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Substrate stiffness-regulated matrix metalloproteinase output in myocardial cells and cardiac fibroblasts: Implications for myocardial fibrosis

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ABSTRACT

Cardiac fibrosis, an important pathological feature of structural remodeling, contributes to ventricular stiffness, diastolic dysfunction, arrhythmia and may even lead to sudden death. Matrix stiffness, one of the many mechanical factors acting on cells, is increasingly appreciated as an important mediator of myocardial cell behavior. Polydimethylsiloxane (PDMS) substrates were fabricated with different stiffnesses to mimic physiological and pathological heart tissues, and the way in which the elastic modulus of the substrate regulated matrix-degrading gelatinases in myocardial cells and cardiac fibroblasts was explored. Initially, an increase in cell spreading area was observed, concomitant with the increase in PDMS stiffness in both cells. Later, it was demonstrated that the MMP-2 gene expression and protein activity in myocardial cells and cardiac fibroblasts can be enhanced with an increase in PDMS substrate stiffness and, moreover, such gene- and protein-related increases had a significant linear correlation with the elastic modulus. In comparison, the MMP-9 gene and protein expressions were up-regulated in cardiac fibroblasts only, not in myocardial cells. These results implied that myocardial cells and cardiac fibroblasts in the myocardium could sense the stiffness in pathological fibrosis and showed a differential but positive response in the expression of matrix-degrading gelatinases when exposed to an increased stiffening of the matrix in the microenvironment. The phenomenon of cells sensing pathological matrix stiffness can help to increase understanding of the mechanism underlying myocardial fibrosis and may ultimately lead to planning cure strategies.

1. Introduction

Myocardial fibrosis, a major biological determinant of mortality in cardiac remodeling following myocardial infarction (MI) [1–3], is characterized mainly by an increase in collagens in the interstitium and perivascular regions of the myocardium [4]. During pathological fibrosis, collagen I, which accounts for over 80% of the total collagen, assembles into thick fibers to convey tensile strength and structural support [5], and the tensile strength of collagen I may increase up to 100 MPa [1,6,7], while collagen III will also be immoderately accumulated [1,8]. An excessive accumulation of collagen components in pathological fibrosis enhances the stiffness of the myocardium matrix [1,2,8]. The elasticity of the fibrosis matrix could be several-fold stiffer than that of normal myocardium [1,8,10]. For example, in bird species, Young's modulus of the normal myocardium is ~14 kPa [9], while at the beginning of fibrosis, Young's modulus could increase as much as threefold [10].

The stiffness of the cardiac extracellular matrix (ECM) has been shown to regulate the fate of cardiac cells [11–19]. Forte et al. [11] revealed that substrate stiffness affected neonatal myocardial cell (MC) maturation, morphology, sarcomere organization, electromechanical coupling and gene expression. Jacot et al. [12] found that rat MC had different morphologies on gels with different stiffness, and they further observed that cells could generate greater mechanical force on gels with stiffness similar to the native myocardium than on stiffer or softer substrates. In embryonic chicken cardiac cells, Engler et al. [9] and Bajaj et al. [13] showed the phenotype and beating rate of MC vs. substrate rigidity. Kharaziha et al. [14] found that substrate stiffness could change

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the distribution density of stress fibers within the cytoskeleton of cardiac fibroblasts (CF) and vary the overall cellular alignment. Smith et al. [15] demonstrated that a substrate stiffness similar to that of heart tissue was more suitable than stiffer substrates to support embryonic MC. Wang et al. [16] confirmed a modulation of alignment, elongation and contraction of MC through a combination of nanotopography and rigidity of substrates. Shapira-Schweitzer and Seliktar [17] also showed that matrix stiffness affected spontaneous contraction of MC cultured within a PEGylated fibrinogen biomaterial. Chiu and Radisic [18] demonstrated that polyacrylamide substrates of intermediate stiffnesses (22 kPa and 50 kPa) best supported the elongation of cardiac cells and improved contractile properties compared with low stiffness (3 kPa) and high stiffness (144 kPa) gel. Furthermore, Arshi et al. [19] showed that their experimental data identified matrix stiffness as an independent factor that instructed not only the maturation of already differentiated MC, but also the induction and proliferation of MC from undifferentiated progenitors. Nevertheless, study of the influence of stiffness of myocardial fibrosis mediated MC and CF on their molecular regulation mechanisms is still limited, and understanding of the mechanism would certainly pave the way to gaining a new insight into pathological processes in cardiac remodeling.

