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Cell-responsive hydrogel for encapsulation of vascular cells

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ABSTRACT

The *in vitro* potential of a synthetic matrix metalloproteinase (MMP)-responsive poly(ethylene glycol) (PEG)-based hydrogel as a bioactive co-encapsulation system for vascular cells and a small bioactive peptide, thymosin β4 (Tβ4), was examined. We show that the physical incorporation of Tβ4 in this bioactive matrix creates a three-dimensional (3D) environment conducive for human umbilical vein endothelial cell (HUVEC) adhesion, survival, migration and organization. Gels with entrapped Tβ4 increased the survival of HUVEC compared to gels without Tβ4, and significantly up-regulated the endothelial genes vascular endothelial-cadherin and angiopoietin-2, whereas von Willebrand factor was significantly down-regulated. Incorporation of Tβ4 significantly increased MMP-2 and MMP-9 secretion of encapsulated HUVEC. The gel acts as a controlled Tβ4-release system, as MMP-2 and MMP-9 enzymes trigger the release. In addition, Tβ4 facilitated HUVEC attachment and induced vascular-like network formation upon the PEG-hydrogels. These MMP-responsive PEG-hydrogels may thus serve as controlled co-encapsulation system of vascular cells and bioactive factors for *in situ* regeneration of ischemic tissues.

1. Introduction

A functional vascular system is essential for the formation and maintenance of most tissues in the body, and the lack of vascularization results in ischemic tissues with limited intrinsic regeneration capacity. In recent attempts to stimulate the regeneration of functional blood vessels in ischemic tissues, endothelial cells and their precursors have been injected into the ischemic site [1–4]. Alternatively, angiogenic cytokines such as vascular endothelial growth factor (VEGF) have been administered by direct injection into the ischemic tissues or into the coronary artery [5–7]. Although improved regional blood flow was reported in preclinical ischemic heart and limb animal models, and also in human clinical trials, a prolonged effect is hampered by low efficiency of incorporation within the recipient's vasculature (less than 3% of injected cells may engraft, mainly due to cell death) [8,9], and rapid clearance of the cytokines from the ischemic site [10,11]. Subsequently, we and others have engineered biomaterials with the goal of preventing *anoikis* and improving functional engraftment, employing biodegradable materials as cell carriers and as cell ingrowth matrices [12–16], or alternatively as a protective environment for the controlled release of active cytokines [17–21]. Although elevated survival and engraftment have been reported, we sought to explore enhancement of cell survival and engraftment by co-encapsulating vascular cells and cytokines in a bioactive hydrogel environment common to both.

We have recently developed a 3D PEG-based synthetic hydrogel material as an extracellular matrix analog with key biochemical characteristics of natural collagenous matrices; MMP-sensitive peptides are used to crosslink telechelically-reactive branched PEG chains, producing a hydrogel matrix capable of cell-mediated proteolytic degradation and remodeling (Fig. 1A) [22]. These characteristics are also relevant in ischemic environments, where increased MMP-expression and activation have been observed [23–25]. Furthermore, the matrix-bound RGDSP adhesion peptide is co-incorporated into the matrix to promote cell adhesion via integrins that are known to be significant in vascular development and maintenance ($\alpha_5\beta_1$, $\alpha_v\beta_3$) [26]. Within these hydrogel matrices, we describe the physical incorporation of Tβ4, a 43-amino acid peptide previously shown to enhance survival of vascular cells and cardiomyocytes in ischemic environments [27–29], stimulate neovascularization after cardiac injury by inducing endogenous endothelial cell migration to the ischemic site [30,31], as well as

