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Engineered endothelial cell adhesion via VCAM1 and E-selectin antibody-presenting alginate hydrogels

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ABSTRACT

Materials that adhere to the endothelial cell (EC) lining of blood vessels may be useful for treating vascular injury. Cell adhesion molecules (CAMs), such as endothelial leukocyte adhesion molecule-1 (E-selectin) and vascular cell adhesion molecule-1 (VCAM1), modulate EC–leukocyte interactions. In this study, we mimicked cell–cell interactions by seeding cells on alginate hydrogels modified with antibodies against E-selectin and VCAM1, which are upregulated during inflammation. ECs were activated with interleukin-1α to increase CAM expression and subsequently seeded onto hydrogels. The strength of cell adhesion onto gels was assessed via a centrifugation assay. Strong, cooperative EC adhesion was observed on hydrogels presenting a 1:1 ratio of anti-VCAM1:anti-E-selectin. Cell adhesion was stronger on dual functionalized gels than on gels modified with anti-VCAM1, anti-E-selectin or the arginine–glycine–aspartic acid (RGD) peptide alone. Anti-VCAM1:anti-E-selectin-modified hydrogels may be engineered to adhere the endothelium cooperatively.

1. Introduction

A fundamental challenge in tissue engineering is modulating cell adhesion, as it affects cell migration and assembly [1]. Integrin–extracellular matrix (ECM) interactions are employed to regulate cell adhesion [2,3]. For example, arginine–glycine–aspartic acid (RGD) peptides are widely used to increase cell attachment to materials that are characterized as non-adhesive, such as hyaluronic acid (HA) [4], polyvinyl alcohol (PVA) [5] and alginate [6].

Engineered substrate surface chemistry has enabled control over cell behavior. The type and number of binding sites have affected cell migration and differentiation [7]. Increasing the molecular surface density beyond the saturation density, however, does not increase adhesion strength [8]. Instead, nature has used cooperativity to control cell adhesion via multiple receptor–ligand interactions [9]. We have shown previously that optimal ratios of cell adhesion molecules (CAMs) cooperatively bind drug delivery vehicles [10–12]. Enhanced cell adhesion was shown to be dependent on the relative molecular surface density; vehicles presenting an optimal anti-VCAM1:anti-E-selectin ratio that complemented EC surface expression showed increased binding [10].

To overcome the saturation of integrin–ECM interactions, we proposed to use dual functionalized hydrogels to strengthen cell adhesion. As a model system, we engineered cellular adhesion by modifying materials with antibodies against CAMs that are upregulated on the surface of inflamed endothelial cells (ECs). CAM expression is regulated by cytokine stimulation, shear stress, substrate mechanical properties and cell–cell interactions [13–15]. CAMs, including endothelial leukocyte adhesion molecule-1 (E-selectin) and vascular cell adhesion molecule-1 (VCAM1), are known to be expressed on ECs in inflammatory diseases such as atherosclerosis [16,17] and cerebral aneurysms [18].

In this study, we hypothesized that alginate hydrogels presenting antibodies against VCAM1 and E-selectin may result in strong, cooperative adhesion. We measured cell retention as a function of time and increasing force via a centrifugation assay. Engineering cell adhesion strength may be useful in the development of materials for vascular remodeling, where binding of materials to the endothelium may be compromised by hemodynamic forces.

2. Materials and methods

2.1. Materials

Calcium sulfate ($CaSO_4$), alginate (medium viscosity), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES), and sodium chloride (NaCl) were purchased from Sigma Aldrich (St. Louis, MO). Hank's Balanced Salt Solution (HBSS) and phosphate buffered saline (PBS) were purchased from Invitrogen (Carlsbad, CA).

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