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The effect of bioengineered acellular collagen patch on cardiac remodeling and ventricular function post myocardial infarction

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ABSTRACT

Regeneration of the damaged myocardium is one of the most challenging fronts in the field of tissue engineering due to the limited capacity of adult heart tissue to heal and to the mechanical and structural constraints of the cardiac tissue. In this study we demonstrate that an engineered acellular scaffold comprising type I collagen, endowed with specific physiomechanical properties, improves cardiac function when used as a cardiac patch following myocardial infarction. Patches were grafted onto the infarcted myocardium in adult murine hearts immediately after ligation of left anterior descending artery and the physiological outcomes were monitored by echocardiography, and by hemodynamic and histological analyses four weeks post infarction. In comparison to infarcted hearts with no treatment, hearts bearing patches preserved contractility and significantly protected the cardiac tissue from injury at the anatomical and functional levels. This improvement was accompanied by attenuated left ventricular remodeling, diminished fibrosis, and formation of a network of interconnected blood vessels within the infarct. Histological and immunostaining confirmed integration of the patch with native cardiac cells including fibroblasts, smooth muscle cells, epicardial cells, and immature cardiomyocytes. In summary, an acellular biomaterial with specific biomechanical properties promotes the endogenous capacity of the infarcted myocardium to attenuate remodeling and improve heart function following myocardial infarction.

1. Introduction

Due to the impaired self-renewal capacity of adult cardiomyocytes and the limited number of cardiac progenitors, the human heart exhibits insufficient ability to restore heart function and structure following injury [1–4]. In recent years, clinical trials to treat severe heart injuries, primarily focused on cell transplantation, have shown modest success due to a number of limitations; these include low cardiac engraftment efficiency, immune system reaction, poor penetration, deficient viability and retaining of the cells at the site of injury, exorbitant costs, and the lack of control of the fate of the grafted cells [5–9]. Therefore, there is a critical need for the development of alternative cardiac regenerative approaches.

The field of cardiovascular tissue engineering is recently receiving growing attention as a new therapeutic modality to treat heart injuries [1,10,11]. This multidisciplinary field applies the principles of engineering, cell biology, and clinical therapies, towards generating tissue replacement (i.e. scaffold or cardiac patch) that maintains, restores, or improves the function of damaged or diseased cardiac tissue [11,12]. Engineered tissue scaffolds are aimed to provide: I) a mechanical support for tissue replacement/regeneration; II) an environment that favors cell migration and integration, proliferation, and differentiation for the desired therapeutic effect; and III) a permeable construct that allows efficient nutrient and waste transfer. The chemical composition, physiomechanical properties (e.g., stiffness), and 3D architecture of the scaffolds have been shown to play key roles in

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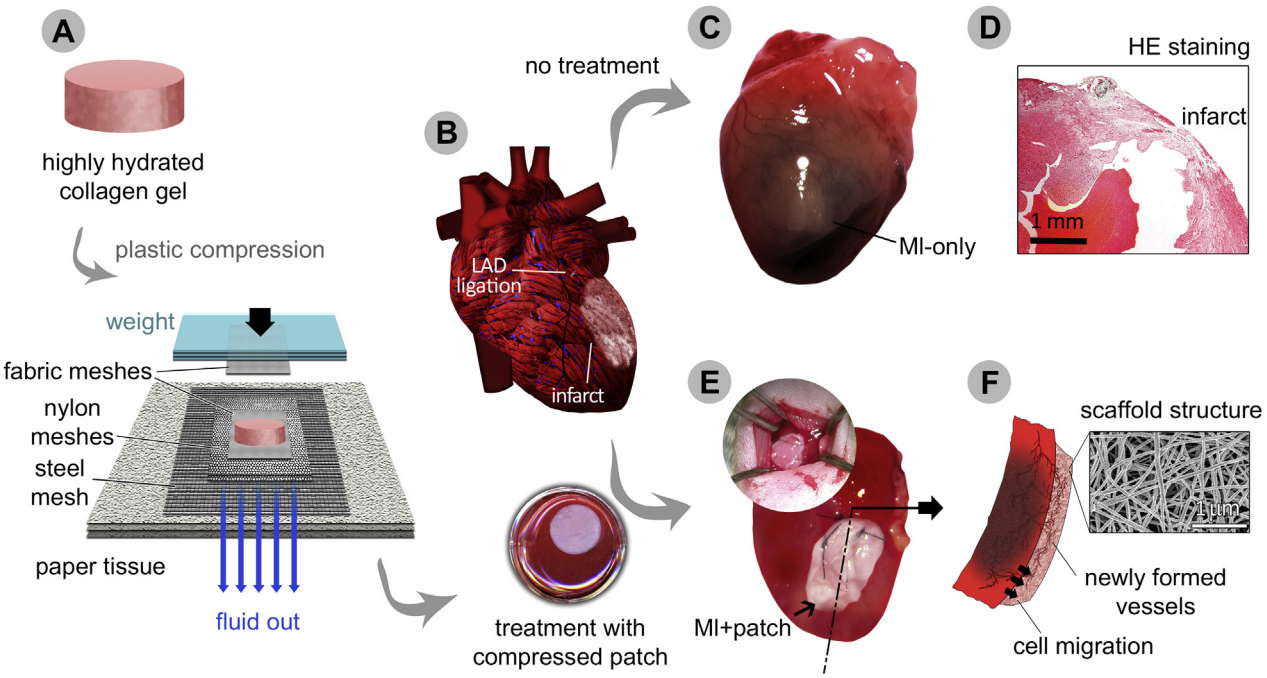

