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Cardiac differentiation of cardiosphere-derived cells in scaffolds mimicking morphology of the cardiac extracellular matrix

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ABSTRACT

Stem cell therapy has the potential to regenerate heart tissue after myocardial infarction (MI). The regeneration is dependent upon cardiac differentiation of the delivered stem cells. We hypothesized that timing of the stem cell delivery determines the extent of cardiac differentiation as cell differentiation is dependent on matrix properties such as biomechanics, structure and morphology, and these properties in cardiac extracellular matrix (ECM) continuously vary with time after MI. In order to elucidate the relationship between ECM properties and cardiac differentiation, we created an in vitro model based on ECM-mimicking fibers and a type of cardiac progenitor cell, cardiosphere-derived cells (CDCs). A simultaneous fiber electrospinning and cell electrospraying technique was utilized to fabricate constructs. By blending a very soft hydrogel with a relatively stiff polyurethane and modulating fabrication parameters, tissue constructs with similar cell adhesion property but different global modulus, single fiber modulus, fiber density and fiber alignment were achieved. The CDCs remained alive within the constructs during a 1 week culture period. CDC cardiac differentiation was dependent on the scaffold modulus, fiber volume fraction and fiber alignment. Two constructs with relatively low scaffold modulus, ~50–60 kPa, most significantly directed the CDC differentiation into mature cardiomyocytes as evidenced by gene expressions of cardiac troponin T (cTnT), calcium channel (CACNA1c) and cardiac myosin heavy chain (MYH6), and protein expressions of cardiac troponin I (cTnI) and connexin 43 (CX43). Of these two low-modulus constructs, the extent of differentiation was greater for lower fiber alignment and higher fiber volume fraction. These results suggest that cardiac ECM properties may have an effect on cardiac differentiation of delivered stem cells.

1. Introduction

Myocardial infarction (MI) affects more than 8 million Americans [1]. MI causes massive heart cell death and heart function decrease. Various therapeutic strategies have been used to treat MI [1–8]. However, normal heart function cannot be restored after MI since cardiomyocytes are less proliferative and endogenous cells are unable to produce sufficient cardiomyocytes for effective regeneration. Stem cell therapy is a potential approach to regenerate heart tissue [2–10]. It delivers stem cells to the damaged areas (scar tissue) where they differentiate into cardiomyocytes. However, various animal studies and clinical trials have shown that the success rate for differentiation is low and it therefore remains impractical for widespread clinical application [2–11]. Identifying and addressing the causes for the low success rate is needed for heart tissue regeneration.

The causes of low differentiation are not completely clear. The harsh biochemical environment initiated by MI compromises both stem cell survival and differentiation [2,3]. This includes low nutrient and oxygen conditions, high concentrations of reactive oxygen species and inflammatory molecules in the infarcted hearts [2,3].

Collagen, a major extracellular matrix (ECM) in the scar tissue, may also contribute to low cell differentiation, although this has not yet been explored in the literature. Following MI, the composition, biomechanics and structure of collagen fibers change

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continuously [12–23]. Their stiffness gradually increases to 3–4 times that of collagen in healthy heart tissue [24]. A hypothesis is that such increases may affect stem cell differentiation, consistent with recent in vitro studies showing that matrix stiffness regulates stem cell differentiation [25–33]. This hypothesis can be examined by studying how collagen at different stages of MI affects stem cell differentiation.

Recent studies in stem cell biology show that stem cells can differentiate into different lineages in vitro when exposed to intrinsic properties of the matrix, such as composition, biomechanics and structure [34]. These parameters modulate the forces exerted between the cells and matrix. Mechanosensitive pathways subsequently convert these forces into biochemical signals that commit the cell to a specific lineage [34]. Each of these attributes is significant. Matrix composition can regulate cell fate by differential integrin binding to the matrix [34]. For example, ECM proteins can interact with a specific subset of integrins on the mesenchymal stem cell (MSC) surface, directing differentiation into heart cells [35]. Specific domains from ECM proteins can dramatically affect cell differentiation by conformational changes that enhance force transmission as integrins bind to these ligands [36–39]. Matrix structure can also affect stem cell differentiation, by affecting integrin binding and the distribution of focal contacts between the matrix and cells [34]. For example, embryonic stem cells show different differentiation behavior in a microporous foam with solid walls compared to fibrous ones [34,40,41]. Finally, matrix mechanical properties can induce stem cell differentiation. Specifically, stem cells show lineage-specific differentiation when cultured on matrices mimicking the stiffness of native tissue [25–33]. MSCs become neurogenic, myogenic and osteogenic on matrices mimicking neural, skeletal muscle and bone stiffness environments, respectively [25]. We have demonstrated that MSCs differentiate into cardiomyocytes in a hydrogel with 45-65 kPa modulus [32], while cardiosphere-derived cells (CDCs) differentiate into cardiomyocytes at 35 kPa modulus [31]. More recently, we demonstrated that fibrous scaffolds mimicking the global mechanical properties of healthy heart tissue can induce MSC differentiation into cardiomyocytes [30].

