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Peptide-grafted poly(ethylene glycol) hydrogels support dynamic adhesion of endothelial progenitor cells

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ABSTRACT

This study investigated the dynamic adhesion of endothelial progenitor cells (EPCs) to peptide-grafted poly(ethylene glycol) diacrylate (PEGDA) hydrogels and determined the relative ability of RGDS, REDV and YIGSRG peptides to reduce the velocity of EPC rolling. Circulating EPCs are key mediators of endothelium repair and have been shown to accelerate re-endothelialization, which is important in reducing the incidence of restenosis following stent placement and occlusion of small diameter vascular grafts. However, to exploit these capabilities for tissue engineering applications, more knowledge is needed about EPC binding to the vascular wall under shear and, in particular, whether the incorporation of peptide ligands into biomaterials can support the process of EPC rolling or maintain EPC adhesion. This study specifically examined one type of EPCs endothelial colony forming cells (ECFCs), based on their ability to be expanded in culture and differentiate into mature endothelial cells. The amount of grafted PEG–peptide was shown to be dependent on the concentration of PEG–peptide grafting solution photopolymerized onto the hydrogel surface. The ECFC strength of adhesion on PEG–RDGS grafted hydrogels exceeded 350 dyn cm^{-2} for 85% of adherent cells. PEG–RGDS grafted hydrogels supported ECFC rolling, whereas ECFC velocity on the negative control PEG–RGES grafted hydrogels and on the "blank slate" PEGDA hydrogels was substantially higher than the cutoff velocity for cell rolling. The ECFC rolling velocity on PEG–RDGS grafted hydrogels depended on the shear rate; as shear rate was increased from 20 s^{-1} to 120 s^{-1}, ECFC rolling velocity increased from 103 ± 3 μm s^{-1} to 741 ± 28 μm s^{-1}. REDV and YIGSRG, which are known to preferentially support endothelial cell adhesion, also supported ECFC rolling. Interestingly, the rolling velocity of ECFCs on PEG–REDV grafted hydrogels was significantly lower than on PEG–YIGSRG or on PEG–RGDS grafted hydrogels. Understanding the dynamic adhesion of ECFCs to peptide-grafted hydrogels is the first step towards understanding the similarities and differences of EPCs from mature endothelial cells and improving the ability to sequester EPCs to biomaterial surfaces in order to promote intravascular re-endothelialization.

1. Introduction

Endothelial progenitor cells (EPCs) are an important autologous cell source for many tissue engineering applications [1]. Understanding the interactions of these cells with materials will be critical, however, in taking advantage of their usefulness. Endothelial cells (ECs) form the lining of blood vessels, making them vital for the prevention of thrombosis in established vessels as well as for new blood vessel formation. Therefore, EPCs could prove useful for endothelializing small-diameter vascular grafts, for vascularizing tissue-engineered constructs, for repairing areas of the vasculature damaged by atherosclerosis, and for preventing restenosis, or the re-narrowing of vessels, following balloon angioplasty.

EPCs are a subpopulation of monocytes that are derived from myeloid cells, which are one type of leukocyte [2]. EPCs that have been isolated from blood and expanded in vitro are frequently called late outgrowth or endothelial colony forming cells (ECFCs) [3]. Expression of surface receptors on these cells differs from other types of monocytes, "early outgrowth" EPCs and mature endothelial cells [4]. Advantages of ECFCs for tissue engineering applications include the relative ease and lack of comorbidity in obtaining autologous cells, the highly proliferative nature of ECFCs and the ability of ECFCs to yield mature endothelial cells [5,6]. To exploit their potential, however, it is necessary first to understand whether ECFCs behave similarly to ECs in their abilities to interact with engineered biomimetic materials and which cell surface receptors mediate these interactions.

Cell rolling is a phenomenon that has been historically observed in the recruitment of leukocytes, such as EPCs during inflamma-

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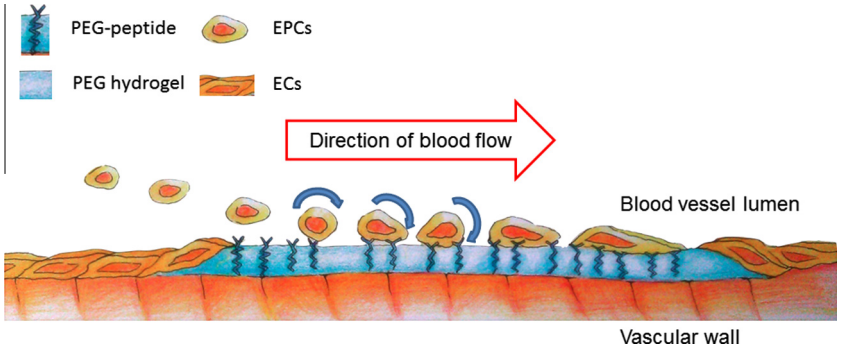

Fig. 1. Schematic of EPC capture on a biomaterial surface for enhancing re-endothelialization of blood vessels and vascular grafts. The goal of this study was to investigate the ability of peptide-grafted hydrogels to support EPC rolling and strength of adhesion for eventual use in facilitating rapid endothelialization of injured vessels.

tion. When a leukocyte comes in contact with the endothelium, surface receptors on the leukocyte interact adhesively with the ligands present on the endothelium. However, this adhesion is transient as a result of the shear force exerted on the cells by flowing blood. This force causes the leukocyte to roll along the endothelium with the flow stream until the overall cellular adhesiveness, or avidity, is strong enough to withstand the shear forces and support firm adhesion [7]. The strength of the adhesion depends on the number and types of bonds formed between the leukocyte and the endothelium. Cell rolling is the result of balanced bond formation and breakage, and it seems to be a necessary step before leukocytes are firmly adhered to the endothelium. Cell rolling velocity can be used to characterize cell rolling [8], and it is determined by finding the mean of instantaneous velocity during rolling.

