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ScienceDirect
Acta Biomaterialia 5 (2009) 865–875
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Production of heparin-containing hydrogels for modulating cell responses ☆

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Received 12 August 2008; received in revised form 14 November 2008; accepted 8 December 2008
Available online 24 December 2008

Abstract

Successful tissue regeneration requires that biomaterials have optimal bioactivity and mechanical properties. Heparin-containing hydrogels that can be crosslinked in situ were designed to contain tunable amounts of biological components (e.g. heparin, arginine–glycine–aspartate (RGD)) as well as to exhibit controlled mechanical properties (e.g. shear modulus). These gel parameters can also be tuned to provide controlled delivery of proteins, such as growth factors, for regulating cellular behavior. Maleimide-functionalized low-molecular-weight heparin (LWMH) was conjugated to a poly(ethylene glycol) (PEG) hydrogel. The elastic shear modulus, as assessed via oscillatory rheology experiments, could be tuned by the concentration of polymer in the hydrogel, and by the end group functionality of PEG. Hydrogels of two different moduli (2.8 and 0.4 kPa) were used to study differences in the response of human aortic adventitial fibroblasts (AoAF) in two-dimensional cell culture experiments. These experiments indicated that the AoAFs show improved adhesion to materials with the higher modulus. Evaluation of cell responses to hydrogels with RGD linked to the hydrogels via conjugation to PEG or to LMWH indicated improved cellular responses to these materials when the bioactive ligands were chemically attached through linkage to the PEG rather than to the LMWH. These results highlight important design considerations in the tailoring of these materials for cardiovascular tissue engineering applications.

Keywords: Cell binding; Heparin; Cell response; Hydrogel

1. Introduction

Controlling cell-mediated remodeling of cardiovascular implants using biomaterials is a concept with great appeal. Biological vessels often become occluded due to maladaptive cellular responses elicited by the tissue injury and hemodynamic changes associated with surgical or catheter-based procedures [1]. Autologous vein grafts used in leg artery bypass surgery, for example, fail in the first few years at a rate approaching 50% primarily due to inappropriate graft remodeling [2]. The development of biomaterials that beneficially influence cell responses and vessel remodeling would have significant clinical impact.

Blood vessel remodeling depends to a great degree on the activation of adventitial fibroblasts (AFs) [3,4], which populate the outermost layer of blood vessels. AFs are the major cell type in the adventitia and critical contributors to the structural integrity, growth and remodeling of blood vessels in vivo [4–6]. AFs not only play a significant role in extracellular matrix production during adventitial remodeling but also help recruit and organize the microvascular blood supply necessary to feed the cells within the vessel [7]. The remodeling and organizing functions of

☆ Part of the Self-Assembling Biomaterials Special Issue, edited by William L. Murphy and Joel H. Collier.

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doi:10.1016/j.actbio.2008.12.004

AFs are largely determined by physical and chemical cues from the extracellular matrix, and AFs undergo phenotypic conversion in response to changes in their local physical and humoral environment [6,8]. Biomaterials that can be placed easily within or immediately surrounding the adventitia of at-risk blood vessels and that are capable of controlling AF function would be useful in attenuating maladaptive vessel remodeling and encouraging the recruitment of microvasculature to improve graft survival.

Hydrogels offer unique opportunities to control the distribution and function of cells in engineered or biological tissues, and several characteristics recommend advanced hydrogels for cardiovascular applications. Hydrogel components are injectable, and gels can be formed rapidly in situ using catheter-based injection methods applied to the target blood vessel at the time of the clinical procedure or subsequently. Since the elastic shear modulus of hydrogels can be easily controlled, gels of relatively low modulus could be designed to optimize cellular responses without adversely affecting intrinsic vessel stiffness. Cellular components (e.g. AFs and microvascular endothelial cells) could be readily encapsulated within gel matrices to help drive the formation of healthy adventitia and would remain localized at the therapeutic target site during the gel erosion process. Precisely designed hydrogels with controlled local mechanical properties and the inclusion of critical growth factors could direct formation of the target vessel's vascular supply (i.e. vaso vasorum) while controlling maladaptive remodeling. Thus, understanding how the chemical and physical characteristics of hydrogels influence AFs is an important goal in the development of hydrogels for cardiovascular applications.

