promote EC adhesion and spreading over SMCs and platelets [22,31]. The REDV peptide has been immobilized onto several polymers such as poly(ethylene glycol) diacrylate hidrogels [32], PET surfaces [33], zwitterionic polycarboxybetaine copolymers [34], and polysaccharide hydrogels [35], aiming at improving the capacity of these surfaces to support endothelialization.

Thus, combining the RGDS sequence with either REDV or YIGSR motifs could potentially lead to improved values of EC adhesion. In this regard, the ability of the peptides YIGSR, PHSRN and RGDS, and their combinations, to selectively affect the adhesion of ECs and SMCs onto PEG had been evaluated by Fittkau et al. [13]. However, the effect of immobilizing mixtures of RGDS, YIGSR and REDV peptides onto CoCr alloys, which are widely used as cardiovascular stents, has not been yet explored. The extent and quality of endothelialization strongly depends on the interactions established between functionalized surfaces and ECs. Such process should enhance ECs adhesion and migration [14], but ideally also reduce SMCs migration and proliferation, and prevent platelet adhesion and thrombogenicity [4].

In this work we evaluate the use of equimolar combinations of specific cell adhesive peptides to improve the endothelialization of CoCr surfaces for cardiovascular applications. To this end, CoCr surfaces were functionalized with the different oligopeptides, and after a thorough characterization of the physicochemical properties of the surfaces, the adhesion and proliferation of ECs, as well as the adhesion of SMCs, were examined at the in vitro level.

2. Materials and methods

2.1. Materials

2.1.1. Metallic surfaces

CoCr alloy (ASTM F90: Co–20Cr–14.6W–10.8Ni–2.5Fe–1.5Mn) (Technalloy, Barcelona, Spain) disks of 8,5 mm diameter and 2 mm thick were subsequently abraded with silicon carbide papers of decreasing grit size (P240, P400, P600, P800 and P1200) and finally polished with suspensions of 1 μm and 0,05 μm alumina powder in distilled water. Prior to the surface treatments, all samples were ultrasonically cleaned with ethanol, distilled water and acetone for 5 min each.

2.1.2. Solid-phase peptide synthesis

The linear peptides RGDS, REDV and YIGSR (Fig. 1(a) and Supplementary material Table S1) were manually synthesized in solid-phase following the Fmoc/tBu strategy and using 2-chlorotrityl-chloride resin (200 mg, loading of 1.0 mmol/g) as previously reported [36]. Briefly, Fmoc-L-amino acids (4 equiv) were sequentially coupled with either ethyl 2-cyano-2-(hydroxyimino)acetate (OxymaPure) (4 equiv), and *N*,*N*′-diisopropylcarbodiimide (DIC) (4 equiv); or *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate (HATU) (4 equiv) and *N*,*N*-diisopropylethylamine (DIEA) (8 equiv) as coupling systems. The efficiency of each reaction was monitored using the Kaiser test and/or by HPLC analysis. Once the peptide sequences were completed, cleavage from the resin was accomplished upon treatment with trifluoroacetic acid (TFA)/water/triisopropylsilane (TIS) (85:10:5, v/v/v) for 1–2 h in the presence of small amounts of dithiothreitol (DTT). The peptides were purified by semi-preparative HPLC and characterized by analytical HPLC and MALDI-TOF (Supplementary material Table S1). All chemicals required for the synthesis, including resins, Fmoc-L-amino acids and coupling reagents, were obtained from Iris Biotech GmbH (Germany) and Sigma-Aldrich (USA).

2.2. Surface functionalization

The peptides covalent immobilization onto CoCr surfaces was achieved through a three-step strategy consisting of (1) activation, (2) silanization and (3) peptide immobilization. Alternatively, peptides were deposited by simple physical adsorption on the CoCr surfaces. The process of CoCr surfaces functionalization is summarized in Fig. 1(b).

2.2.1. Surface activation

The surface of CoCr samples was activated by basic etching with 5 M NaOH solution during 2 h at room temperature (RT) (samples NA). Samples treated with the alkaline solution were cleaned twice in distilled water during 30 min. Non-activated CoCr samples were used as controls (CT).

2.2.2. Silanization

Activated samples were silanized by immersing the substrates in a 10 ml solution of 0.5 M 3-chloropropyltriethoxysilane (CPTES) (Sigma-Aldrich) and 0.05 M *N*,*N*-diisopropylethylamine (DIEA) in anhydrous toluene under nitrogen atmosphere for 1 h at 90 °C under vigorous stirring. After silanization was completed, the disks were ultrasonically washed with cyclohexane, isopropanol, distilled water, and acetone, for 15 min each, and finally dried with nitrogen. The CPTES-modified substrates were stored under vacuum. Silanized samples were coded as NA-CP.

2.2.3. Peptide attachment

Finally, 100 μl of peptide solutions (RGDS, REDV and YIGSR) at 100 μM in PBS at pH 13.0 (adjusted with Na_2CO_3) and their combinations (1:1) were deposited on the CPTES-grafted surfaces overnight at RT. The immobilization of the peptides by physical adsorption was done under the same conditions but using PBS at pH 7.0 instead. After the immobilization protocol, samples were washed three times with distilled water. Prior to cell adhesion assays, functionalized samples were blocked for 1 h at