International Journal of BioMedicine 4(1) (2014) 40-45

INTERNATIONAL JOURNAL OF BIOMEDICINE

EXPERIMENTAL RESEARCH

Examination of Bone Marrow Mesenchymal Stem Cells Seeded onto Poly(3-hydroxybutyrate-co-3-hydroxybutyrate) Biological Materials for Myocardial Patch

Junsheng Mu[1]*; Hongxing Niu[1]; Fan Zhou[2]; Jianqun Zhang[1] ; Ping Hu[3]; Ping Bo[1]; Yan Wang[3]

[1]Department of Cardiac Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases

[2]Department of Echocardiagraphy, General Hospital of the Chinese People's Armed Police Forces

[3]Polymer Research Institute of the Department of Chemical Engineering, Tsinghua University Beijing , P.R. China

Abstract

The implantation of the Bone Marrow Mesenchymal Stem Cells (BMSCs) into the heart has been reported to be effective for the treatment of myocardial infarction; however, the methods most suitable for supporting stem cell growth in a myocardial patch, still remain unknown. We used a new polymer material composed of poly(3-hydroxybutyrate-co-3-hydroxybutyrate) [P(3HB-co-3HB)] co-cultured with BMSCs to create a myocardial patch. The BMSCs were obtained from healthy male BSL-C57 mice. The cells were treated with 5-azacytidine to investigate their differentiation into cardiomyocytes. The cells were seeded for 24 hours onto the P(3HB-co-3HB) biological material films (n=8). Cell-biomaterial constructs were fixed and analyzed using different methods. BMSCs were $CD34^-$, $CD45^-$, $CD90^+$ (low) and $CD73^+$. The cells were stained with anti-cardiac troponin T (cTnT) and anti-connexin 43 (CX43) antibodies after 5-azacytidine treatment. Scanning electron microscopy revealed that the morphology of the BMSC was normal and that cell numbers were more abundant on the P(3HB-co-3HB) material surfaces. The growth curve of the BMSCs on the biomaterial patches showed the P(3HB-co-3HB) material enhanced good stem cell growth. Owing to its excellent biocompatibility and biodegradability properties, in particular its porosity, the P(3HB-co-3HB) is hailed as an optimal material to support myocardial cell growth and to create a myocardial patch in patients with myocardial infarction.

Keywords: *myocardial regeneration; myocardial infarction; poly(3-hydroxy-butyrate-co-3-hydroxybutyrate) [P(3HB-co-3HB)]; bone marrow mesenchymal stem cell (MSC).*

Introduction

Cell therapy is believed to be a promising candidate treatment for severe heart failure [1]. Various types of cell sources, including skeletal myoblasts, bone marrow mononuclear cells and MSCs, have been examined in both basic and clinical studies [2]. Although reports showing that transplanted cells improve the function of the ischemic heart are in existence, the effects of cell therapy are variable in clinical trials and the beneficial effects and types of cells suitable for myocardial repair still remain unknown [3,4]. The efficiency of cell transplantation is hindered by the low rate of engraftment of the transplanted cells caused by leakage during injections and massive cell death within the host [5,6]. Biological scaffolds are expected to circumvent the loss of the grafted cells as they confer a three-dimensional (3D) microenvironment for the cells that support their survival, proliferation and function [7]. Several types of scaffolds have been designed to date; however, there are many limitations that still need to be addressed in terms of their correct matching with the engrafted cells, compatibility with the host tissue, and clinical safety.

Self-assembling nanopeptides consist of alternating hydrophilic and hydrophobic amino acid residues that can

**Corresponding author:* *Jun-sheng Mu. Department of Cardiac Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing 100029, China.Telephone: +86-10-64456341 .Fax: +86-10-64456341.E-mail: wesleymu@hotmail.com*

adopt the ß-sheet structures and form a stable three-dimensional hydrogel, consisting of > 99.5% water depending upon the pH, salt and time [8]. Hydrogels have been shown to promote cell survival, proliferation and differentiation of many different cell types *in vitro*, including neural stem cells [9], osteocytes [10] and endothelial cells [11]. When self-assembling peptides are injected into a tissue, they form a nanofiber network, which creates three-dimensional microenvironments for the endogenous cells, leading to angiogenesis and axonal regeneration [12,13].

