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Reprint of: Development of bioactive peptide amphiphiles for therapeutic cell delivery

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ABSTRACT

There is great clinical interest in cell-based therapies for ischemic tissue repair in cardiovascular disease. However, the regenerative potential of these therapies is limited due to poor cell viability and minimal retention following application. We report here the development of bioactive peptide amphiphile nanofibers displaying the fibronectin-derived RGDS cell adhesion epitope as a scaffold for therapeutic delivery of bone marrow derived stem and progenitor cells. When grown on flat substrates, a binary peptide amphiphile system consisting of 10 wt.% RGDS-containing molecules and 90 wt.% negatively charged diluent molecules was found to promote optimal cell adhesion. This binary system enhanced adhesion 1.4-fold relative to substrates composed of only the non-bioactive diluent. Additionally, no enhancement was found upon scrambling the epitope and adhesion was no longer enhanced upon adding soluble RGDS to the cell media, indicating RGDS-specific adhesion. When encapsulated within self-assembled scaffolds of the binary RGDS nanofibers in vitro, cells were found to be viable and proliferative, increasing in number by 5.5 times after only 5 days, an effect again lost upon adding soluble RGDS. Cells encapsulated within a non-bioactive scaffold and those within a binary scaffold with scrambled epitope showed minimal viability and no proliferation. Cells encapsulated within this RGDS nanofiber gel also increase in endothelial character, evident by a decrease in the expression of CD34 paired with an increase in the expression of endothelial-specific markers VE-Cadherin, VEGFR2 and eNOS after 5 days. In an in vivo study, nanofibers and luciferase-expressing cells were co-injected subcutaneously in a mouse model. The binary RGDS material supported these cells in vivo, evident by a 3.2-fold increase in bioluminescent signal attributable to viable cells; this suggests the material has an anti-apoptotic and/or proliferative effect on the transplanted bone marrow cells. We conclude that the binary RGDS-presenting nanofibers developed here demonstrate enhanced viability, proliferation and adhesion of associated bone marrow derived stem and progenitor cells. This study suggests potential for this material as a scaffold to overcome current limitations of stem cell therapies for ischemic diseases.

1. Introduction

Despite advances in modern therapy, ischemic tissue disease remains one of the foremost causes of morbidity and mortality [1]. Interest in the regenerative potential of cell-based therapies for ischemic tissue has gained momentum through the description of endothelial progenitor cells (EPCs), lineage-committed precursors of mature endothelial cells that combine endothelial and stem cell characteristics [2]. It has been shown that EPCs are mobilized from the bone marrow in response to an ischemic trigger and home to the ischemic zone where they participate in repair of ischemic tissue through paracrine effects and de novo blood vessel formation [3,4]. Subsequent studies have attempted to leverage the endogenous stem/progenitor cell mechanism through therapeutic applications of various cell types to the region of interest in cardiovascular diseases. In the clinical setting, the application of

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unselected bone-marrow mononuclear cells (BMNCs) is most advanced, showing promise in the treatment of acute [5] and chronic [6] myocardial infarction and peripheral arterial disease [7]. It remains uncertain, however, which subtypes of BMNC-derived cells elicit this regenerative effect and comparative studies of different cell lineage are pending. The excitement surrounding these cell-based therapies has been tempered, however, by several practical limitations, including limited local retention and poor viability of transplanted cells within the ischemic tissue [8,9]. In order to exploit the full regenerative potency of these cell therapies, it is crucial to overcome these limitations; especially when considering the observed dose–response relationship found in preclinical and clinical studies coupled with the known dysfunctionality and pro-apoptotic state of cells isolated from older multimorbid cardiovascular patients in autologous strategies [6,10,11]. Thus, promoting retention and viability of transplanted cells within the target region is of particular clinical interest to improve the efficacy of cell-based therapies.

Synthetic cell delivery scaffolds, often polymer-based systems, have been developed for cell-based therapies in order to enhance their retention at the treatment site [12]. Attempts to incorporate a signaling capacity into these otherwise non-bioactive materials has led to the incorporation of epitopes for cellular interaction, particularly motifs that foster cell adhesion [13]. Biological adhesion to native extracellular matrix (ECM) occurs, in part, through binding of integrin proteins on the cell surface to specific epitopes present on proteins of the ECM, creating a focal adhesion, anchoring the cell and allowing for communication with the surrounding environment [14–16]. One such ECM protein responsible for biological adhesion, fibronectin, binds to integrins through a domain containing Arg-Gly-Asp-Ser (RGDS) [17,18]. Previous studies have shown that RGDS epitope-spacing is a crucial factor for cell recognition and response [19–22]. The RGDS sequence, or sometimes the abbreviated RGD sequence, has been incorporated into a variety of synthetic materials to promote cell interaction and adhesion. Recently, alginate scaffolds presenting an RGD epitope were used as a depot for therapeutic applications of vascular progenitor cells to a hind-limb ischemia model, with results showing enhanced efficacy when using this biomaterial delivery vehicle [23].

