Cytotherapy, 2014; 0: 1–10

International Society for Cellular Therapy
ISCT®

Injection of mesenchymal stromal cells into a mechanically stimulated *in vitro* model of cardiac fibrosis has paracrine effects on resident fibroblasts

PETER A. GALIE & JAN P. STEGEMANN

Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA

Abstract

Background aims. Myocardial infarction results in the formation of scar tissue populated by myofibroblasts, a phenotype characterized by increased contractility and matrix deposition. Mesenchymal stromal cells (MSC) delivered to the myocardium can attenuate scar growth and restore cardiac function, though the mechanism is unclear. *Methods.* This study describes a simple yet robust three-dimensional (3D) *in vitro* co-culture model to examine the paracrine effects of implanted MSC on resident myofibroblasts in a controlled biochemical and mechanical environment. The fibrosis model consisted of fibroblasts embedded in a 3D collagen gel cultured under defined oxygen tensions and exposed to either cyclic strain or interstitial fluid flow. MSC were injected into this model, and the effect on fibroblast phenotype was evaluated 48 h after cell injection. *Results.* Analysis of gene and protein expression of the fibroblasts indicated that injection of MSC attenuated the myofibroblast transition in response to reduced oxygen and mechanical stress. Assessment of vascular endothelial growth factor and insulin-like growth factor-1 levels demonstrated that their release by fibroblasts was markedly upregulated in hypoxic conditions but attenuated by strain or fluid flow. In fibroblast-MSC co-cultures, vascular endothelial growth factor levels were increased by hypoxia but not affected by mechanical stimuli, whereas insulin-like growth factor-1 levels were generally low and not affected by experimental conditions. *Conclusions.* This study demonstrates how a 3D *in vitro* model of the cardiac scar can be used to examine paracrine effects of MSC on the phenotype of resident fibroblasts and therefore illuminates the role of injected progenitor cells on the progression of cardiac fibrosis.

Key Words: *cardiac fibrosis, cell therapy, co-culture, hypoxia, mesenchymal stromal cells*

Introduction

The benefits of injecting adult stem cells into the myocardium after infarction have been widely examined, but there is little consensus on the degree, duration and mechanism of cardiac improvement that can be consistently achieved with the use of such treatment (1,2). Moreover, the success of such cell therapy can vary, depending on the timing and mode of cell delivery (3,4). Variability in the cell source may be influenced by hypertension, diabetes, and other pathologies that have comorbidity with heart disease. Additionally, a myriad of biochemical and mechanical factors within the microenvironment of the post-infarcted myocardium can affect the response of the transplanted cells. The scar environment is characterized by dense extracellular matrix, decreased capillary density and the presence of contractile myofibroblasts (5–7). Mechanically, the scar is stiffer than surrounding tissue, and the degree of cyclic deformation from systole and diastole is also reduced. It is therefore important to characterize and understand the response of injected stem cells to these exogenous stimuli to optimize cell therapy for the treatment of heart damage.

It is likely that transplanted adult stem cells benefit the myocardium through a paracrine mechanism (8). Originally, adult stem cells transplanted into the myocardium were thought to differentiate into cardiomyocytes (9) or fuse with damaged cells to restore function (10). However, bulk regeneration of damaged heart muscle in this manner is unlikely because it would require the transplanted cells to differentiate into a fully functional cardiomyocyte phenotype as well as to self-assemble in the correct mechanical and electrical micro-architecture. Improperly integrated heart muscle could give rise to systolic dysfunction or entry points for arrhythmias. More recently, there is a growing consensus that implanted stem cells release paracrine factors that

Correspondence: **Jan P. Stegemann**, PhD, Department of Biomedical Engineering, University of Michigan, 1101 Beal Avenue, Ann Arbor, MI 48109, USA.
E-mail: jpsteg@umich.edu

(Received 3 July 2013; accepted 31 January 2014)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2014.01.416

have cardioprotective and angiogenic effects on the damaged myocardium (11). Identification of these factors would allow their therapeutic delivery in the absence of cells. However, it is likely that stem cell–mediated paracrine release has both a spatial and time dependence, which complicates delivery of a growth factor cocktail. Model systems are therefore required to characterize this paracrine action of the implanted stem cells and their interaction with resident cardiac cell types.