In this manuscript, we examined a 3D patch material capable of imitating the aspects of the extracellular stroma, and providing a suitable environment for the survival and proliferation of the seed cells. The optimal myocardial patch material should be able to provide good biocompatibility, biodegradability and safety, reduce cell loss following transplantation and promote cell growth for tissue repair [14,15]. Current studies are developing poly(3-hydroxybutyrate-co-3-hydroxybutyrate) [P(3HB-co-3HB)] biomaterials for medical applications. However, very little research has been performed regarding the combination of P(3HB-co-3HB) biomaterials with the BMSCs for their application as a patch material for myocardial regeneration. This study was aimed at isolating and expanding the BMSCs *in vitro*, which would subsequently be seeded onto three patch biomaterials to observe the influence of these biomaterials on cell growth and proliferation. From the results of this study, it is our hope that a biological material with good compatibility and physical properties suitable for BMSC growth and proliferation will be identified for *in vivo* applications [16].

Material and Methods

The experiment was conducted from July 2011 to April 2012 in the Cardiovascular Disease Research Institute of Beijing and in the main laboratory for cardiovascular remodeling. BSL-C57 mice were bought from Wei Tong Lihua Experimental Animal Laboratory, Beijing. Fetal calf serum and low glucose Dulbecco's modified Eagle's medium (LG-DMEM) were purchased from Gibco Life Technologies. Antibodies were purchased from BD Biosciences and DAPI reagents were purchased from Sigma. Biological materials composed of a thin film of PU, P(3HB-co-3HB) and PPC were provided and detected by the High Polymer Institute Laboratory of the Chemical Department in Qinghua University. The porosity was analyzed using a micrometrics ASAP 2020 instrument and the BET method was used to determine the surface Area (31.2899 m2/g).

Cell Isolation and cultivation

Four-week-old C57 mice were euthanized by cervical dislocation. Both tibial and femoral bones were excised and separated. Muscle tissues were removed and the bone marrow cavity was exposed under aseptic conditions. The capital medical university institutional animal care and use committee was approved. All animal welfare were taken to ameliorate suffering. Sterile DMEM was used to repeatedly flush cells from the bone marrow cavity, prior to filtering through a 200 mesh nylon net to remove large tissue fragments. The cell suspension was collected in liquid and centrifuged at 1000 rpm for 5 min. Cells were re-suspended and cultured in LG-DMEM containing 10% FBS. The culture medium was changed after 24 h and every other day thereafter. Cells were incubated in 5% CO_2 at 37°C, with a volume fraction of 0.05. Original generation cells (P0) were harvested on reaching 90% confluence by digestion with 0.25% EDTA-pancreatic enzymes and passaged at a ratio of 1:2 for subsequent cultures (P1-P5). Culture medium was changed every other day and cell growth was observed using an inverted microscope.

Flow cytometry

BMSCs (P5) were harvested using 0.25% EDTA-Pentazyme and adjusted to a cell concentration of 1×10^7/ml with phosphate-buffered saline (PBS). Cells were added to five flow tubes containing 100µl of cell-suspension. PE-anti-CD90, PE-anti-CD34, FITC-anti-CD45, and FITC-anti-CD73 were each added to one tube of cells and the remaining tube represented a blank control [17 , 18 , 19, 20]. After stirring, cells were incubated at 4°C for 30 min, washed twice with PBS and analyzed using flow cytometry.

Differentiation in vitro and immunohistochemistry

BMSCs of all five passages were re-suspended after trypsin treatment and washed three times with PBS. The cells were re-suspended in complete medium and seeded into a 6-well dish at a density of 5×10^5 cells/dish. Twenty-four hours after seeding, the medium was changed to complete medium containing 5-azacytidine (10 µmol/l). After incubating for another 24 h, the medium was changed to complete medium without 5-azacytidine. The medium was changed twice a week thereafter until the experiment was terminated two weeks after drug treatment. After completing the protocol, the cells were mounted for microscopic examination, and the cells were prepared for immunohistochemical analysis using fluorescence microscopy.