The use of supramolecular self-assembly to create biomaterials offers the possibility of controlling the architecture, shape and dimensions of bioactive nanostructures, as well as the spatial display and density of bioactive signals. This is made possible by the local order in the assembled one-dimensional structures [24,25]. Previously, our laboratory developed several classes of self-assembling biomaterials [26–30] including a class of synthetic peptide amphiphiles (PA). PAs contain a hydrophobic alkyl segment covalently grafted to an amino acid sequence composed of a domain controlling self-assembly of the molecules into nanofibers through hydrogen bonding and a domain allowing for presentation of cell-signaling sequences or protein-binding sequences. The assembly of molecules into nanofibers emulates ECM architecture, and by design allows the bioactive domain to be presented on the surface of the nanostructures as the alkyl tail is buried in the core of the fiber through hydrophobic collapse. Electrostatic screening of charged amino acids on these molecules by electrolytes in physiologic media triggers the self-assembly into high aspect-ratio nanofibers that form gel networks at relatively low concentrations, on the order of 1 wt.% [31,32]. PA nanofibers have been used previously for a host of biological applications. When presented on a PA, the laminin-derived IKVAV epitope showed differentiation of neural progenitor cells [33] and inhibition of glial scar formation while promoting axon elongation in a spinal cord injury model [34]. Another PA was designed to bind heparin for the delivery of angiogenic growth factors [35,36], while still others have been used for applications as magnetic resonance imaging contrast agents [37,38]. RGDS has been previously incorporated into PAs using various covalent architectures including linear, branched and cyclic epitope presentations [22,39,40]. Different PA molecules are capable of co-assembly, allowing for a specific bioactive molecule to be mixed with a different bioactive molecule or a non-bioactive diluent molecule to vary the epitope density on the assembled nanostructure for optimized cell signaling [41,42]. The optimal composition of an RGDS-presenting PA co-assembled with a diluent molecule was previously determined to be between 2.5% and 10% for 3T3 fibroblasts, depending on the covalent architecture used [22].

In this work, we investigate RGDS-presenting PA nanostructures as a potential bioactive vehicle for BMNC delivery. We first explore optimization of BMNC biological adhesion in vitro and then assess the feasibility of this RGDS nanofiber gel to support these cells in vivo. Since the limitations of BMNC-based therapies for ischemic cardiovascular diseases center around cell viability and retention following targeted application, our goal is to develop a bioactive RGDS-presenting nanofiber matrix that could serve as a cell delivery system for ischemic tissue therapies.

2. Materials and methods

2.1. Synthesis and purification of peptide amphiphiles

We synthesized five different PAs for this study having the following amino acid sequences covalently linked to a 16-carbon alkyl segment: C_{16}-$V_3A_3K_3$RGDS (RGDS), C_{16}-$V_3A_3K_3$DGSR (scrambled), C_{16}-$V_3A_3K_3$ (K_3 diluent), C_{16}-$V_3A_3R_3$ (R_3 diluent) and C_{16}-$V_3A_3E_3$ (E_3 diluent). Structures for the primary PAs used in this study are shown in Fig. 1. All PAs were synthesized by standard solid-phase Fmoc chemistry on a CS Bio automated peptide synthesizer. Fmoc-protected amino acids, MBHA rink amide resin and HBTU were purchased from NovaBiochem and all reagents were purchased from Mallinckrodt. The resulting product was purified using standard reversed-phase high performance liquid chromatography. TFA counter-ions were exchanged by sublimation from 0.1 M hydrochloric acid. All PAs were dialyzed against deionized water using 500 MWCO dialysis tubing and isolated by lyophilization. The purity and accurate mass for each PA was verified using liquid chromatography/mass spectrometry on an electrospray ionization quadripole time-of-flight mass spectrometer (Agilent).

2.2. BMNC isolation and culture

Total bone marrow was obtained from 8 week old male FVB/N wild-type mice (Charles-River). The mononuclear cell fraction was isolated by density gradient centrifugation using Histopaque (Sigma). Isolated BMNCs were plated on rat fibronectin-coated (Sigma–Aldrich) culture dishes (Nunc) and maintained in endothelial basal medium-2 supplemented with EGM-2 SingleQuots (Lonza) containing FBS and VEGF-1, FGF-2, EGF, IGF-1 and ascorbic acid, in accordance with previously established culture methods [43]. Cells were cultured in this way to enrich for subpopulations of endothelial character while preventing differentiation into other lineages. After 4 days of culture, non-adherent cells were removed by exchanging the culture medium. Cells were used for experiments after 7 days in culture. This isolation protocol was approved by the Northwestern University Animal Care and Use Committee.

2.3. Diluent charge preference screening

Solutions of E_3, K_3 and R_3 diluent molecules were prepared at 0.01 wt.% in water and 100 μl was added to wells of a 96-well tissue culture plate. To coat the surfaces, the PA solution was