High-efficiency matrix modulus-induced cardiac differentiation of human mesenchymal stem cells inside a thermosensitive hydrogel

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Abstract

Mesenchymal stem cells (MSCs) experience an extremely low rate of cardiac differentiation after transplantation into infarcted hearts, in part due to the inability of stiff scar tissue to support differentiation. We hypothesized that delivering MSCs in a hydrogel with a modulus matched to that of native heart tissue should stimulate MSC differentiation into cardiac cells. We have developed a thermosensitive and injectable hydrogel suitable for the delivery of cells into the heart, and found that the appropriate gel modulus can differentiate MSCs into cardiac cells with high efficiency. The hydrogel was based on N-isopropylacrylamide, N-acryloyxysuccinimide, acryl acid and poly(trimethylene carbonate)-hydroxyethyl methacrylate. The hydrogel solution can be readily injected through needles commonly used for heart injection, and is capable of gelling within 7 s at 37 °C. The formed gels were highly flexible, with breaking strains (>300%) higher than that of native heart tissue and moduli within the range of native heart tissue (1–140 kPa). Controlling the concentration of the hydrogel solution resulted in hydrogels with three different moduli: 16, 45 and 65 kPa. The moduli were decoupled from the gel water content and oxygen diffusion, parameters that can also influence cell differentiation. MSCs survived in the hydrogels throughout the entire culture period, and it was observed that gel stiffness did not affect cell survival. After 14 days of culture, more than 76% of MSCs had differentiated into cardiac cells in the 45 and 65 kPa gels, as confirmed by the expression of cardiac markers at both the gene and protein levels. MSCs in the hydrogel with the 65 kPa modulus had the highest differentiation efficiency. The differentiated cells also developed calcium channels that imparted an electrophysiological property, and gap junctions for cell–cell communication. The efficiency of differentiation reported in this study was much higher than for the differentiation approaches described in the literature, such as chemical induction and co-culture of MSCs and cardiomyocytes. These results indicate that the novel hydrogel holds great promise for delivering MSCs into an infarcted heart for the generation of new heart tissue.

1. Introduction

Myocardial infarction (MI) is one of the major causes of heart damage [1,2]. It leads to cell death on a massive scale and the eventual loss of heart function. Current approaches used to treat MI can only re-establish blood supply to the infarcted area to prevent further damage to the heart; they are unable to regenerate lost heart muscle, so the lost heart function cannot be regained. To address this issue, cell-based therapies have been used to treat MI [1–3]. After delivery of stem cells that are capable of differentiating into cardiac cells into the infarcted heart, new heart muscle can be generated to compensate for lost heart function [4].

Mesenchymal stem cells (MSCs) are considered to be a suitable cell type for cardiac cell therapy [3,4]. Previous results have demonstrated that injection of MSCs directly into the infarct site improved heart function and stimulated local angiogenesis [2,3]. However, the transplanted MSCs did not efficiently differentiate into cardiomyocytes for the generation of new heart tissue [1]. In fact, only a low percentage of them can differentiate into cardiomyocytes [5]. One of the possible reasons for this behavior stems from the fact that the stiff scar tissue to which the MSCs were applied does not favor cardiac differentiation [6,7]. This was confirmed by various studies which demonstrated that MSC differentiation is modulus-responsive [8–10]. The culture of MSCs on hydrogels with different mechanical moduli led the cells to differentiate into different lineages [9]. Current approaches to differen-