Induced and uninduced cells grown on glass coverslips in a 24-well plate, were fixed in 4% formaldehyde for 10 min. washed three times with PBS for 3 min each at room temperature. Non-specific binding was prevented by several washes with PBS. Then, cells were permeabilized with 0.2% Triton X-100 for 10 min, and washed three times with PBS for 10 min at room temperature. After blocking with 5% BSA in PBS for 0.5 h at room temperature, the cells were incubated with primary antibodies directed against anti-cTnT or anti-CX43 overnight at 4°C. After three washes in PBS for 5 min each, cells were incubated with goat anti-rabbit IgG/RBITC or goat anti-rabbit IgG/FITC as the secondary antibody for 45 min at room temperature. Finally, after washing three times with PBS, they were incubated with 0.5µg/mL DAPI for 10 min, washed with PBS three times, and mounted for microscopic examination under fluorescence microscopy. Cells with green or red antibody staining in the cytoplasm were considered positive for the protein in question. Cells with blue staining in the nucleus were considered healthy.

Preparation and identification of a myocardial patch

Myocardial patch biomaterials and puncher were sterilized with epoxyethane. Circular discs (0.5 cm diameter) were punched out and soaked in culture medium. Patches were rinsed three times in PBS before placing them flattened

on a 96-hole board for use. BMSCs (P5) were seeded onto the patches at a density of 1×10^5 cells per construct.

A) Preparation for scanning electronic microscopy (SEM)

Cell-constructs were cleaned three times with deionized water and fixed with 500 μL 0.25% glutaraldehyde at 4°C. With the cell patches totally submerged, samples were analyzed under the microscope.

B) Preparation for fluorescent staining and cell counting

BMSCs were re-suspended at 1×10^5 cells/mL and transferred in triplicate into the 24-hole board (500 μL/hole). Cells were cultured on the board for 24 h in the incubator at 5% CO_2 and 37°C. Medium was removed after cells had adhered to the surface. Cells were fixed with paraformaldehyde for 10 min, washed three times with PBS and placed on a glass slide. DAPI was dropped onto the patch to immerse the cell-construct, after which it was covered with a glass coverslip. Slides were mounted reversely under a fluorescent microscope for observation and photography. Average cell counts were obtained from 10 fields of vision for each material.

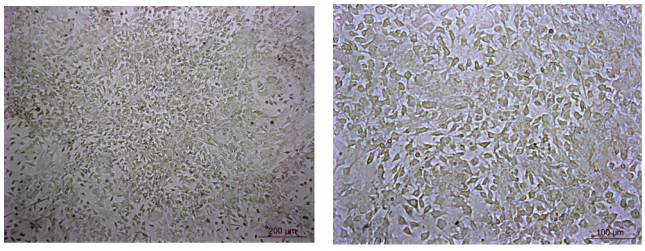
Data processing

Cell counts were analyzed with SPSS 12.0 statistics software and variance analysis was determined for inter-group comparison. A value of $P < 0.05$ was found to be statistically significant. Flow cytometry results were analyzed using EXPOTM 32 ADC software.

Results

BMSC Morphology

Original generation BMSCs were observed in various shapes including circular, elliptical, rod and shuttle-like morphologies. These cells grew in colonies after 4-6 days, extending outwards in a radial or vortex arrangement. By to three weeks, 80-90% confluence was achieved. Non-adherent cells were removed with media changes and the cells were distributed more evenly on the surface, with spindle-like morphology and rapid growth (Fig.1).

