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Matrix stiffness affects spontaneous contraction of cardiomyocytes cultured within a PEGylated fibrinogen biomaterial

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Abstract

Successful implementation of cardiac cell transplantation for treating damaged myocardium relies on the development of improved injectable biomaterials. A novel biomaterial technology using PEGylated fibrinogen has been developed with controllable physicochemical properties based on the poly(ethylene glycol) (PEG) constituent. In addition, the fibrinogen backbone of the material confers inherent bioactivity to cells. The purpose of this investigation was to explore by in vitro techniques the use of this biomaterial as a scaffold for cardiac tissue regeneration. To this end neonatal rat cardiomyocytes were cultivated in PEGylated fibrinogen constructs. The cell-seeding density and biomaterial composition were optimized to obtain maximum spontaneous contraction of the constructs. Quantitative characterization of the contraction pattern was accomplished by video image analysis. It was possible to demonstrate an inverse correlation between the material stiffness and the amplitude of contraction of the tissue constructs by changing the modulus of the matrix using different compositions of PEG and fibrinogen. The relationship between matrix stiffness, cell density and tissue contraction also provided some insight into the mechanism of cellular remodeling that ultimately leads to synchronized contraction of the constructs. These findings indicate that PEGylated fibrinogen hydrogels can be used as a scaffold for cardiomyocytes, and offer the possibility of controlling cellular remodeling via simple compositional modifications to the matrix.

Keywords: Cardiac cell therapy; Mechanical properties; Fibrin; Polyethylene glycol; Image analysis; Hydrogel

1. Introduction

The limited regenerative capacity of heart muscle after a myocardial infarction (MI) remains the main reason for myocardial scarring, aneurismal thinning and progressive heart failure [1]. As new treatment options emerge with the success of stem cell technologies in cardiac regeneration [2], the search continues for an effective technique to implant cardiomyocyte grafts into an infarcted myocardium. Two approaches for myocardial grafting have been explored recently: a saline suspension of differentiated cardiac cells [3] and a tissue-engineered three-dimensional patch with myocardial features [4]. Minimally invasive surgical techniques favor a cardiac cell suspension approach but recent studies have indicated that few of the transplanted cells can survive and functionally integrate into the myocardium [5,6]. Transplanted cardiomyocytes probably need the physical support of a biomaterial scaffold to maintain their placement in the injury site, protect the cells from host inflammation and facilitate functional integration with the injured myocardium. Alternatively, surgical implantation of a robustly engineered cardiac patch comprised of a biomaterial scaffold and cultivated cardiomyocytes is possible [7,8], yet can be difficult to effectively implement at a clinically relevant scale. The use of an in situ polymerizable (injectable) biomaterial scaffold for cardiac cell delivery can address these limitations and provide valuable alternatives for myocardial cell therapy and tissue engineering [9].

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Identifying an appropriate biomaterial for cardiomyocyte transplantation is currently an important area of research in cardiac tissue engineering. An injectable biomaterial must undergo in situ liquid-to-solid transition with cardiomyocytes in suspension without harming the cells or the surrounding host myocardium. After polymerization, the cells should be able to readily remodel the polymer so that true engraftment is possible by natural, cell-mediated pathways. A biomaterial possessing susceptibility to tissue remodeling enzymes would be advantageous in this regard [10]. At the same time, the injectable polymer must not obstruct cellular remodeling [11], or distort the myocardial geometry [12]. For this reason it is important to consider the impact that material compliance has on cardiomyocyte phenotype [13]. Finally, the biomaterial needs to be a suitable growth environment for myocardial cells to survive and express a contracting cardiac phenotype so that they can functionally integrate upon implantation.

A number of recent cardiomyocyte transplantation studies using injectable polymers such as fibrin glue [14], matrigel [12] and self-assembling peptide hydrogels [15] have demonstrated improvements in cell transplant survival, vasculogenesis and even cardiac function. Despite encouraging results, there are still unanswered questions about how the composition and structure of injectable biomaterials will affect cardiomyocyte remodeling and functional integration of the cell graft. To this end, it would be useful to study cardiomyocyte remolding with injectable polymers using in vitro cultures and clinically relevant endpoints such as cell and tissue contraction. This could readily be done with a new biosynthetic material based on fibrinogen [16], which is an injectable polymer with bioactivity similar to native fibrin and the added advantage of controllable physical properties and biodegradation [17,18]. This unique biomaterial is made by conjugating poly(ethylene glycol) (PEG) with denatured fibrinogen in solution to form a liquid PEGylated fibrinogen precursor. The liquid precursor is assembled into a three-dimensional hydrogel scaffold using non-toxic photo-polymerization [19]. Several mammalian cell types have been tested with this biomaterial and have shown an ability to remodel the biosynthetic matrix in vitro [16,17,20] and in vivo [21].