Although many studies have investigated the relationship among collagen global biomechanics, collagen organization and heart function [12–23], the current literature lacks systematic studies of the effect of collagen fibers in scar tissue on stem cell differentiation. Specifically, how do collagen composition, single-fiber modulus, fiber density and alignment evolve at different stages of MI and how do these changes affect cell differentiation? After being delivered into the scar tissue, stem cells adhere to collagen fibers. Fiber properties such as composition, single-fiber modulus, fiber density and alignment determine the forces that a stem cell can exert on collagen fibers. Cells contact single fibers and thus sense the single fiber rather than global modulus. Fiber density and alignment determine the distribution of focal contacts between fibers and cells. The evolution in collagen composition, biomechanics and structure due to MI introduces the possibility that differentiation might be stimulated or inhibited during certain stages. This may impact both the optimal timing for stem cell delivery into infarcted hearts and the design of matrices to promote cardiac differentiation. However, these studies cannot be conducted in vivo, as other parameters such as cytokines and glycosaminoglycans (GAGs) may concurrently affect cell differentiation, making it difficult to isolate the effects of collagen fibers [2,3].

Towards the goal of elucidating the relationship between stem cell differentiation and changes in collagen fiber properties after MI, we created a novel in vitro model based on fibrous scaffolds that mimic structure of the collagen matrix. By modulating fiber composition and fabrication parameters, scaffolds with different single-fiber moduli, global moduli, alignment and fiber density were obtained. CDC cardiac differentiation in the scaffolds was then investigated and correlated to these properties.

2. Materials and methods

2.1. Materials

N-Isopropylacrylamide (NIPAAM, TCI) was recrystallized with hexane three times before use. 2-Hydroxyethyl methacrylate (HEMA, Alfa Aesar) and acrylic acid (AAc, Acros) were purified by passing through an inhibitor-remover column. Polycaprolactone (PCL) diol (Mn = 2000, Acros) was dried under vacuum at 55 °C overnight before use. β-butyrolactone (VWR), gelatin type A (Acros), hexafluoroisopropanol (HFIP, Oakwood), tin(II) trifluoromethanesulfonate ($Sn(OTf)_2$,VWR), 1,6-diisocyanatohexane (HMDI, Acros), 1,4-diaminobutane (DAB, Acros), stannous octoate ($Sn(Oct)_2$, Pfaltz & Bauer) and dimethyl sulfoxide (DMSO, Fisher) were used as received.

2.2. Poly(ester urethane) urea synthesis

Poly(ester urethane) urea (PU) was synthesized from PCL, HMDI and DAB according to our previously established method [42]. In brief, 12.37 g of PCL was dissolved in 150 ml DMSO in a three-necked flask with nitrogen protection. Following addition of 2 ml HMDI and six drops of $Sn(Oct)_2$, the mixture was heated to 70 °C to start the reaction. After 3 h, the solution was cooled to room temperature. A solution of DAB (0.544 g) in 100 ml DMSO was then added dropwise to the flask. The reaction was conducted overnight under stirring. The resulting PU solution was precipitated in excess cool deionized (DI) water and dried under vacuum. The synthesized PU has a glass transition temperature (T_g) of −46 °C, tensile stress of 4.7 ± 0.6 MPa, breaking strain of 946 ± 78%, and Young's modulus of 2.2 ± 0.3 MPa.

2.3. Hydrogel synthesis

The hydrogel was based on NIPAAM, AAc, and a macromer based on HEMA and oligo (β-butyrolactone). The macromer was synthesized by reacting HEMA with β-butyrolactone (molar ratio 1:6) under 110 °C with nitrogen protection for 1 h. $Sn(OTf)_2$ was used as a catalyst. After the reaction, the mixture was cooled to room temperature, dissolved in THF, and precipitated in cool DI water. The precipitate was then dissolved in ethyl acetate, dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure. The resulting macromer was abbreviated as HEMA-oHB6.

The hydrogel was synthesized by free radical polymerization using benzoyl peroxide (BPO) as the initiator [43,44]. The molar ratio of NIPAAM/AAc/HEMA-oHB6 was 86/4/10. Stoichiometric amounts of NIPAM, AAc and HEMA-oHB6 were dissolved in dioxane in a three-necked flask. The monomer concentration was controlled at ~5 wt.%. The solution was bubbled with nitrogen for 15 min before BPO solution (in dioxane) was injected into the flask. After 24 h of reaction at 70 °C, the solution was cooled to room temperature and precipitated with hexane. The polymer was purified twice using THF/diethyl ether. The resulting polymer was finally dried under vacuum overnight.

The synthesized hydrogel had a sol–gel temperature of ~14 °C as determined by differential scanning calorimetry. This allows the hydrogel to maintain a solid state at body temperature. The degradation product poly (NIPAM-co-AAc-co-HEMA) has 4% AAc, which has been demonstrated to have a lower critical solution temperature above 37 °C [45]. Therefore, the degraded polymer

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