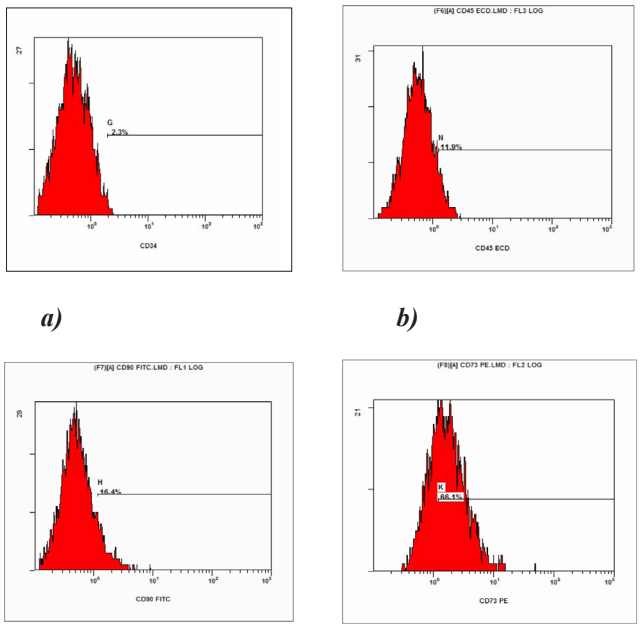


a) ***b)***

Fig 1. I*nverted microcopy image of BMSCs (P5) at: ×100 (a) and ×200 (b)*

Flow cytometry

The BMSCs (P3) were analyzed using flow cytometry to characterize their cell surface marker profile. As shown in Fig. 2, the BMSCs were $CD34^-$ and $CD45^-$, $CD90^+$ (low) and $CD73^+$ (Fig. 2).



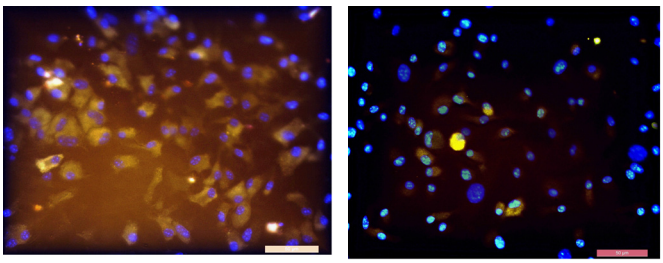
a) ***b)*** ***c)*** ***d)***

Fig. 2. *Flow cytometry for surface characteristics of BMSCs. BMSCs were CD34- and CD45- negative, CD90-positive (low) and CD73-positive*

Differentiation into cardiomyocytes

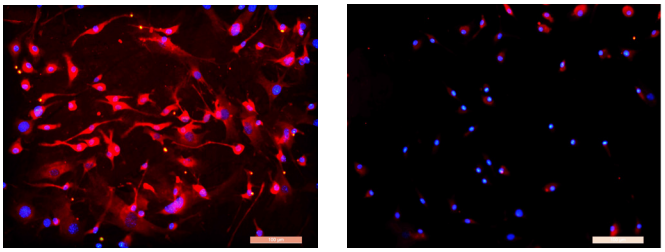
To determine the morphological changes in the BMSCs induced by the 5-azacytidine treatment, phase-contrast microscopy and/or immunostaining with anti-CX43 or anti-cTnT antibodies were performed. The morphological differentiation from MSCs to myogenic-like cells evolved gradually. During exposure to 5-azacytidine, some adherent cells died, while the surviving cells began to proliferate and differentiate. One week later, approximately 30% of all of the remaining adherent cells had enlarged and assumed ball-like or stick-like morphologies. Within two weeks, the cells had connected with the adjoining cells and formed myotube-like structures. The cells looked like long spindle-shaped fibroblastic cells when observed under a microscope.