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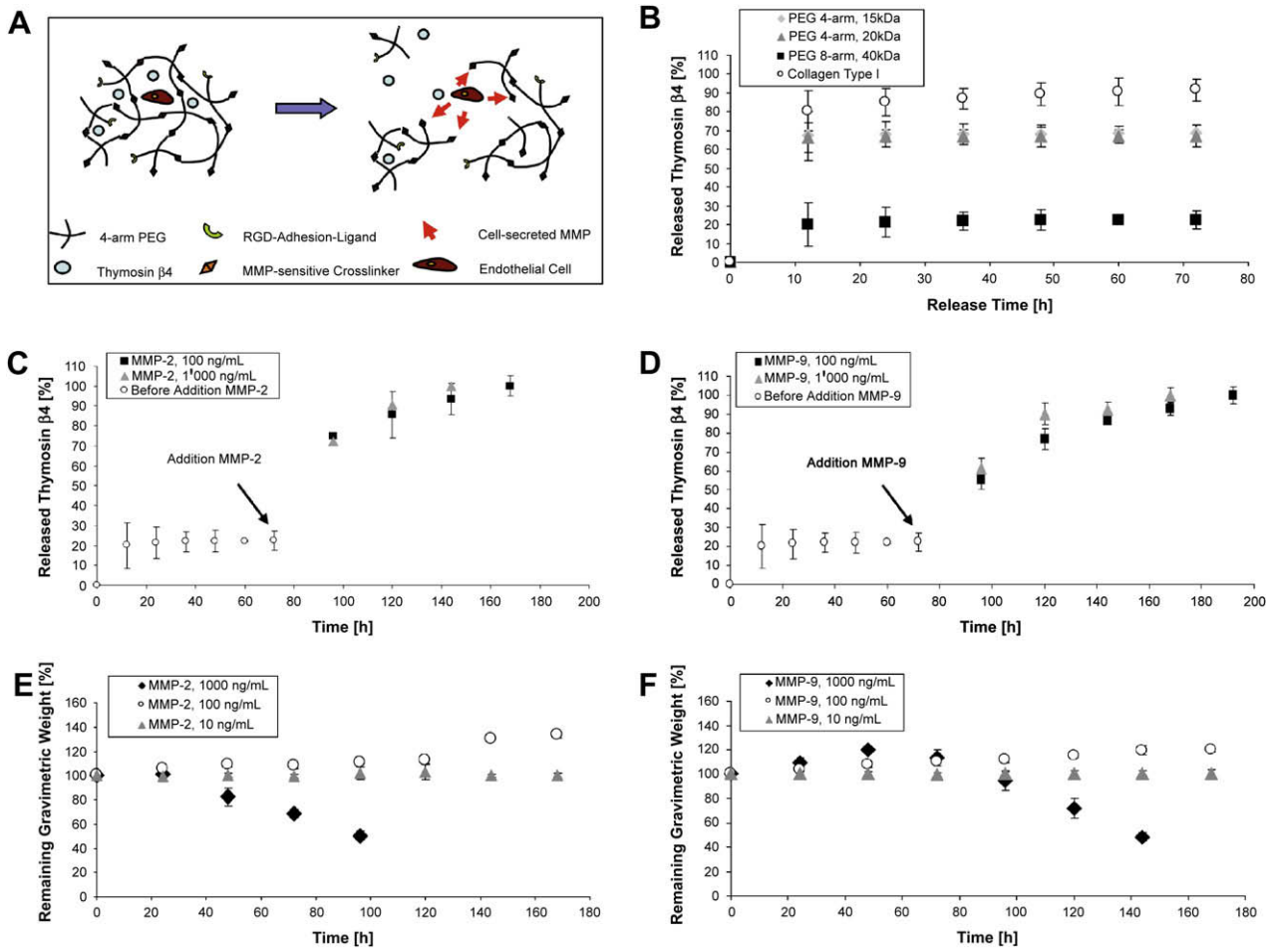

Fig. 1. (A) Scheme of co-encapsulation of HUVECs with Tβ4 in 3D MMP-responsive PEG-hydrogels. Reactive branched PEGs are crosslinked with bifunctional peptides, which are designed to be MMP substrates. The crosslinked gels that result are also functionalized with integrin-binding peptides. (B) Passive release (i.e., in the absence of MMPs) of Tβ4 from PEG-hydrogels and collagen type I gels. 8-arm PEG-gels ($M_w = 40$ kDa) were able to retain the physically incorporated Tβ4 peptide over time, whereas collagen type I gels released around 80% in an initial burst within the first 12 h. Crosslinking of branched PEGs with fewer arms was also less effective for entrapment. (C,D) Both MMP-2 (C) and MMP-9 (D) addition resulted in gel degradation. (E,F) MMP exposure resulted in Tβ4 release, for both MMP-2 (E) and MMP-9 (F).

play a key role in down-regulating expression of inflammatory molecules [32]. In this paper, we examined the *in vitro* potential of these synthetic MMP-responsive gels as a bioactive co-encapsulation system of HUVEC and Tβ4.