A variety of methods have been used to examine the paracrine effects of transplanted stem cells on cardiac cells. A common approach involves culturing cardiomyocytes or cardiac fibroblasts in conditioned media from adult stem cells (12,13), and conditioned media from human mesenchymal stromal cells (MSC) has also been injected into animal models to analyze its effect on infarcted heart function (14). Although useful for studying the potential for stem cell–mediated benefit, these experiments cannot capture the full spectrum of molecular communication between injected stem cells and cardiac cell types. To address this issue, cell co-culture systems have been created (15). Such systems yield insight into the effect of stem cells on cardiomyocytes and illuminate how the presence of myocytes affects stem cell response. However, these simple systems typically do not include the potentially very important effects of mechanical and biochemical stimuli present in microenvironment of the native myocardium.

Animal models have also been used to study the paracrine effects of injected stem cells. These experiments have the advantage that they include the relevant myocardial architecture, presence of cardiomyocytes, fibroblasts and vascular cell types, as well as mechanical stresses during systole and diastole. A recent study showed that MSC injected into the myocardium improved wall thickness and left ventricular ejection fraction by stimulating angiogenesis (16), and improvement in left ventricular function has also been observed in human studies (17), as well as after intracoronary cell delivery (18). MSC delivered by intramyocardial injection were shown to increase the presence of myofibroblasts in the scar region (19), whereas intracoronary delivery of MSC embedded in alginate attenuated the presence of myofibroblasts (20). Genetic modification to induce overexpression of key paracrine factors such as vascular endothelial growth factor (VEGF) (21), Akt (22), CXCR4 (23) and Wnt (24) has yielded some insight into the mechanisms of therapeutic action. However, isolating the effects of specific cell-to-cell interactions and discerning the responses to particular exogenous stimuli remains a challenge when animal models are used.

The goal of the present study was to create a co-culture system that mimics key features of the myocardial scar environment but still allows resolution of the stem cell response to specific mechanical and biochemical stimuli. Our focus in this study was on the paracrine effects of MSC on resident cardiac fibroblasts because this interplay has direct relevance to the etiology and treatment of cardiac fibrosis. Fluorescently labeled MSC were injected into a cardiac fibroblast–seeded three-dimensional (3D) collagen gel to create a co-culture system, and these tissue constructs were then exposed to defined levels of fluid shear, solid stress and hypoxia. The effect of MSC injection on the phenotype of the resident fibroblasts was evaluated through the use of immunohistochemistry and analysis of gene expression at a distance of 250 μm from the injection site. Secretion of VEGF and insulin-like growth factor (IGF) by both the injected MSC and resident fibroblasts was also examined by immunoassay. These studies provide insight into how stem cells injected into the myocardium interact with resident cardiac fibroblasts under mechanical and biochemical conditions that are found in the heart.

Methods

Cell culture and collagen gel fabrication

Cardiac fibroblasts were isolated from the ventricles of 2- to 3-day-old Sprague-Dawley rats with the use of procedures approved by the Institutional Animal Use and Care Committee at the University of Michigan. Briefly, isolated ventricles were minced and digested in 0.125% trypsin and 0.15% pancreatin (Sigma-Aldrich, St Louis, MO, USA) at 37°C in consecutive 15-min steps. Fibroblasts were separated from myocytes in a pre-plating step and were culture-expanded. Fibroblasts to be used in experiments were cultured in M199 containing 5% fetal bovine serum and 1% penicillin/streptomycin (Sigma-Aldrich) for 1 week.

Three-dimensional collagen hydrogels were fabricated through the use of acid-solubilized bovine collagen type I (MP Biomedicals, Solon, OH, USA) with the use of techniques similar to those used in previous studies (25). Briefly, isolated cardiac fibroblasts were suspended at a concentration of 5.0×10^5 cells/mL in a gel precursor solution composed of collagen type I (2.0 mg/mL), 10% fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM) and 0.1 mol/L NaOH at physiological pH. Gelation was then initiated by incubation at 37°C in a humidified 5% CO_2 incubator for 45 min. The resulting collagen hydrogels had an open fibrillar structure with cells embedded directly inside the matrix, as characterized in previous studies (26,27). In this study, fibroblast-