Figs. 3 and 4 show fluorescence microscopy (FM) images of the BMSCs immunostained with anti–CX43 or anti-cTnT antibodies in two weeks after 5-azacytidine treatment. The CX43-positive or cTnT-positive cells gradually connected with the neighboring CX43-positive or cTnT-positive cells. The uninduced cells were negative for both of these cardiomyocyte markers.



a) ***b)***

Fig. 3. a). *Induced cells: under FM, cells with the CX43 green antibody binding in the cytoplasm, cells with blue reaction product in the nuclear.* ***b).*** *Uninduced cells:under FM. Few cells with the CX43 green antibody bi nding in the cytoplasm, cells with blue reaction product in the nuclear (x400).*



A B

Fig. 4. A. *Induced cells: under fluorescence microscopy. Cells with cTnT red antibody binding in the cytoplasm; Cells with blue reaction product in the nuclear.* ***B.*** *Uninduced cells: under fluorescence microscopy. Few cells with cTnT red antibody binding in the cytoplasm; Cells with blue reaction product in the nuclear (x400).*

SEM analysis

Using SEM, the patch was observed to have a normal surface, comprising high numbers of granule-like cell aggregates with typical cell morphology. P(3HB-co-3HB) is a 3D network-structure with a fiber intertexture and even pore size, into which the BMSCs flourished, showing normal morphology and an absence of cell lysate or debris (Fig.5).

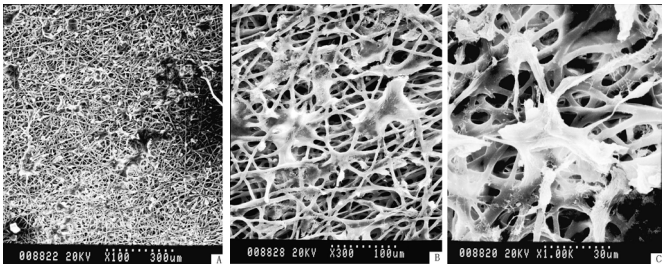


Fig.5. *SEM images of P(3HB-co-3HB) polymer biomaterial patches A (×100), B (×300), C (×1000).*

Fluorescence microscopy and cell counting

Cell-seeded patches, stained with DAPI (Fig.6), were mounted and examined under a microscope to obtain the average cell counts and growth curves on the biomaterial patches (Fig.7).

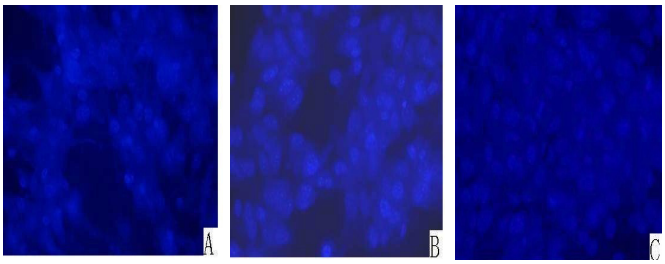


Fig.6. *Fluorescent microscopy, DAPI staining of BMSCs on P(3HB-co-3HB) patches. A (48h×400), B (72h×400), C (96h×400).*

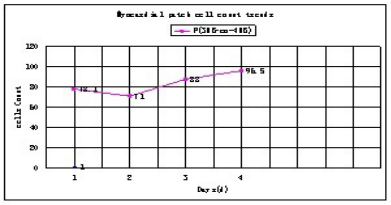


Fig. 7. *Growth curve on P(3HB-co-3HB) biomaterial patches.*

Discussion

The BMSCs are relatively primitive, with a strong potential for multi-lineage cell differentiation. They have been shown to differentiate into cardiac cells, through transverse differentiation and cell integration, and into endothelial and smooth muscle cells, directly participating in the formation of blood vessels [21,22]. The paracrine action of the BMSCs activates an endogenous rehabilitation mechanism to repair a damaged heart [23] and this ability has drawn scientific attention towards their clinical application in tissue engineering and gene therapy. Due to the advantages of repairing cardiac muscle and the associated vessels, MSCs have been the preferred cell source for cardiac tissue engineering applications. The formation of cellularized cardiac muscle is the primary goal for the tissue engineering treatments of myocardial infarct. However, a reduction in the blood flow and heart mechanical activity may cause the loss of the transplanted cells [24]. It is therefore necessary to identify materials with excellent physical characteristics, biological compatibility and biodegradability properties to provide a good environment for the transplanted cells, as well as for cell survival and growth in the infarct area, ultimately improving the post-treatment conditions.

