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Enzymatic degradation of heparin-modified hydrogels and its effect on bioactivity

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ABSTRACT

The extracellular matrix is continually remodelled by the action of various enzymes such as heparanase, which specifically targets heparan sulfate (HS) and is found in human platelets at high levels. The activity of heparin-containing hydrogels following incubation with platelet extract (PE) was investigated in order to simulate the responses that might occur when the hydrogels, as tissue engineered scaffolds, come in contact with blood products at the site of an injury. The heparanase activity of PE on heparin, used as a model of HS, was confirmed by the decrease in molecular weight. PE treatment diminished heparin's anticoagulation property but increased its FGF-2 signalling activity, suggesting that the PE's heparanase activity cleaves at the 3-*O*-sulfated glucosamine to produce large fragments that can signal cell receptors. The dual effect observed when poly(vinyl alcohol)/heparin co-hydrogels were incubated with PE supports the hypothesis of platelets having the capacity to limit anticoagulation and thus promote blood clot formation, which may be critical in the process of tissue repair.

1. Introduction

Tissue engineered scaffolds are designed to mimic the extracellular matrix (ECM) by their structure and interactions with the surrounding host environment. Heparan sulfate (HS) molecules, which are a major part of the ECM, are continually synthesised and replaced in the body, a process that helps maintain homeostasis and regulate angiogenesis. HS is synthesised as a part of HS proteoglycan (HSPG), which consists of a protein core that carries linear HS chains composed of alternating hexuronic acid and glucosamine residues. These residues are differentially sulfated to create polydisperse chains with variably charged regions [1]. The HSPG sequesters numerous enzymes, growth factors and cytokines on the cell surface and in the ECM, often acts as an inactive reservoir [2]. Cleavage of the HSPG or HS chains releases fragments of these proteins or polysaccharides from the ECM and can affect their bioactivity [3]. In humans, the protein core of HSPG is susceptible to degradation by several proteases while the HS chains are cleaved by a single heparanase enzyme, an endo-β-glucuronidase that is often referred to as heparanase-1.

Heparanase cleaves HS at specific sites to release HS fragments as well as proteins such as growth factors that are trapped within the ECM or on cell surfaces [4,5], making them available to the cell surface receptors and causing a change in the environmental conditions that may modulate local tissue responses. One of the primary functions of heparanase is to degrade basement membrane HSPG at sites of injury or inflammation [6–8], allowing the migration of inflammatory cells and releasing HS-bound growth factors that promote the migration and survival of endothelial cells [9]. The release of basic fibroblast growth factor (FGF-2) by the heparanase action on HS has traditionally been correlated with cell invasion during angiogenesis and inflammation [10].

Platelets have been shown to be a good source of heparanase and the heparanase activity has been reported to be optimum at a pH range of 5.1–6.8 [6,11–13]. Other sources of heparanase include endothelial cells (EC) and smooth muscle cells (SMC), although the HS-degrading activity from these cells has been reported to be less that 10% that of platelet extract [13]. Nevertheless, cell lysates from both EC and SMC have been shown to contain factors that can further activate platelet heparanase.

Enzymatic degradation studies of biologically derived hydrogels have been performed to examine substrate availability on the crosslinked biopolymers for enzyme digestion. Collagen, for example, is degraded by metalloproteases and serine proteases, allowing degradation to be locally controlled by cells present in the engineered tissue [14]. Most of the studies have often used bacterial enzymes instead of those from mammalian sources, such

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