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In vitro maturation of large-scale cardiac patches based on a perfusable starter matrix by cyclic mechanical stimulation

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ABSTRACT

The ultimate goal of tissue engineering is the generation of implants similar to native tissue. Thus, it is essential to utilize physiological stimuli to improve the quality of engineered constructs. Numerous publications reported that mechanical stimulation of small-sized, non-perfusable, tissue engineered cardiac constructs leads to a maturation of immature cardiomyocytes like neonatal rat cardiomyocytes or induced pluripotent stem cells/embryonic stem cells derived self-contracting cells. The aim of this study was to investigate the impact of mechanical stimulation and perfusion on the maturation process of large-scale (2.5 × 4.5 cm), implantable cardiac patches based on decellularized porcine small intestinal submucosa (SIS) or Biological Vascularized Matrix (BioVaM) and a 3-dimensional construct containing neonatal rat heart cells. Application of cyclic mechanical stretch improved contractile function, cardiomyocyte alignment along the stretch axis and gene expression of cardiomyocyte markers. The development of a complex network formed by endothelial cells within the cardiac construct was enhanced by cyclic stretch. Finally, the utilization of BioVaM enabled the perfusion of the matrix during stimulation, augmenting the beneficial influence of cyclic stretch. Thus, this study demonstrates the maturation of cardiac constructs with clinically relevant dimensions by the application of cyclic mechanical stretch and perfusion of the starter matrix.

Statement of significance

Considering the poor endogenous regeneration of the heart, engineering of bioartificial cardiac tissue for the replacement of infarcted myocardium is an exciting strategy. Most techniques for the generation of cardiac tissue result in relative small-sized constructs insufficient for clinical applications. Another issue is to achieve cardiomyocytes and tissue maturation in culture. Here we report, for the first time, the effect of mechanical stimulation and simultaneous perfusion on the maturation of cardiac constructs of clinical relevant dimensions, which are based on a perfusable starter matrix derived from porcine small intestine. In response to these stimuli superior organization of cardiomyocytes and vascular networks was observed in contrast to untreated controls. The study provides substantial progress towards the generation of implantable cardiac patches.

1. Introduction

More people die annually from cardiovascular diseases (CVDs) than from any other cause [1]. Acute myocardial infarction (MI) is usually the result of an occlusion of a coronary artery followed by ischemia or reperfusion injury and necrosis of the tissue. In the best of cases, patients face gradual deterioration of their condition over several years, ultimately resulting in heart failure and death. This failure results from a lack of intrinsic regenerative

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responses [2] unable to replenish up to one billion lost cardiomyocytes and entails scar formation and rapidly compromised heart function in mammals [3].

Cardiac tissue engineering (TE) is an evolving technology, which is aiming to create functional tissue that can reconstruct the structure and function of injured myocardium. Many groups have established myocardial TE protocols utilizing collagen or gelatin, as those composites are biodegradable and readily available [4,5]. Our group focuses on the engineering of cardiac constructs based on decellularized small intestinal submucosa (SIS), which is one of the most broadly utilized biological matrices. In numerous studies, SIS showed the potential to support cell attachment, proliferation, migration and differentiation (reviewed in Andrée et al. [6]). Additionally, we utilize porcine SIS with preserved mesenteric arterial and venous pedicles – named Biological Vascularized Matrix (BioVaM), which presents a new approach in tissue engineering [7]. This preparation allows the perfusion of the vessel bed of small intestinal segments and offers an option to create an artificial vascularization *in vitro*. For a better comparison of each of the preparations similarities and differences of SIS and BioVaM are summarized in Table 1. Different approaches for the generation of vascularized, cardiac tissue have been reported employing either *in vivo* systems like arteriovenous loop chamber [8] or *in vitro* systems containing native blood vessels [9,10] or microchannels [11,12].

