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Original article

Enhanced potency of cell-based therapy for ischemic tissue repair using an injectable bioactive epitope presenting nanofiber support matrix

Jörn Tongers [a,d,1], Matthew J. Webber [b,1], Erin E. Vaughan [a], Eduard Sleep [b], Marie-Ange Renault [a], Jerome G. Roncalli [a], Ekaterina Klyachko [a], Tina Thorne [a], Yang Yu [a], Katja-Theres Marquardt [a,d], Christine E. Kamide [a], Aiko Ito [a], Sol Misener [a], Meredith Millay [a], Ting Liu [a], Kentaro Jujo [a], Gangjian Qin [a], Douglas W. Losordo [a], Samuel I. Stupp [b,c,*], Raj Kishore [a,e,**]

[a] Feinberg Cardiovascular Research Institute, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[b] Institute for Bionanotechnology in Medicine, Department of Medicine, Northwestern University, Chicago, IL, USA
[c] Department of Materials Science and Engineering, Department of Chemistry, Northwestern University, Evanston, IL, USA
[d] Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany
[e] Center for Translational Medicine, Temple University School of Medicine, Philadelphia, PA, USA

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ABSTRACT

The translation of cell-based therapies for ischemic tissue repair remains limited by several factors, including poor cell survival and limited target site retention. Advances in nanotechnology enable the development of specifically designed delivery matrices to address these limitations and thereby improve the efficacy of cell-based therapies. Given the relevance of integrin signaling for cellular homeostasis, we developed an injectable, bioactive peptide-based nanofiber matrix that presents an integrin-binding epitope derived from fibronectin, and evaluated its feasibility as a supportive artificial matrix for bone marrow-derived pro-angiogenic cells (BMPACs) used as a therapy in ischemic tissue repair. Incubation of BMPACs with these peptide nanofibers *in vitro* significantly attenuated apoptosis while enhancing proliferation and adhesion. Pro-angiogenic function was enhanced, as cells readily formed tubes. These effects were, in part, mediated *via* p38, and p44/p42 MAP kinases, which are downstream pathways of focal adhesion kinase. In a murine model of hind limb ischemia, an intramuscular injection of BMPACs within this bioactive peptide nanofiber matrix resulted in greater retention of cells, enhanced capillary density, increased limb perfusion, reduced necrosis/amputation, and preserved function of the ischemic limb compared to treatment with cells alone. This self-assembling, bioactive peptide nanofiber matrix presenting an integrin-binding domain of fibronectin improves regenerative efficacy of cell-based strategies in ischemic tissue by enhancing cell survival, retention, and reparative functions.

1. Introduction

To combat the rising burden of ischemic cardiovascular disease [1], including myocardial and critical limb ischemia (CLI), new regenerative strategies must be explored. While endogenous repair mechanisms have been described, the preponderance of evidence indicates a limited capacity for self-repair in the cardiovascular system. Early clinical trials have suggested that the therapeutic application of adult stem and progenitor cells derived from the bone marrow may improve the repair and function of ischemic tissue following acute myocardial infarction [2–4], chronic myocardial ischemia [5], and CLI [6,7]. Cumulatively, these findings support the consideration of cell-based therapies as a treatment modality for ischemic cardiovascular disease [8]. However, the excitement from these early clinical studies has been tempered by uncertainties and practical limitations that have been encountered in the translation of cell-based therapy [9].

Evidence from preclinical tracking studies suggests that the majority of transplanted cells do not remain at the site of injury for more than a few hours following injection [10,11]. In patients, only ~2% of unselected bone marrow-derived mononuclear cells and 14–39% of CD34-enriched progenitor cells remain in the myocardium 1 h following intracoronary infusion, while the remainder localize to the spleen and liver [12,13]. A further reduction in the number of detectable cells is observed over the following 3–4 days [14]. The disease microenvironment, characterized by ischemia, acidosis, oxidative stress, and inflammation, likely contributes significantly to reduced viability and retention of transplanted

* Correspondence to: S. I. Stupp, Northwestern University, Cook Hall, Room 1127, 2220 Campus Drive, Evanston, IL 60208, USA. Tel.: +1 847 491 3002; fax: +1 847 491 3010.
** Correspondence to: R. Kishore, Center for Translational Medicine, Department of Pharmacology, Temple University School of Medicine, MERB-953, 3500 N Broad Street, Philadelphia, PA 19140, USA. Tel.: +1 215 707 2523; fax: +1 215 707 9890.
E-mail addresses: s-stupp@northwestern.edu (S.I. Stupp), Raj.kishore@temple.edu (R. Kishore).
[1] JT and MJW contributed equally to this work.

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cells. This is particularly problematic for autologous cell-therapy in older patients with severe cardiovascular disease and associated co-morbidities, as stem and progenitor cells isolated from these patients are reduced in number, prone to apoptosis, and show impaired functionality [15–17]. Cells are also altered during isolation through detachment from native extracellular matrix (ECM), which can trigger anoikis and compromise viability and therapeutic potential. Cumulatively, the number of viable cells immediately following transplant ranges from ~30% to merely 1%, with further decline over the next 7 days following application [18,19]. Additionally, the effects of cell-based therapies appear to be dose-dependent, with more transplanted cells resulting in a more robust therapeutic effect [20]. Taken together with the given challenges, this suggests strategies that improve viability, retention, and bioactivity of applied stem and progenitor cells could serve to overcome some of these obstacles in order to exploit the full regenerative potential of cell-based therapies to treat ischemic tissue repair including CLI.

