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Combining self-assembling peptide gels with three-dimensional elastomer scaffolds

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ABSTRACT

Some of the problems raised by the combination of porous scaffolds and self-assembling peptide (SAP) gels as constructs for tissue engineering applications are addressed for the first time. Scaffolds of poly(-ethyl acrylate) and the SAP gel RAD16-I were employed. The in situ gelation of the SAP gel inside the pores of the scaffolds was studied. The scaffold-cum-gel constructs were characterized morphologically, physicochemically and mechanically. The possibility of incorporating an active molecule (bovine serum albumin, taken here as a model molecule for others) in the gel within the scaffold's pores was assessed, and the kinetics of its release in phosphate-buffered saline was followed. Cell seeding and colonization of these constructs were preliminarily studied with L929 fibroblasts and subsequently checked with sheep adipose-tissue-derived stem cells intended for further preclinical studies. Static (conventional) and dynamically assisted seedings were compared for bare scaffolds and the scaffold-cum-gel constructs. The SAP gel inside the pores of the scaffold significantly improved the uniformity and density of cell colonization of the three-dimensional (3-D) structure. These constructs could be of use in different advanced tissue engineering applications, where, apart from a cell-friendly extracellular matrix -like aqueous environment, a larger-scale 3-D structure able to keep the cells in a specific place, give mechanical support and/or conduct spatially the tissue growth could be required.

1. Introduction

Regenerative purposes impose multiple requirements on the material constructs intended to help those aims. In cardiac tissue engineering, for instance, the material vehicle for an efficient cell supply should ideally, among other things, host a large number of cells, guarantee their viability in situ, allow a rapid vascularization and maybe give mechanical support to the infarcted myocardial tissue; this last requirement, in the case of neural tissue engineering, must be replaced by the appropriate guidance of axonal growth. It would be unlikely that the multifunctionality demanded for tissue engineering constructs by these applications could be achieved by simple, one-component material structures. Here we explore the extent to which the combination of two different materials imparts definite advantages to the resulting construct from a tissue engineering point of view.

Synthetic or natural hydrogels possess many properties similar to those of the extracellular matrix (ECM), which makes them good candidates for engineered cellular niches. However, these gels cannot meet demanding mechanical requirements, which greatly limits their uses. Mechanical strength and geometrical definiteness should be provided by porous scaffolds. In a previous work [1] we studied how to incorporate a hyaluronic acid hydrogel into the porous structure of poly(ethyl acrylate) (PEA) elastomer scaffolds. Problems related to the filling technique, to the in situ cross-linking of the gel and to the degree of pore coating or filling depending on the gel concentration, had to be addressed there. Other scaffolds with internal gel coating, such as the couples chitosan/fibrin [2] or polylactide/HA [3], have also been studied; in each case, the filling and the gelation or cross-linking inside the pores represented specific difficulties to overcome. In the present paper we study constructs consisting of PEA scaffolds with the self-assembling peptide (SAP) RAD16-I hydrogel filling the pores. Some general questions raised by the combination of these very different materials are addressed here. How should the scaffold be filled? Can the peptide solution within the pores be gelled in situ? How should the cells be incorporated into the scaffold? Will the cells diffuse through the SAP lodged previously in the scaffold? Does

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the construct indeed show better biological performance than the empty scaffold?

SAP hydrogels are a family of promising synthetic materials. They are injectable in aqueous solution and undergo spontaneous assembling into ordered nanostructures [4,5]. In this work, the RAD16-I peptide (commercially available as BD™ PuraMatrix™) has been used; it consists of a sequence of 16 aminoacids AcN-$(RADA)_4$-$CONH_2$ (R: arginine, A: alanine and D: aspartic acid), which forms β-sheet nanofiber-like gels as a response to pH or ion concentration changes [4,6–9]. In vitro, it encourages the adhesion of endothelial cells, their proliferation and formation of capillaries [10,11] to enhance cardiac myocyte survival [12], and to support the survival, differentiation capacity and migration of neural stem cells [13] and the formation of synapses [14]. In mice, when injected with cardiac progenitors, it promotes angiogenesis and differentiation into cardiomyocytes and vascular smooth muscle cells [15]. Furthermore, as a wholly synthetic ECM-like medium, it presents the advantages, over other protein gels, of being easily functionalizable and of being free from pathogenicity worries. However, this SAP gel is only slightly more consistent than an aqueous solution [16]; it does not meet the demands of shape stability and mechanical resistance of many tissue engineering applications. Thus, interest has focused in its possible combination with other structurally more consistent materials. In Ref. [17] a RAD16-I solution containing Schwann cells was employed to fill the lumen of 2.2 mm diameter cellulose channels, to study regeneration across 10 mm gaps after peripheral nerve injury.