It has been well documented that variations in the mechanical properties of matrices result in significant differences in cell behavior [9]. Matrix stiffness affects cell adhesion, proliferation, and differentiation [10,11]. The actin–myosin cytoskeleton, whose contractile forces are transmitted through transcellular structures, may play an important role in transferring information about the mechanical properties of matrices to cell nuclei. In previous work, primate aortic AFs (AoAFs) and smooth muscle cells (SMCs) were found to mediate matrix contraction of collagen-hyaluronan (HA) gels. In particular, the results showed that HA-promoted contraction of collagen gels by AFs and SMCs led to changes in collagen organization and cell shape [12].

The further development of the above and other hydrogel materials, which have water content and mechanical properties that are often comparable to that of soft tissue, has remained an area of great research interest owing to their potential clinical application in drug delivery, tissue augmentation, and tissue repair and regeneration [9,13]. Strategies to integrate synthetic and biomolecular materials into delivery vehicles have remained an important goal. The cell-adhesive character of hydrogels can be readily manipulated via the inclusion of integrin-binding ligands, such as arginine–glycine–aspartate (RGD). It is well recognized that the presentation of RGD alone, within intact cell adhesion proteins or within matrices containing multiple adhesion sequences, can alter the effectiveness of RGD-binding by cells [14–17]. The evaluation of these events in a given material of interest is necessary for implementation. The use of polysaccharides, particularly glycosaminoglycans such as heparin, offers opportunities to tailor the hydrophilicity of the gel, to modulate mechanical properties via noncovalent interactions with proteins and peptides, and to sequester and protect growth factors [18,19]. Accordingly, heparin has been incorporated covalently into numerous diverse drug delivery vehicles in which its interactions with proteins have permitted the controlled release of growth factors [20–22]. Our group and others have been investigating the noncovalent assembly, in addition to or instead of covalent crosslinking, of heparinized materials as a route to responsive, reversible and injectable drug delivery systems, with main interests in protein delivery and the production of extracellular matrix (ECM)-mimetic materials [19,23–26]. We have demonstrated multiple methods for producing noncovalently assembled hydrogel materials that are capable of either passive [19,24,27,28] or targeted delivery of growth factors in response to the VEGFR-2 receptor [25], and have developed covalent strategies to expand the versatility of these materials [19]. We have investigated methods to reliably functionalize heparin with chemically reactive groups at controlled degrees of substitution, and have studied the rapid in situ crosslinking between maleimide-functionalized heparin with thiol-functionalized poly(ethylene glycol) (PEG)-based polymers of various molecular weights and structures to produce hydrogels with controlled growth factor delivery profiles [19].

In our work here, we report modifications of the hydrogel synthesis to increase its compositional flexibility, to reduce the necessary chemical functionalization of heparin, to permit secondary noncovalent crosslinking by interactions with heparin-binding molecules, to allow for growth factor sequestration and delivery, and to render the resulting hydrogels more cell-adhesive for potential use in cardiovascular applications. Our current approaches utilize hydrogels comprising mainly star PEGs (both maleimide- and thiol-functionalized); such strategies permit increased independence in the tuning of modulus and composition of the hydrogels. A variety of hydrogels have been formed with the star PEGs to produce gels containing maleimide-functionalized heparin, thiol-functionalized RGD peptide and/or fibronectin (FN; fibronectin reacting presumably via reaction of thiol groups [10]). Oscillatory rheology indicates the flexibility in tuning of the hydrogel elastic shear modulus. The development of these new functionalization strategies also broadens the flexibility of the hydrogel synthesis and provides facile routes to tune the cell adhesion to the hydrogels; these materials can be applied as substrates for endothelial cells and fibroblasts in the design of therapeutic materials in cardiovascular applications. In particular, here we show that human aortic adventitial fibroblasts

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