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Functional porous hydrogels to study angiogenesis under the effect of controlled release of vascular endothelial growth factor

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ABSTRACT

Angiogenesis occurs through a cascade of events controlled by complex multiple signals that are orchestrated according to specific spatial patterns and temporal sequences. Vascularization is a central issue in most tissue engineering applications. However, only a better insight into spatio-temporal signal presentation can help in controlling and guiding angiogenesis in vivo. To this end, versatile and accessible material platforms are required in order to study angiogenic events in a systematic way. In this work we report a three-dimensional porous polyethylene glycol (PEG) diacrylate hydrogel bioactivated with heparin that is able to deliver vascular endothelial growth factor (VEGF) in a sustained and controlled manner. The efficiency of the material has been tested both in vitro and in vivo. In particular, the VEGF released from the hydrogel induces cell proliferation when tested on HUVECs, retains its bioactivity up to 21 days, as demonstrated by Matrigel assay, and, when implanted on a chorion allantoic membrane, the hydrogel shows superior angiogenic potential in stimulating new vessel formation compared with unfunctionalized hydrogels. Moreover, in the light of potential tissue regeneration studies, the proposed hydrogel has been modified with adhesion peptides (RGD) to enable cell colonization. The porous hydrogel reported here can be used as a valid tool to characterize angiogenesis, and, possibly, other biological processes, in different experimental set-ups.

1. Introduction

Recovering the physiological functions of degenerated or injured tissues is one of the most critical aspects in tissue engineering and regenerative medicine [1,2]. Vascular integration between the implant and the surrounding tissues is key to achieving this goal. This requires the rapid formation of new capillaries to supply oxygen and the necessary nutrients and to remove waste products from cells [3,4]. In this field angiogenesis, i.e. blood vessel formation from pre-existing ones, plays a pivotal role. It is a very complex phenomenon which is regulated by several biochemical and biophysical factors. Several issues must be addressed in the promotion of angiogenesis in biological matrices such as porous scaffolds and hydrogels. Among these, tight control of the dose and temporal evolution of bioactive signals is fundamental to guide and direct proper cell functions [5]. In particular, for functional angiogenesis pore dimension and the spatial arrangement of bioactive molecules within the matrix play a critical role in blood vessel formation in vitro and vessel invasion in vivo. It is known that the minimum porosity required to regenerate blood vessel is generally considered to be 30–40 μm [6], in order to enable the transport of metabolic components and the induction of endothelial cell invasion. Furthermore, signals presented by the extracellular matrix (ECM), such as soluble macromolecules (e.g. growth factors (GF), chemokines, and cytokines) and insoluble factors (e.g. ECM proteins, glycoaminoglycans, and proteoglycans), also play a major role in tissue regeneration. Accordingly, angiogenic processes are guided by various growth factors whose spatial and temporal presentation is strictly regulated by various ECM components. For example, heparin molecules are known to bind various angiogenic growth factors, such as for vascular endothelial growth factor (VEGF), *basic fibroblast growth factor* (bFGF), and transforming growth factor *beta* (TGFβ), through non-covalent and reversible interactions [7]. Such an interplay brings two major benefits: first, bound GFs are less prone to degradation; second, the spatial arrangement of heparin molecules and their binding affinity for GFs provides cells with directional and temporal cues which guide and direct the process of new vessel formation. Cells are very sensitive to both the local concentration of VEGF and to the way it is delivered. High doses of VEGF elicit an evident tissue response, but generally lead to dysfunctional growth. Indeed, undesired

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