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Heterogeneous micromechanical properties of the extracellular matrix in healthy and infarcted hearts

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ABSTRACT

Infarcted hearts are macroscopically stiffer than healthy organs. Nevertheless, although cell behavior is mediated by the physical features of the cell niche, the intrinsic micromechanical properties of healthy and infarcted heart extracellular matrix (ECM) remain poorly characterized. Using atomic force microscopy, we studied ECM micromechanics of different histological regions of the left ventricle wall of healthy and infarcted mice. Hearts excised from healthy ($n = 8$) and infarcted mice ($n = 8$) were decellularized with sodium dodecyl sulfate and cut into 12 μm thick slices. Healthy ventricular ECM revealed marked mechanical heterogeneity across histological regions of the ventricular wall with the effective Young's modulus ranging from 30.2 ± 2.8 to 74.5 ± 8.7 kPa in collagen- and elastin-rich regions of the myocardium, respectively. Infarcted ECM showed a predominant collagen composition and was 3-fold stiffer than collagen-rich regions of the healthy myocardium. ECM of both healthy and infarcted hearts exhibited a solid-like viscoelastic behavior that conforms to two power-law rheology. Knowledge of intrinsic micromechanical properties of the ECM at the length scale at which cells sense their environment will provide further insight into the cell–scaffold interplay in healthy and infarcted hearts.

1. Introduction

Heart failure due to myocardial infarction (MI) is a major healthcare issue worldwide [1]. The myocardial tissue that is damaged during MI lacks the ability to significantly regenerate itself, which leads to adverse left ventricular (LV) remodeling and eventual heart failure [2,3]. While total heart transplantation remains the only successful treatment for end-stage post-MI heart failure, this approach is limited by the lack of donors [4]. Thus, tissue engineering and cell therapy strategies to repair and regenerate the area of infarct are essential to prevent heart failure post-MI. New approaches to treat heart failure, such as recellularization and the design of biomimetic scaffold materials for cardiac tissue engineering, show great potential [5–7].

Following acute MI and loss of necrotic cardiomyocytes a complex process of cardiac repair and remodeling begins. Structural remodeling is initiated by an inflammatory reaction followed by scar formation in the extracellular matrix (ECM) at the infarct site, which is associated with interstitial fibrosis and vascular remodeling in areas of non-infarcted myocardium [8]. The increase in collagen content, collagen crosslinking, fiber structure and variation in the ratio between collagen I and collagen III after remodeling are associated with an increase in stiffness [9,10].

Microenvironment mechanical cues have been demonstrated to mediate cardiac cell behavior [11–13]. Functional maturation of neonatal rat ventricular cardiomyocytes has been shown to be affected by substrate stiffness [14]. Microenvironmental stiffness has also been reported to influence the phenotype and contractile properties of heart cells [12]. Engler et al. [13] observed that

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embryo-derived cardiomyocytes beat best on substrates with heart-like stiffness. Jacot et al. [11] reported highest contractile forces along with enhanced myocyte structure on substrates that match tissue stiffness. Moreover, Hersch et al. [15] found that cardiomyocytes adapt their contractile forces to substrate stiffness. Therefore, a precise definition of the local mechanical properties of heart ECM is needed to further our understanding of the cell–microenvironment interplay. A better knowledge of the connection between material properties, tissue structure and functionality of the heart, and how their alterations lead to dysfunctional behavior of the organ, will provide know-how for further steps in tissue engineering scaffolds.

Mechanical properties of heart tissue have been studied by means of atomic force microscopy (AFM) in fresh samples of healthy and infarcted hearts excised from mice [16] and rats [14]. Since fresh tissue contains cells and ECM, it is difficult to separate their relative contribution to tissue mechanics. The mechanical properties of the heart ECM scaffold have been studied in decellularized rat hearts by subjecting tissue strips excised from the LV wall to tensile assays [6,17]. However, intrinsic mechanical properties are difficult to obtain in samples thick enough to contain the 3-D architectural structure of the tissue. Therefore, a direct approach to characterize the intrinsic micromechanical behavior of the ECM is to probe thin decellularized tissue slices with AFM, as has been done for lung [18].

This work studies the local micromechanical properties of healthy and infarcted heart ECM by AFM in thin slices of decellularized heart scaffolds of mice. ECM stiffness of the healthy LV was studied by measuring the effective Young's modulus (E) of the endocardium, myocardium and epicardium. Measurements in the myocardium were performed both in collagen- and elastin-rich regions, which are major components of the heart ECM. The effect of MI was assessed by measuring E in the scar produced in the myocardium. To associate mechanical behavior with ECM composition, stiffness maps were computed in non-fixed immunostained slices. The viscoelastic properties were characterized by the complex shear modulus (G^*) measured by applying small-amplitude oscillatory indentations over a wide frequency range. The viscoelastic behavior of both healthy and infarcted ECM was interpreted in terms of the two power law rheological model.