At body temperature, PEA is rubber-like when cross-linked, and its mechanical properties (elastic and loss moduli) have values not dissimilar to those of soft biological tissues. It has shown excellent compatibility with different types of cells in vitro—chondrocytes [18], keratinocytes [19], endothelial cells [20], neural cells [21–24], osteoblasts [25] or dental pulp stem cells [26]—and has been recently proposed as a feeder-free platform for the maintenance and growth of human embryonic stem cells [27]. Due to its ease of polymerization, PEA can be prepared in the form of scaffolds with different architectures, liable to invasion by cells and intended as guiding support for cell growth [1,19,20,22,23,28–31].

Here we establish a protocol for the assembly of the RAD16-I peptide gel within the pores, having diameters smaller than 100 μm, of hydrophobic PEA scaffolds, and characterize the performance of the combination as delivery platforms and as cell growth niches. The present work is, to the best of our knowledge, the first work that studies such a hydrophobic scaffold plus RAD16-I hydrogel system as an in situ assembling filler of micropores. Though these constructs are intended for future tissue engineering applications, the first studies presented here, due to their preliminary methodological nature, were performed using a commercial line of mouse fibroblasts; they have served to choose the main assembly conditions and to understand the role of the gel as a cell diffusion and encapsulation medium. The system here presented with adipose-tissue-derived stem cells (ASCs) is currently being employed in animal experiments as a cell carrier to support the regeneration of cardiac tissue after myocardial infarction. In the present paper we characterize the assembly and cell seeding of these constructs.

2. Materials and methods

2.1. Preparation of the bare elastomer scaffolds

PEA scaffolds with interconnected spherical pores were obtained by a radical polymerization of the monomer mixture and a template leaching technique, following a procedure analogous to that used in Ref. [29]. Briefly, ethyl acrylate (99%, Aldrich) monomer was mixed with 1 wt.% of benzoin (98%, Scharlau) as photo-initiator and 2 wt.% of ethyleneglycol dimethacrylate (98%, Aldrich) as cross-linker, stirred for 15 min, injected in the corresponding porogen template, polymerized for 24 h under a UV source and post-polymerized for 24 h more in an oven at 90 °C. 5×5 cm^2 templates were previously obtained by sintering poly(methyl methacrylate) microspheres of 90 ± 10 μm in diameter (PMMA; Colacryl dp 300). After polymerization, the PMMA templates were removed by soxhlet extraction with acetone (Scharlab). After this, the solvent was slowly exchanged with water, and the scaffolds were dried under vacuum and stored until use. Their final thickness was ~0.8 mm.

Bulk 0.8 mm thick PEA sheets (hereafter 2DPEA) were synthesized by an analogous procedure, to be used as two-dimensional controls. The monomeric mixture was allowed to polymerize in a mold consisting of two glass plates with a rubber separator in between. Once polymerized, the sheets were rinsed in boiling acetone for 48 h with a solvent renewal and finally dried.

Samples for the different experiments were cut from the scaffolds or the bulk sheets as 8 mm diameter circles. Bulk samples were employed to determine the water contact angle and swelling of the PEA matrix, because in its porous format the pores would interfere with such measurements.

2.2. Preparation of the scaffold-cum-gel constructs

The SAP hydrogel RAD16-I (PuraMatrix™ 1% (w/v), BD Biosciences) was used as a filler of the scaffolds' pores. In order to reduce the viscosity of the stock solution, the original package of RAD16-I was placed in a bath sonicator (Bandelin) for 30 min at 25 °C and 30 W was applied. The stock solution was then diluted with water (extra pure, Scharlau) to a ready-to-use 0.15% (w/v) concentration (high enough to reach percolation of its network) one and vortexed (Elmi SkyLine) to ensure homogeneization. In order to facilitate the penetration of the still viscous SAP solution into the micropores of the hydrophobic PEA scaffolds, three different procedures were followed. The first method involved the incorporation of the peptide solution, loaded in a syringe, into the scaffolds by applying some pressure. The second method consisted in improving the wettability of the PEA scaffolds by a plasma treatment, and afterwards soaking them with the peptide solution. A Plasma-Electronic Piccolo chamber was employed, evacuated to 4×10^{-2} mbar before generation of Ar/O_2 (1:1) plasma at 25 W for 5 min. The third filling procedure consisted in the introduction of the scaffolds, together with the SAP solution, in a tube closed with a septum rubber stopper, pierced by a syringe's needle, and the application of vacuum performing successive movements of the piston.

Once filled with the peptide solution, the scaffolds were placed in a Petri dish and drops of phosphate-buffered saline (PBS) solution or culture medium were added to their boundaries to induce the self-assembly of the peptides. The SAP was allowed to gel in the pores of the scaffolds for 30 or 60 min, which are the limits of the time range recommended by the manufacturer to obtain free gels. The obtained constructs were used for measurements immediately to avoid drying. Bare PEA scaffolds were kept as controls.

2.3. Morphology of the scaffold-cum-gel constructs

Bare scaffold samples were examined by scanning electron microscopy (SEM) in a JSM 6300 (JEOL Ltd, Tokyo, Japan) device, with the samples previously sputter-coated with gold, 15 kV of acceleration voltage and 15 mm of working distance. The samples were fractured in liquid nitrogen in order to obtain images of the surface and the cross-sections.