Acta Biomaterialia 58 (2017) 102–112

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Angiogenic peptide nanofibers repair cardiac tissue defect after myocardial infarction

Abdul Jalil Rufaihah [a,1], I. Ceren Yasa [b,1], Vaibavi Srirangam Ramanujam [a], Suganya Cheyyatraivendran Arularasu [a], Theo Kofidis [a,c,*], Mustafa O. Guler [b,d,*], Ayse B. Tekinay [b,e,*]

[a] Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[b] Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara 06800, Turkey
[c] Dept of Cardiac, Thoracic & Vascular Surgery, National University Heart Centre Singapore, National University Health System, Singapore
[d] Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA
[e] Neuroscience Graduate Program, Bilkent University, Ankara 06800, Turkey

ARTICLE INFO

Article history:
Received 6 October 2016
Received in revised form 1 June 2017
Accepted 5 June 2017
Available online 13 June 2017

Keywords:
Myocardial infarction
Peptide nanofibers
Neovascularization
Cardiomyocyte
VEGF

ABSTRACT

Myocardial infarction remains one of the top leading causes of death in the world and the damage sustained in the heart eventually develops into heart failure. Limited conventional treatment options due to the inability of the myocardium to regenerate after injury and shortage of organ donors require the development of alternative therapies to repair the damaged myocardium. Current efforts in repairing damage after myocardial infarction concentrates on using biologically derived molecules such as growth factors or stem cells, which carry risks of serious side effects including the formation of teratomas. Here, we demonstrate that synthetic glycosaminoglycan (GAG) mimetic peptide nanofiber scaffolds induce neovascularization in cardiovascular tissue after myocardial infarction, without the addition of any biologically derived factors or stem cells. When the GAG mimetic nanofiber gels were injected in the infarct site of rodent myocardial infarct model, increased VEGF-A expression and recruitment of vascular cells was observed. This was accompanied with significant degree of neovascularization and better cardiac performance when compared to the control saline group. The results demonstrate the potential of future clinical applications of these bioactive peptide nanofibers as a promising strategy for cardiovascular repair.

Statement of Significance

We present a synthetic bioactive peptide nanofiber system can enhance cardiac function and enhance cardiovascular regeneration after myocardial infarction (MI) without the addition of growth factors, stem cells or other biologically derived molecules. Current state of the art in cardiac repair after MI utilize at least one of the above mentioned biologically derived molecules, thus our approach is ground-breaking for cardiovascular therapy after MI. In this work, we showed that synthetic glycosaminoglycan (GAG) mimetic peptide nanofiber scaffolds induce neovascularization and cardiomyocyte differentiation for the regeneration of cardiovascular tissue after myocardial infarction in a rat infarct model. When the peptide nanofiber gels were injected in infarct site at rodent myocardial infarct model, recruitment of vascular cells was observed, neovascularization was significantly induced and cardiac performance was improved. These results demonstrate the potential of future clinical applications of these bioactive peptide nanofibers as a promising strategy for cardiovascular repair.

1. Introduction

Cardiovascular diseases constitute one of the major life threatening diseases and are among the leading causes of morbidity and mortality worldwide [1]. The prevailing cause of heart failure is the death of heart muscle tissue. This irreplaceable cardiomyocyte loss caused by arrested blood and oxygen flow to the heart is detrimen-

* Corresponding authors at: Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore (T. Kofidis). Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA (M.O. Guler). Institute of Materials Science and Nanotechnology, UNAM-National Nanotechnology Research Center, Bilkent University, Ankara 06800, Turkey (A.B. Tekinay).
E-mail addresses: surtk@nus.edu.sg (T. Kofidis), mguler@uchicago.edu (M.O. Guler), atekinay@bilkent.edu.tr (A.B. Tekinay).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.actbio.2017.06.009
1742-7061/

tal as it leads to pathological remodeling, reduced myocardial function and inevitable progression to heart failure [2]. Current treatments for salvaging the jeopardized myocardium include pharmacological therapy and percutaneous coronary intervention or coronary artery bypass surgery. The limited efficiency of current medical treatments might result in final stage heart failure, and such heart failure patients require heart transplantation, which has limited availability, or the support of ventricular assisted devices, which have limited efficacy [3]. These limitations plummet the patient's odds of survival and have led to the development of novel therapeutic strategies such as regenerative medicine and tissue engineering approaches for the repair of damaged myocardial tissues.

Induction of angiogenesis is a vital mechanism that may alleviate heart dysfunction after myocardial infarction (MI). Increased vascularization and oxygen supply to the affected ischemic area can reduce the degree of cardiomyocyte apoptosis and fibrosis [4,5]. It is also essential to prevent the transition of infarction to heart failure by providing long term left ventricular remodeling. Pro-vascularization signals including biomolecules such as extracellular matrix elements (e.g. collagen, elastin, laminin and fibronectin), growth factors (e.g. VEGF and FGF2) and GAGs (e.g. heparan sulfate and chondroitin sulfate), make up the structural framework that regulates the behavior of vascular cells (*i.e.* endothelial and smooth muscle cells) in order to establish stable and functional vascular networks [6].