2. Materials and methods

2.1. Subjects

The study was carried out on healthy ($n = 8$) and infarcted hearts ($n = 8$) excised from 12–14 week old Bl6/C57 male mice. The infarction protocol is described elsewhere [19]. Briefly, 8–10 week old animals were anesthetized with 2% isofluorane, intubated, connected to a ventilator for small animals and placed on a heating table in a supine position. A thoracotomy was then performed at the left fourth intercostal space. Next the pericardium was opened and the left anterior descending coronary artery was ligated using a 7.0 absorbable suture. The pericardial incision was closed in layers with a 6.0 absorbable suture and the skin incision with 6.0 sutures. Finally, the endotracheal tube was removed and spontaneous breathing restored. The animals were kept in a cage, lying on a heating blanket for several hours until recovery from surgery. The infarcted animals were kept alive for 4 additional weeks to ensure that tissue scar was present. Healthy and infarcted mice were killed and hearts were excised after phosphate-buffered saline perfusion. The experiments were performed in accordance with the principles of laboratory animal care formulated by the National Society for Medical Research and the guide for care and use of laboratory animals of the Institute of Laboratory Animal Resources (Commission on Life Science, National Research Council). All animal procedures were approved by the University of Navarra Institutional Committee on Care and Use of Laboratory Animals.

2.2. Decellularization

The decellularization protocol used in the study was based on that described by Ott et al. [6]. Hearts were continuously perfused through the antegrade coronary artery with 1% sodium dodecyl sulfate (SDS) for 12 h. Perfusion pressure was maintained at 70 mmHg to avoid damaging the 3-D structure of the ECM. After decellularization, SDS was washed by perfusing Dulbecco's phosphate-buffered saline (DPBS). Subsequently, the hearts were embedded in tissue freezing medium (OCT compound, Sakura, Torrance, CA) and 12 μm thick transverse slices of the LV scaffold were cut with a cryostat. The slices were immobilized onto positively charged glass slides and OCT was washed with DPBS. The samples were kept in DPBS during the AFM measurements.

2.3. AFM measurements

Mechanical measurements were carried out with a custom-built atomic force microscope attached to an inverted optical microscope (TE2000, Nikon, Tokyo, Japan). Measurements were performed at room temperature with four-sided pyramidal tips attached to cantilevers with a nominal spring constant (k) of 0.03 N m^{-1} (MLCT, Bruker, Mannheim, Germany). The spring constant of the cantilevers was calibrated by thermal tuning using the simple harmonic oscillator model. Photodiode calibration to obtain cantilever deflection (d) was performed on a bare region of the glass coverslip to which samples were attached. The force exerted by the tip was computed as $F = k \cdot d$ [20].

To compute the effective Young's modulus of the ECM 10 force–displacement curves (F–z) were recorded at 1 Hz with a peak-to-peak amplitude of 5 μm. The last force curve was used to calculate an operating indentation of 500 nm. Subsequently, the viscoelastic properties of the ECM were measured by applying low-amplitude (75 nm) multifrequency oscillations to the sample around the operating indentation (500 nm). The multifrequency signal was composed of five sinusoidal components with frequencies of 0.1, 0.35, 1.15, 3.55 and 11.45 Hz. The viscous drag of the cantilever was measured by oscillating the tip 12 μm above a bare region of the coverslip.

In healthy hearts, measurements were taken in four regions of the LV scaffold: endocardium, regions of the myocardium rich in collagen, regions of the myocardium rich in elastin located around the vessels, and in the epicardium. In the infarcted hearts, measurements were taken in the central area of the myocardium scar.

The regions of interest for AFM measurements were identified by means of phase-contrast images recorded with the inverted optical microscope. The endocardium, epicardium and collagen-rich regions of the myocardium were localized by morphology. Elastin-rich regions in the tunica intima of the vessels were identified by autofluorescence in a wavelength that included the elastin autofluorescence range, i.e. 410/440 excitation/emission [21,22]. The localization of elastin around the vessels was confirmed by epifluorescence imaging [21]. For each region, AFM measurements were taken in two areas. In each area the measurements were performed in four points separated by 5 μm. To assess the relationship between mechanical properties and histology, AFM stiffness maps were recorded in non-fixed slices immunostained for elastin and collagen. In each region, measurements were taken over a square grid of 9×9 points defining an area of 40 μm $\times$ 40 μm.

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