We have established those BMSCs that can be induced to differentiate into cardiomyocytes *in vitro* by 5-azacytidine induction. CX43 and cTnT are well recognized early markers of myogenic differentiation. They are also involved in muscle cell contraction. Other markers include alpha-cardiac actin and beta-MHC. In this study, CX43 and cTnT were found in the cytoplasm of the cells induced with 5-azacytidine. In the ventricular muscle of small mammals, a developmental switch from the expression of CX43 and cTnT, which is the predominant fetal form, is observed [25,26]. The differentiated BMSCs were mainly expressed as CX43 and cTnT, indicating the activation of the transcriptional cascade regulating cardiomyocyte differentiation.

Many 3D materials have been investigated for myocardial scaffolds, including fibrin glue, poly (lactic-co-glycolic) acid, gelatin hydrogels and hyaluronic acid (HA) hydrogels [7]. Once the cells adapt to the 3D milieus, the cells migrate, proliferate and function according to the multi-directional molecular and mechanical signals, similar to organized tissue. However, there are substantial challenges for the existing materials, including stimulation of the host inflammatory response, toxic degradation and excessively large pore sizes and fiber diameters [27]. Self-assembling nanopeptides, such as P(3HB-co-3HB), are completely artificial, containing no animal-derived proteins. Hydrogels formed by self-assembling peptides have very small pore sizes that promote endothelial adhesion and capillary formation, while still enabling the rapid migration of cells [28]. This study also determined the porosity and surface area using the BET method of P(3HB-co-3HB). When implanted into the myocardium, the nanofibers rapidly polymerize at the physiological pH and osmolality, resulting in the entrapment of the transplanted cells and prevention of leakage. In addition, the rigidity of the hydrogel can be regulated by the concentration of the nanopeptide

solution, enabling the delivery of the BMSCs more easily. These properties of the nanopeptides, especially their porosity, may enhance the engraftment of a larger number of cells.

Self-assembling nanopeptides can be modified in a variety of ways that allow for cell-specific signals to be delivered. For example, nanofiber scaffolds containing RGD-binding sequence have been shown to significantly promote the proliferation of mouse pre-osteoblasts [29]. Moreover, the alkaline phosphatase activity and osteocalcin secretion, which are the early and late markers for osteoblastic differentiation, respectively, were also significantly increased [29]. Silva *et al.,* synthesized self-assembling peptides containing an IKVAV laminin motif, in which the neuronal precursors differentiated into neurons with extensive processes, while very few cells differentiated into astrocytes [30]. These tailor-made peptides have the ability to modulate bidirectional signals between the nanopeptides and transplanted cells, enabling us to create a 3-D cardiac graft, which substitutes the scar tissue after myocardial infarction, leading to the prevention of cardiac remodeling and improvement of cardiac function.

In this work we have shown that the P(3HB-co-3HB) can be a useful biomaterial, which assures safe and effective delivery of cells into the myocardium. Therefore, the P(3HB-co-3HB) may be suitable for supporting long-term proliferation, engraftment and differentiation of the transplanted cells in infarcted heart tissue. The P(3HB-co-3HB) is a new third generation polyhydroxyalkanoate material that has received wide attention due to its good physical properties, in particular its porosity. The P(3HB-co-3HB) is a new material synthesized by the Polymer Research Institute in the Department of Chemical Engineering, Tsinghua University, which has been shown to have better biological compatibility and biodegradability properties than polyhydroxybutyrate (PHB).