EPCs are thought to act similarly to other monocytes in that they originate from the bone marrow, circulate in the blood, roll along the vessel wall, and eventually firmly adhere [4]. EPCs can then participate in local repair of the vascular endothelium [9] or extravasate and participate in neovascularization [10]. EPC rolling has been observed both in vitro on extracellular matrix (ECM) proteins [11] and in vivo on the endothelium [12]. For tissue engineering applications, designing materials that exploit the natural mechanisms for EPC rolling and capture could substantially advance the ability of these materials to be endothelialized intravascularly, as illustrated in Fig. 1. Ideally, ECFCs could be expanded in vitro from circulating EPCs injected into the blood vessel upstream of a location needing endothelialization, and then captured on the intravascular material surface. Even if the risk of angiogenesis-mediated tumor growth [13–15] or other logistical considerations preclude distal injection of ECFCs, designing materials that support firm ECFC adhesion in a relatively short time will still be highly advantageous for use in enhancing endothelialization.

To understand the interactions of specific material-bound ligands with cell surface receptors on ECFCs, it is important to eliminate the confounding effects of adsorbed proteins on the surface with which the cells are interacting. Poly(ethylene glycol) diacrylate (PEGDA) hydrogels have been demonstrated to be an effective "blank slate" material in vitro for this purpose [16]. Using a grafting technique, peptides of interest can be immobilized on PEGDA hydrogels. These peptide-grafted PEGDA hydrogels can then be used to evaluate the peptides' potential to support ECFC adhesion and rolling. Other types of ECs are known to interact preferentially with the peptide YIGSR [17], which is recognized by the 67 kDa laminin binding protein cell adhesion receptor, and the peptide REDV, which is recognized by the $\alpha_4\beta_1$ integrin receptor [18,19]. ECs have been shown to bind REDV selectively over smooth muscle cells, fibroblasts and platelets [18]. YIGSR is known to enhance EC migration [17] and has been shown to promote endothelialization [20], EC adhesion and spreading [21]. Research has shown that YIGSR modified poly(ethylene glycol) (PEG)/polyurethaneurea gels support EC adhesion, but not platelet adhesion [22]. In addition, ECs are known to interact with the peptide RGDS, which is widely used in tissue engineering as a cell adhesion ligand, because it is recognized by many integrin receptors [23]. RGDS is present in various ECM proteins including fibronectin, vitronectin, laminin and collagen [24].

PEG–peptide copolymers can be coated on the interior surface of blood vessels and vascular grafts through interfacial photopolymerization [25,26] and can also be used as matrices for tissue engineering [27]. Specific applications include vascular grafts [28], vascularization of synthetic biomaterial [29] and next-generation stenting [30,31]. To support vascular re-endothelialization, biomimetic materials should be able to maintain adherent cells on their surfaces under physiological shear stress, support migration and proliferation of mature ECs from the ends of the lesion, and provide appropriate ligands for the rolling and eventual firm adhesion of ECFC.

This study examined the grafting of PEG–peptides onto PEGDA hydrogels and evaluated the ability of these coupled peptides to support ECFC adhesion and rolling. First, the capacity of PEGDA alone to serve as a viable "blank slate" material for testing the interactions of coupled peptides with ECFCs was assessed. Concentration dependence of PEG–peptide grafting to PEGDA hydrogels was then investigated. The ability of PEG–RGDS grafted hydrogels to support adherent ECFCs when subjected to superphysiological shear stress was next characterized using a parallel-plate flow chamber. Finally, ECFC rolling velocity on PEG–RGDS, PEG–YIGSRG and PEG–REDV was quantified.

2. Materials and methods

2.1. ECFC culture

Human umbilical cord blood derived ECFCs (Poietics Human Endothelial Colony Forming Cells, Lonza) were expanded in collagen (BD)-coated tissue culture polystyrene flasks using EGM-2 growth media (Lonza). When ECFCs reached 80–90% confluency, they were subcultured using trypsin (0.025%, Lonza) at 37 °C for 1.5 min. Trypsin was neutralized by the addition of ECFC media, followed by centrifugation at 200*g* for 5 min. Cells were resuspended in ECFC media and subcultured at a ratio of 1:3 or used for experiments (ECFCs were received at passage 6 and used at passage 8–16).

2.2. PEG acrylation

PEG (6 kDa; Sigma) was acrylated to form PEGDA following methods that have been previously described [32]. In brief, PEG