During the process of myocardial fibrosis, over-stiffened myocardium is believed to be correlated with increased ECM turnover [20,21]. The stiffened myocardium disrupts the dynamic equilibrium between degradation and synthesis of the ECM. Similarly, it upsets the secretion of the ECM-mediated enzyme metalloproteinases (MMP) and their endogenous inhibitors [22]. Gelatinase, one of the MMP members, plays a potential role in the homeostasis of the ECM [23,24]. Previous data confirmed that mutations in collagen I can result in an increase in the left ventricle wall stress, which was proved to be associated with gelatinase A (MMP-2) activities, using animal models [25]. The adverse remodeling, a major factor for morbidity and mortality in cardiac pathologies, also inferred that MMP-2 was an important contributor after gene deletion in mice [26,28]. Meanwhile, gelatinase B (MMP-9) also plays an important role in the process of ECM remodeling [27–29]. Inhibiting MMP-9 reduced myocardial inflammation and remodeling in a variety of animal models, suggesting that MMP-9 was also a key contributor to adverse myocardial remodeling [27,28,30]. However, the influence of direct crosstalk between cell and matrix stiffness on gelatinases in the pathological myocardium needs to be explored further in vitro.

Therefore, in the present study, MC and CF were first cultured on silicon-based elastomer polydimethylsiloxane (PDMS) substrates with various stiffness similar to that of physiological and pathological heart tissues. The morphologies, MMP-2 and MMP-9 gene expressions, and MMP-2 and MMP-9 protein activities were further compared between the two cell types of the myocardium. The phenomenon of cells sensing physiological and pathological matrix stiffness can help to increase understanding of the mechanism underlying fibrosis and may ultimately lead to planning cure strategies.

2. Materials and methods

2.1. Preparation of PDMS substrates

PDMS has been used as a principal candidate in many biomaterial studies because of its flexibility, optical clarity and elastic tenability [31,32]. PDMS elastomer was prepared by mixing Sylgard 184 (Corning, NY, USA) in four different ratios of mass of curing agent to oligomeric base (i.e. 1:5, 1:15, 1:30 and 1:45), which was subsequently cast onto a single-well Petri dish (35 × 10 mm, Corning) and cured at 60 °C for 3 h. This was followed by a sterilization step, carried out by exposing samples to UV radiation for 2 h, and then cell culturing was performed.

2.2. Surface topography characterization of PDMS substrates

The deposited PDMS membranes with different stiffness were imaged by atomic force microscopy (AFM) (Nanoscope IIIa, Digital Instruments, Santa Barbara, CA) in tapping mode with 512 × 512 pixel data acquisition. The scan speed was 1 Hz at ambient conditions. The topographic images were recorded with a standard silicon tip on a cantilever beam. The spring constant of the cantilever was 50 pN nm^{-1}, and its length was 125 μm, with a resonant frequency of 300 kHz.

2.3. Young's modulus measurements of PDMS substrates

Young's modulus measurement was carried out by the spherical indentation method to characterize the stiffness of different PDMS substrates, as described previously [33]. The ElectroForce® 3100 test instrument (Bose, Shanghai, China) was used for the indentation tests. A spherical indenter 3 mm in radius was used, while the measurements were performed in ambient conditions. Loading procedures were undertaken with displacement controlled, and the loading rate was set at 2 mm s^{-1}, while the maximum indentation depth was 3 mm. Samples had a diameter of 55 mm and height 15 mm. Six measurements at different positions of the sample were conducted, and the depth–indentation load curves were recorded. The initial shear modulus was determined by fitting the load curves up to different ratios of h/R using the Hertzian solution and the hyperelastic solution [33], as follows

$$P = \frac{16}{9} E \sqrt{Rh} h \left(1 - 0.15 \frac{h}{R}\right) \quad (1)$$

where E represents Young's modulus, P is the indentation load, h is the indentation depth, and R is the indenter radius.

2.4. Isolation and culture of MC and CF

White leghorn chicken embryos were obtained from China Agricultural University, Beijing, China. The animal materials used for this study were obtained according to ethical principles, and the protocol was reviewed and approved by the Institutional Review Board (IRB) of both Tsinghua University and China Agricultural University.

Ventricles, dissected under sterile conditions from chicken embryos 10–12 days old, were washed three times with 1 × phosphate buffered saline (PBS) to avoid red blood cell contamination and were further cut into small pieces and subsequently used to collect cell suspensions by being exposed to nominally Ca^{2+}–Mg^{2+}-free saline containing 2 mg ml^{-1} collagenase (Sigma, St Louis, MO) every 10 min until they were digested, then centrifuged at 1000 rpm for 5 min. The cells at the bottom of centrifuge tube were collected by discarding the supernatant and adding culture medium (high glucose DMEM, Corning, supplemented with 1% penicillin/streptomycin and 10% heat-inactivated horse serum (HS-DMEM), and the HS-DMEM was selected to inhibit proliferation of low-ratio CF coexisting in isolated MC). The cell suspensions were plated onto a single-well Petri dish. After 2 h attachment, the non-adherent cells in suspensions (MC) were collected and transferred to other dishes with 10% HS-DMEM (The cells within the 2 h attachment were discarded to avoid CF contamination.) The MC were maintained in an atmosphere of 95% air −5% CO_2 at 37 °C and 95% relative humidity. Passage 0 was used in all experiments.