2. Materials and methods

2.1. Synthesis of PEG-vinylsulfone and peptides (RGDSP, MMP substrate, Tβ4)

PEG-vinylsulfone was synthesized adapting our previous protocol [33]. In brief, branched 8- or 4-arm PEG-OH ($M_w = 40{,}000$ g/mol for 8-arm PEG; $M_w = 20{,}000$ g/mol and $M_w = 15{,}000$ g/mol for 4-arm PEG) (Shearwater Polymers, Huntsville, AL) was dried by azeotropic distillation in toluene (VWR, Nyon, Switzerland) for 4 h. Toluene was distilled off and the residue dissolved in dichloromethane (Fisher Scientific, Wohlen, Switzerland). Sodium hydride (Sigma–Aldrich, Buchs, Switzerland) was added at 20-fold molar excess over OH-groups. Divinylsulfone (Fluka, Buchs, Switzerland) was added at a 50-fold molar excess over OH-groups. The reaction was carried out at room temperature under argon with constant stirring for 24 h. After the addition of acetic acid (Fluka, Buchs, Switzerland), the mixture was filtered and concentrated by rotary evaporation. The polymer was then isolated by precipitation in ice-cold diethylether (Brunschwig, Basel, Switzerland) and filtered. Finally, the product was dried under vacuum, yielding 85%. The degree of PEG functionalization with vinylsulfone was determined by proton NMR spectroscopy (in $CDCl_3$) using a Bruker 400 spectrometer (Bruker BioSpin, Faellanden, Switzerland). Characteristic vinylsulfone peaks were observed at 6.1, 6.4, and 6.8 ppm. The degree of end group conversion was found to be ≈95%.

The integrin ligand peptide (Ac-GCGYG*RGDSP*G-NH_2), the substrates for MMP (Ac-GCRD*GPQGIWGQ*DRCG-NH_2) and the Tβ4 peptide (Ac-SDKPDMAEIEKFDKSKL-KKTETQEKNPLPSKETIEQEKQAGES-NH_2), were synthesized by solid phase peptide synthesis using NovaSyn TGR resin (Merck Biosciences, Laeufelfingen, Switzerland) with an automated peptide synthesizer (Chemspeed, Augst, Switzerland) with standard Fmoc chemistry. Purification was performed by mass-directed reverse phase-C_{18} HPLC using a Waters Autopurification System. Separation and collection were performed by UV and mass-directed software. Peptide sequences were confirmed by ion trap ESI mass spectrometry (all Waters, Baden-Daettwil, Switzerland).

2.2. Formation of PEG-hydrogels

Gel formation was done under physiological conditions as described elsewhere [33]. Briefly, the synthesis was carried out through Michael-type addition reaction of thiol-containing peptides onto vinylsulfone-functionalized PEG. PEG-vinylsulfone was dissolved in 0.3 M triethanolamine buffer pH 8.0 (Sigma Aldrich, St. Louis, MO, USA) to give a 10% (w/v) solution. A solution of the integrin ligand peptide (100 μM) in the same buffer was added to the PEG-vinylsulfone solution. After 10 min, the cell suspension, MMP-sensitive peptide in triethanolamine buffer was added (stoichiometric ratio between the crosslinking MMP-peptide and the PEG-arms: 1.2). The crosslinking reaction was continued for around 30 min, at 37 °C. To enable cell survival analysis through flow cytometry, the cells were extracted from the matrix after controlled degradation with collagenase type IV. Gel degradation was carried out at standard incubator conditions (37 °C, 5% CO_2) for 30 min with 2 ml collagenase type IV of 1.0 mg/mL in PBS (both from Invitrogen, Carlsbad, CA, USA).

2.3. Release of Tβ4 from PEG- and collagen-gels

Tβ4 (40,000 ng/mL gel) was physically entrapped into 25 μL PEG- or collagen-gels (Vitrogen 100, Cohesion, Palo Alto, CA, USA, at 2 mg/mL) by mixing it into the PEG-precursor solution before gelation, or collagen solution before initiating solidification by the addition of 0.1 M NaOH. Tβ4 release from both materials was analyzed with a competitive ELISA kit (Bachem, Peninsula Laboratories, San Carlos,