To date, several strategies have been tested including using cytokines to modulate inflammation, introducing angiogenic growth factors and transplantation of stem cells within various matrices [7–9]. Previous studies also demonstrated the potential of using heparin to deliver growth factors. Heparin preserves growth factors in their active form by protecting them from proteolysis, and enhances the growth factors' affinity to their respective receptors, enabling consistent release of growth factors for an extended period and optimizing their local concentration [10–16]. The ability of heparin to bind to several angiogenesis promoting growth factors, such as VEGF and FGF2, chemokines and cell adhesion molecules via specific electrostatic interactions mediated by their heparin binding domains or sulfated sequences have previously been identified. This binding boosts cell signaling, inducing the formation of new blood vessels [17,18]. On the other hand, the use of heparin in tissue engineering applications could trigger immune reactions due to its animal origin [19].

Supramolecular scaffolds formed by self-assembling peptides could address unmet needs of cardiac regenerative medicine by providing a structural and functional recapitulation of native tissue elements. Their biocompatibility and versatility for attachment of discrete bioactive chemical groups make peptide based scaffolds suitable for various tissue engineering applications [20–22]. Molecular stacking of these motifs through non-covalent interactions gives rise to the formation of high-aspect-ratio nanofibers at physiological conditions. These nanofibrous scaffolds can have the advantage of slow degradation, hypo-immunogenicity, and suitability for sustained release of specific growth factors immobilized through covalent bonding. Recently, multidomain VEGF mimetic peptides have been shown to promote an increase in the microvessel density in an *in vivo* model in which hydrogels were subcutaneously injected to the animals [23]. In another study, nanofibers presenting VEGF-mimetic peptide at high density were shown to induce phosphorylation of VEGF receptors and promote proangiogenic behavior in endothelial cells [24]. In a model of acute myocardial infarction, significant preservation of hemodynamic functions was demonstrated after injection of heparin binding self-assembling peptide nanofibers, which were capable of binding to VEGF and bFGF [25]. Other studies also used injectable in situ self-assembling peptide gels to recruit endogenous endothelial and smooth muscle cells and to stimulate angiogenesis by slow release of angiogenic factors [26,27].

Recently, we have developed a GAG mimetic peptide nanofiber scaffold that interacts with endogenous growth factors and induces angiogenesis without the addition of external growth factors or other supplements [6,28]. Through an innovative design, the GAG mimetic peptide amphiphiles (PA) were decorated with a sulfonate, hydroxyl and carboxylic acid groups in order to mimic heparan sulfate glycosaminoglycans, which are fundamental constituents of the extracellular matrices of many tissues and known to regulate growth factor binding and distribution [29]. In addition to VEGF, GAG mimetic nanofibers were demonstrated to bind to other angiogenesis related growth factors including hepatocyte growth factor (HGF) and fibroblast growth factor-2 (FGF-2) [6,28].

Here, we show that the injection of this previously described angiogenic GAG mimetic peptide nanofibers led to better cardiac function via preservation of more cardiac muscle and formation of new blood vessels after MI in a rat cardiac infarct model. Cardiac functional parameters were monitored through echocardiography and hemodynamics and cellular level remodeling was assessed through histological and immunofluorescence assessment. In addition to *in vivo* MI model, adhesion and differentiation of cardiomyoblast cells were studied *in vitro*.

2. Materials and methods

2.1. Materials

All 9-fluorenylmethoxycarbonyl (Fmoc) and *tert*-butoxycarbonyl (Boc) protected amino acids, lauric acid, [4-[α-(2 0,40-dimethoxyphenyl) Fmoc-aminomethyl] phenoxy] acetamidonorleucyl-MBHA resin (Rink amide MBHA resin) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and diisopropylethylamine (DIEA) were purchased from NovaBiochem, Merck and ABCR. The other chemicals for peptide synthesis were analytical grade and purchased from Fisher, Merck, Alfa Aesar, or Sigma Aldrich. All other materials used in this study were purchased from Sigma–Aldrich, Invitrogen, Bio-Rad, Fisher and Merck.

2.2. Synthesis and characterization of peptide amphiphiles

PA molecules were synthesized by using solid phase peptide synthesis method with Rink amide MBHA resin. For coupling of amino acids, 1.95 equivalents of HBTU, 3 equivalents of DIEA and 1 equivalent of starting resin were used with 2 equivalents of amino acid in 10 mL of dimethylformamide (DMF). Coupling of each amino acid takes 2 h and Fmoc removal was performed with 20% (v/v) piperidine/dimethylformamide solution for 20 min. Lauric acid addition was performed similarly to amino acid coupling except that coupling time was 4 h. In order to acetylate the free amine groups after each coupling step, 10% (v/v) acetic anhydride–DMF solution was used after each coupling. Dichloromethane (DCM) and DMF were used for washing steps. The *p*-sulfobenzoic acid addition to ε-amine of lysine was done after cleavage of Mtt protecting group of Fmoc-Lys(Mtt)-OH residue with 5% of cleavage cocktail in DCM 3 times for 5 min. Peptide cleavage from the resin and deprotections were carried out with 95% cleavage cocktail (95:2.5:2.5 trifluoroacetic acid (TFA): triisopropylsilane (TIS):water) for 2 h at room temperature. Excess TFA was removed by rotary evaporation. Remaining PA solution was precipitated in ice-cold diethyl ether overnight at −20 °C. Centrifugation was used to collect the precipitate and ultrapure water was used to dissolve the pellet. The solution was frozen at −80 °C and