Following the initial culturing, the BMSCs are heterogeneous in shape, with circular, elliptical, rod and shuttle-like morphologies. During culture, the non-adherent cells were removed and the remaining adherent cells grew into characteristic MSCs, with spindle-shaped morphology. The BMSCs (P3) include $CD34^-$ and $CD45^-$, $CD90^+$ (low) and $CD73^+$. These cell surface marker characteristics are similar to those reported for the MSCs. Twenty-four hours after seeding the BMSCs onto the patch biomaterials, cell growth and morphology on all the three biomaterials were observed using SEM. The results indicated that there were higher cell numbers, lesser cell debris and good cell morphology on the P(3HB-co-3HB) scaffold compared with the other materials, indicating that it was most effective in supporting the growth and morphology of the BMSCs. The P(3HB-co-3HB) is a highly porous material, highly effective for good cell attachment, tissue in-growth and cell maintenance. These findings were supported by cell counting using the DAPI staining and by fluorescent microscopy. The P(3HB-co-3HB) has excellent tissue compatibility and biodegradability properties and can be well absorbed into the body.

However, some limitations were observed in this study. Although the cells can survive *in vitro* on the P(3HB-co-3HB) myocardial patches, their influence on the degradation and absorption of the patch materials within the body and the influence of the transplanted patch on the immune system is yet to be determined. Further studies are required to assess the feasibility of using the P(3HB-co-3HB) as a cardiac patch in the clinic, as well as its biotoxicity and *in vivo* tissue repair function.

In summary, we have investigated several potential biomaterials for application as a myocardial patch. In this study, the BMSCs were readily transplanted onto the P(3HB-CO-3HB) patch biomaterials. However, due to the non-absorbability property of the former, it is limited in its use, in the fields of medical and biological materials. The P(3HB-CO-3HB) is a new material with excellent biological tissue compatibility and biodegradability, has good potential for application as a myocardial patch. Further studies are required to determine the feasibility of using the P(3HB-co-3HB) as a myocardial patch *in vivo*, as well as to identify its biotoxicity and tissue repair function.

Competing interests

The authors declare that they have no competing interests.

References

1. Passier R, van Laake LV, Mummery CL. Stem-cell-based therapy and lessons from the heart. Nature 2008; 453(7193):322–9.
2. Dimmeler S, Burchfield J, Zeiher AM. Cell-based therapy of myocardial infarction. Arterioscler Thromb Vasc Biol 2008; 28(2):208-16.
3. Menasché P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, *et al.* The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation 2008; 117(9):1189–200.
4. Rosenzweig A. Cardiac cell therapy-mixed results from mixed cells. N Engl J Med 2006; 355(12):1274-7.
5. Dow J, Simkhovich BZ, Kedes L, Kloner RA. Washout of transplanted cells from the heart: a potential new hurdle for cell transplantation therapy. Cardiovasc Res 2005; 67(2):301-7.
6. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE, *et al.* Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. J Mol Cell Cardiol 2001; 33(5):907-21.
7. Eschenhagen T, Zimmermann WH. Engineering Myocardial Tissue. Circ Res 2005; 97(12): 1220-31.
8. Zhang S, Holmes T, Lockshin C, Rich A. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. Proc Natl Acad Sci USA 1993; 90(6):3334-8.
9. Thornhoff JR, Lou DI, Jordan PM, Zhao X, Wu P. Compatibility of human fetal neural stem cells with hydrogel biomaterials in vitro. Brain Res 2008; 1187:42–51.
10. Hamada K, Hirose M, Yamashita T, Ohgushi H. Spatial distribution of mineralized bone matrix produced by marrow mesenchymal stem cells in self-assembling peptide hydrogel scaffold. J Biomed Mater Res 2008; 84(1):128-36.
11. Narmoneva DA, Oni O, Sieminski AL, Zhang S, Gertler JP, Kamm RD, *et al.* Self-assembling short oligopeptides and the promotion of angiogenesis. Biomaterials 2005;