In order to increase cardiomyocyte maturation *in vitro*, different conditioning methods have been developed. Controlled electrical stimulation (pacing) to induce active muscle contraction and mechanical stimulation (stretch) to alter passive muscle loading are most commonly utilized to imitate the most important aspects of cardiomyocyte work. The initial evidence of effectiveness of mechanical stimulation protocols in cardiac TE dates back to 1996, when Vandenburgh et al. demonstrated that unidirectional mechanical stretch initiated *in vitro* a number of morphological alterations in a confluent cardiomyocyte population which were similar to those occurring during *in vivo* heart growth [13]. In 2000 Fink et al. proposed an *in vitro* model of stimulating engineered heart tissue (EHT) allowing morphological, molecular, and functional consequences of stretch to be studied under defined conditions [14]. In the following years this model revealed that unidirectional cyclic stretch improved cardiomyocyte organization, force development, and number and distribution of adherens junctions, gap junctions and desmosomes [5,14]. Additionally, cyclic mechanical stretch has been reported to upregulate the secretion of various autocrine and paracrine factors, such as insulin-like growth factor-I (IGF-I), adenosine mono- and tri-phosphate (AMP, ATP), reactive oxygen species (ROS), and prostaglandins (PG) [15]. Finally, Zimmermann et al. provided the evidence that EHT generated with the aid of mechanical strain *in vitro* could survive after implantation and support contractile function of infarcted rat hearts [16,17].

However, most of these *in vitro* generated cardiac tissues are small in size and lack an appropriate vascularization. Therefore, we developed a large-scale cardiac patch based on SIS and the perfusable starter matrix BioVaM and a 3-dimensional neonatal rat heart cardiomyocyte construct [18]. To allow the perfusion of the starter matrix and mechanical stimulation of cardiac constructs a bioreactor was developed. The data provided in this study suggests, that cyclic mechanical stretch improves cardiovascular properties of large-scale constructs regarding contractility, cell alignment, cardiac gene expression and endothelial cell network formation. In addition, this study demonstrates the simultaneous perfusion of the starter matrix resulting in further improvement of cardiomyocyte and endothelial cell properties. Thus, this study demonstrates a major step towards surgically applicable tissue engineered cardiac constructs.

2. Material and methods

2.1. Animal care

This work was approved by the Institutional Review Board and the local Animal Care. It was conducted according to local government policy (#10/0214) and Committee protocols of Hannover Medical School and the Research Advisory Committee. All animals received humane care in compliance with the European Convention on Animal Care.

2.2. Isolation of neonatal rat heart cells

Neonatal rat heart cells (nrHC) were isolated as previously reported [19]. In brief, hearts from 1- to 3-day old Sprague Dawley rats were minced and enzymatically digested with 0.44 mg/mL type II collagenase (Worthington Laboratories) and 0.1 mg/mL pancreatin (Sigma–Aldrich) at 37 °C. Neonatal rat cardiomyocyte (nrCM) population within the primary isolate was enriched either by centrifugation through a discontinuous Percoll gradient using two density solutions, 1.062 and 1.082 g/mL, made from Percoll reagent (GE Healthcare) or using a preplating method, as previously described [20]. In brief, isolates were cultivated for 1 h in cardio medium (DMEM:M199 in ratio 1:4 supplemented with 10% fetal calf serum (PAA), 5% horse serum (Gibco), 2 mM L-Glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin) in a humidified 37 °C atmosphere with 5% CO_2. Following the incubation period, the supernatant enriched for nrCM was collected. As described before, the resulting cell isolates contained 65% nrCM after preplating and 77% nrCM after Percoll gradient [18].

Table 1
Comparison of SIS and BioVaM.

	SIS	BioVaM
Origin	Porcine small intestine	Porcine small intestine
Availability	Large amounts from one pig (average 10 m)	In average 3 BioVaMs from one pig
Preparation	Simple removal	Cannulation of mesenteric artery and vein of an intestinal segment
Mechanical decellularization	Removal of mucosa and serosa	Removal of mucosa only
Chemical decellularization	1% Triton X-100, 24 h	1% Triton X-100, 48 h
Composition	Mainly collagen I	Mainly collagen I
Thickness	100–200 µm	400–500 µm
Possibility for perfusion *in vitro*	No	Yes
Possibility of connection to host circulatory system after transplantation *in vivo*	No	Yes