The design of bioactive biomaterials for cell delivery could be important in improving efficacy of cell-based therapies [21–23]. In this context, a material would provide a microenvironment for cells resembling native tissue mechanics and architecture, facilitate cell–matrix interactions, and actively support cellular homeostasis through functional signaling. A class of bioactive materials composed of nanofibers that emulate extracellular matrix architecture has been developed based on synthetic self-assembling molecules known as peptide amphiphiles (PA) [24,25]. Preclinical efforts in a variety of disease models have demonstrated that this class of materials could have broad application to regenerative medicine [26–28] although their use to enhance stem cell retention and function in ischemic tissue repair models is not yet reported.

The molecules that form these bioactive materials consist of a hydrophobic alkyl segment attached to a customizable oligopeptide (Fig. 1). This amphiphilic molecular design guides self-assembly into nanofibers in aqueous environments. The filamentous architecture and mechanical properties can be controlled through the use of peptide sequences that form β-sheet hydrogen bonds, while bioactive sequences can be inserted at the terminal end of the peptide for display at high density on the nanofiber surface and can facilitate interaction with soluble proteins, receptors, and biopolymers, thus enabling the creation of an active signaling niche for cells [29–32]. The filamentous nanofibers can form three-dimensional gels upon electrostatic screening of their charged residues by electrolytes in physiologic fluids. Therefore, a viscous solution of PA nanofibers can be combined with cells and delivered *via* syringe injection for gelation in situ, obviating surgical implantation and minimizing associated tissue damage. The assembly and composition of PA molecules facilitate biodegradation into natural amino acids and lipids over weeks following injection [33]. In native ECM, integrin–matrix interactions enable a cell to communicate with its environment and help to promote cellular homeostasis and functionality [34]. One important ECM protein involved in cell–matrix interaction is fibronectin, which signals through integrins to control cellular functions that are important in the context of cell-based therapy, such as adhesion, survival, proliferation, and motility. Interestingly, fibronectin adhesion to integrins can be recreated using only a short segment of the whole protein, the Arg-Gly-Asp-Ser (RGDS) peptide [35]. Here we report that RGDS-PA nanofibers presenting this integrin-binding epitope facilitate improved efficacy of cell-based therapy in a murine model of hind limb ischemia. This represents a novel application of PA nanofiber technology to augment cell-based therapy.

2. Materials and methods

2.1. Preparation of peptide amphiphiles

PAs used in these studies were prepared identically to those reported previously [30]. Nanofiber composition consisted of 100% of a diluent sequence C16–V3A3E3 (diluent PA) or a binary mixture consisting of 90% of this same diluent with 10% C16–V3A3K3RGDS (RGDS-PA), based on the optimal ratio for this system [30]. A scrambled epitope control, C16–V3A3K3DGSR (DGSR-PA), was used at this same ratio with the diluent PA.

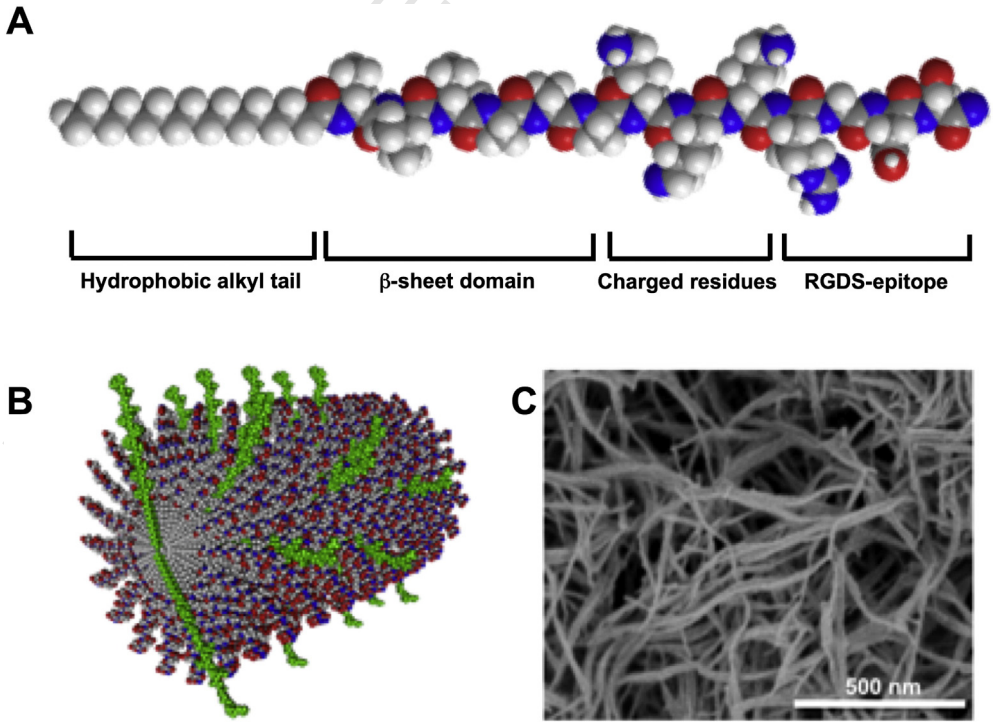

Fig. 1. (A) Molecular structure of an RGDS-PA molecule, indicating the specific design elements within the molecule. (B) In aqueous environments, PA molecules assemble into cylindrical nanofibers, with the RGDS-epitope-bearing molecules (green) distributed throughout the nanofiber. (C) Assembled nanofibers can form a three-dimensional nanofiber gel network, verified by a SEM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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