Fig. 1. Schematic representation of inducing myocardial infarction (MI) via permanent ligation of LAD artery (A) which was either treated with patch (B,C) or left untreated (D,E). Panel C depicts a cross-sectional view of the patch located on the infarct, describing various processes involved in protective role of engineered patch. The inset on the right shows electron microscopy of the dense collagen scaffold fibrillar structure (reconstructed from Ref. [44]). Panel E shows HE staining of the MI heart with no treatment.

determining cell–microenvironment interactions and the fate of stem cells [13–15].

Whereas the outcomes of the studies utilizing various systems of cardiac patches have been modest but promising [16–19], the clinical application of these techniques has been limited. The main obstacles include poor control of the patch's structure and properties, insufficient vascularization, infection, immunogenicity, and lack of appropriate physiomechanical cues to promote cardiogenesis [11,20–22]. Moreover, incorporating exogenous cells into the patch can cause additional complications including diffusional limitations of oxygen/nutrients transport, the necessity of an extensive, functional vascular network, poor integration and differentiation of the cells, and immune system responses [23–25].

Considering the challenges and restrictions on the *in vivo* application of cellular patches, *acellular* biomaterial matrices have been recently evaluated as mechanical-structural supports for lesions, as well as for their potential to provide a favorable physiological milieu for new tissue formation [26–30]. Among various biomaterials, *in vitro* reconstituted three-dimensional (3D) collagen type I scaffolds are increasingly being used as protein substrates in diverse biomedical applications, particularly, in tissue repair, via providing a biomimetic environment for cell growth [15,31–33]. Their main attributes include favorable fibrous structure, mechanical properties, biocompatibility, and biodegradability [20,31,32].

The goal of this study is to engineer a myocardial-friendly environment through the use of biologically compatible, type I collagen gel with optimized physiomechanical properties in order to organize and condition the cardiac cells already present in the damaged heart tissue. Plastic compression technique was used to rapidly generate dense tissue scaffolds with optimal elastic modulus to support myocyte contractility [34–37]. We assessed the epicardial delivery of the engineered collagen scaffold to treat acute transmural myocardial infarction (MI) in the mouse.

2. Materials and methods

2.1. Collagen gel preparation–plastic compression

Highly hydrated collagen gels were produced by adding 0.5 and 0.6 ml of 10X and 1X DMEM (Sigma, MO, US), respectively, to 0.9 ml of sterile rat tail-derived type I collagen solution in acetic acid (3.84 mg/ml, Millipore, MA, US) and neutralizing with 5 M NaOH. The collagen solution (0.9 ml) was then cast into 24-well plates (15.6 mm in diameter) and placed in a tissue culture incubator for 30 min at 37 °C for polymerization. Compressed gels–used as cardiac patch in this study–were produced as previously described [34–36]. Briefly, as cast highly hydrated collagen gels were transferred to a porous support comprising (bottom to top) absorbent paper blot layers, a steel mesh, two polymer meshes, and two fabric meshes (Fig. 1A). Subsequently, a static compressive stress of either 340, 1000, or 1400 N/m^2 was applied to the hydrated scaffolds for 2–5 min in order to remove water and produce a dense biomaterial with improved biological and mechanical properties.

2.2. Atomic force microscopy–mechanical characterization of the patch

Elastic moduli of the compressed collagen gels were assessed by atomic force microscopy (AFM) in nanoindentation mode, using a force trigger that resulted in a minimal local strain of less than 10% (indentation of ~100 nm) in order to minimize the effect of substrate-related artifacts. For this purpose, a custom-designed flat AFM tip was fabricated using focused ion beam milling and utilized to probe the gel stiffness by scanning areas of 90 μm × 90 μm (Fig. 2). A minimum number of ($n > 3$) gels were tested per each condition. For each gel specimen, a minimum of 3 areas were probed.

The surface topography and phase contrast images of the compressed collagens were also assessed by AFM, in semi-contact mode with rounded cantilevers ($R_{tip} = 40$ nm) for prolonged scanning and minimal surface damage.

2.3. Mouse model of myocardial infarction–epicardial application of the patch

All procedures involving animal use, housing, and surgeries were approved by the Stanford Institutional Animal Care and Use Committee (IACUC). Animal care and interventions were provided in accordance with the Laboratory Animal Welfare Act. Male 10–13 weeks old C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and studied in four different groups: I) control mice underwent sham ligation with no infarct/patch, II) MI-only group which had infarct with no treatment (Fig. 1B–D), III) healthy myocardium treated with the patch, and IV) infarcted myocardium grafted with patch ($n > 5$, Fig. 1B, E–F). For this purpose, mice

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