In this study, we used neonatal rat cardiomyocytes in PEGylated fibrinogen constructs to investigate a relationship between the material modulus and spontaneous contraction of the constructs. We have previously reported that the modulus of the PEGylated fibrinogen hydrogels can be precisely controlled based on the composition of the matrix [17], and now we examine how changing the modulus affects the ability of cardiomyocytes to spontaneously contract in the biosynthetic matrix. We applied video analysis algorithms to characterize spontaneous contraction and to correlate spatial and temporal displacement patterns to the material composition and cell density, both of which can affect hydrogel stiffness and cardiac remodeling. These results validate the use of PEGylated fibrinogen biomaterials for cardiomyocyte culture and represent the initial optimization of such a material for its eventual use in cardiac repair strategies.

2. Materials and methods

2.1. Hydrogel preparation

PEG-diacrylate (PEG-DA) with an average molecular weight of 10 kDa was prepared by reacting linear PEG-OH (Aldrich, Sleeze, Germany) and acryloyl chloride (Merck, Darstadt, Germany) at a molar ratio of 1.5:1 relative to –OH groups in a dichloromethane and triethylamine (Fluka) solution. The final product was precipitated in ice-cold diethyl ether, dried under vacuum, and the end-group conversion of the product was confirmed by proton nuclear magnetic resonance spectroscopy. PEGylation of bovine fibrinogen (Sigma–Aldrich, St Louis, MO) was done in 150 mM phosphate-buffered saline (PBS) containing 8 M urea at a fibrinogen concentration of 7 mg ml^{-1}. The reaction was carried out with tris(2-carboxyethyl)phosphine hydrochloride (TCEP–HCl) (Sigma–Aldrich) and PEG-DA at a molar ratio of 1.5:1 TCEP to fibrinogen cysteines and 4:1 PEG to fibrinogen cysteines for 3 h in the dark at room temperature (pH 8). The PEGylated protein solution was purified by acetone precipitation and overnight dialysis against PBS (12–14 kDa MW cutoff) (pH 7.4) at 4 °C. The dry weight of the PEGylated fibrinogen precursor solution was measured and the product was characterized by BCA protein assay using an Albumin standard (Pierce Biotechnology, Rockford, IL). To initiate gel formation by photopolymerization of the precursor solution, 0.1% (w/v) Irgacure™ 2959 (Ciba Specialty Chemicals, Tarrytown, NY) photoinitiator was added to the precursor and the mixture exposed to ultraviolet (UV) light (365 nm, 5 mW cm^{-2}) for 5 min.

2.2. Cardiomyocyte isolation and culture

Cardiomyocytes from whole hearts of neonatal Sprague–Dawley rats (1–3 days old) were isolated according to published protocols [22]. Briefly, neonatal rat ventricular tissue was digested with Trypsin (1:250, MP Biomedicals, Inc., Solon, OH) and DNase II (2130 units/mg solid, Sigma–Aldrich) in calcium and bicarbonate free Hanks with Hepes solution (CBFHH). Cells were immediately suspended in 40 μl PEGylated fibrinogen precursor and polymerized into disc-shaped constructs 5 mm in diameter and 2 mm thick. The PEGylated fibrinogen constructs were cultured for up to 4 weeks in Dulbecco's modified Eagle's medium (DMEM, 1000 mg l^{-1} glucose) (Gibco, UK) containing 10% donor horse serum (DHS) (Biological Industries, Israel), 1% penicillin–streptomycin (Biological Industries) and 2% chicken embryo extract. In order to identify the optimal cardiomyocyte culture conditions in the PEGylated fibrinogen constructs, the following parameters were tested: (1) cell density (4, 8 and 16×10^6 cells/ml); (2) precursor concentration (6, 7, 8, and 9 mg ml^{-1}); (3) PEG to fibrinogen molar ratio (25:1 and 50:1).

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