26(23):4837-46.
12. Davis ME, Motion JP, Narmoneva DA, Takahashi T, Hakuno D, Kamm RD, *et al.* Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. Circulation 2005; 111(4):442-50.
13. Ellis-Behnke RG, Liang YX, You SW, Tay DK, Zhang S, So KF, *et al.* Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision. Proc Natl Acad Sci USA 2006;103(13):5054-9.
14. Zhou Q, Zhou JY, Zheng Z, Zhang H, Hu SS. A novel vascularized patch enhances cell survival and modifies ventricular remodeling in a rat myocardial infarction model. J Thorac Cardiovasc Surg 2010; 140(6):1388-1396. e1-3.
15. Valarmathi MT, Goodwin RL, Fuseler JW, Davis JM, Yost MJ, Potts JD. A 3-D cardiac muscle construct for exploring adult marrow stem cell based myocardial regeneration. Biomaterials 2010; 31(12):3185-200.
16. Köse GT, Korkusuz F, Korkusuz P, Purali N, Ozkul A, Hasirci V. Bone generation on PHBV matrices: an in vitro study. Biomaterials 2003; 24(27):4999 ~ 5007.
17. Shachar M, Tsur-Gang O, Dvir T, Leor J, Cohen S. The effect of immobilized RGD peptide in alginate scaffolds on cardiac tissue engineering. Acta Biomater 2011; 7(1):152-62.
18. Park BW, Kang EJ, Byun JH, Son MG, Kim HJ, Hah YS, et al. In vitro and in vivo osteogenesis of human mesenchymal stem cells derived from skin, bone marrow and dental follicle tissues. Differentiation 2012; 83(5):249–59.
19. Sapir Y, Kryukov O, Cohen S. Integration of multiple cell-matrix interactions into alginate scaffolds for promoting cardiac tissue regeneration. Biomaterials 2011; 32(7):1838-47.
20. Saeed H, Taipaleenmäki H, Aldahmash AM, Abdallah BM, Kassem M. Mouse embryonic fibroblasts (MEF) exhibit a similar but not identical phenotype to bone marrow stromal stem cells (BMSC). Stem Cell Rev 2012; 8(2):318-28.
21. Zhang S, Ge J, Sun A, Xu D, Qian J, Lin J, et al. Comparison of various kinds of bone marrow stem cells for the repair of infarcted myocardium: single clonally purified non-hematopoietic mesenchymal stem cells serve as a superior source. J Cell Biochem 2006; 99(4):1132-47.
22. Jain M, Pfister O, Hajjar RJ, Liao R. Mesenchymal stem cells in the infarcted heart. Coron Artery Dis. 2005; 16(2):93-97.
23. Martens TP, See F, Schuster MD, Sondermeijer HP, Hefti MM, Zannettino A, et al. .Mesenchymal lineage precursor cells induce vascular network formation in ischemic myocardium. Nat Clin Pract Cardiovasc Med 2006; 3(1):S18-22.
24. Al Kindi AH, Asenjo JF, Ge Y, Chen GY, Bhathena J, Chiu RC, et al. Microencapsulation to reduce mechanical loss of microspheres: implications in myocardial cell therapy. Eur J Cardiothorac Surg 2011; 39(2):241-7.
25. Antonitsis P, Ioannidou-Papagiannaki E, Kaidoglou A, Cardiomyogenic potential of human adult bone marrow mesenchymal stem cells in vitro Thorac Cardiovasc Surg 2008; 56(2):77-82.
26. Antonitsis P, Ioannidou-Papagiannaki E, Kaidoglou A, Papakonstantinou C. In vitro cardiomyogenic differentiation of adult human bone marrow mesenchymal stem cells. The role of 5-azac ytidine. Interact Cardiovasc Thorac Surg 2007; 6(5):593-7.
27. Davis ME, Hsieh PC, Grodzinsky AJ, Lee RT. Custom design of the cardiac microenvironment with biomaterials. Circ Res 2005; 97(1):8–15.
28. Rando TA, Blau HM. Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy. J Cell Biol 1994; 125(6):1275-87.
29. Horii A, Wang X, Gelain F, Zhang S. Biological designer self-assembling peptide nanofiber scaffolds significantly enhance osteoblast proliferation, differentiation and 3-D migration. PLoS ONE.2007; 2:190.
30. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, *et al.* Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science 2004; 303(5662):1352-5.