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Textile-templated electrospun anisotropic scaffolds for regenerative cardiac tissue engineering

H. Gözde Şenel Ayaz [a], Anat Perets [a], Hasan Ayaz [a], Kyle D. Gilroy [b], Muthu Govindaraj [c], David Brookstein [c], Peter I. Lelkes [d, *]

[a] Drexel University, School of Biomedical Engineering, Science and Health Systems, Philadelphia, PA 19104, USA
[b] Temple University, Dept. Mechanical Engineering, College of Engineering, Philadelphia, PA 19122, USA
[c] Philadelphia University, School of Engineering & Textiles, Philadelphia, PA 19144, USA
[d] Temple University, Dept. Bioengineering, College of Engineering, Philadelphia, PA 19127, USA

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ABSTRACT

For patients with end-stage heart disease, the access to heart transplantation is limited due to the shortage of donor organs and to the potential for rejection of the donated organ. Therefore, current studies focus on bioengineering approaches for creating biomimetic cardiac patches that will assist in restoring cardiac function, by repairing and/or regenerating the intrinsically anisotropic myocardium. In this paper we present a simplified, straightforward approach for creating bioactive anisotropic cardiac patches, based on a combination of bioengineering and textile-manufacturing techniques in concert with nano-biotechnology based tissue-engineering stratagems. Using knitted conventional textiles, made of cotton or polyester yarns as template targets, we successfully electrospun anisotropic three-dimensional scaffolds from poly(lactic-co-glycolic) acid (PLGA), and thermoplastic polycarbonate-urethane (PCU, Bionate®). The surface topography and mechanical properties of textile-templated anisotropic scaffolds significantly differed from those of scaffolds electrospun from the same materials onto conventional 2-D flat-target electrospun scaffolds. Anisotropic textile-templated scaffolds electrospun from both PLGA and PCU, supported the adhesion and proliferation of H9C2 cardiac myoblasts cell line, and guided the cardiac tissue-like anisotropic organization of these cells *in vitro*. All cell-seeded PCU scaffolds exhibited mechanical properties comparable to those of a human heart, but only the cells on the polyester-templated scaffolds exhibited prolonged spontaneous synchronous contractility on the entire engineered construct for 10 days *in vitro* at a near physiologic frequency of ~120 bpm. Taken together, the methods described here take advantage of straightforward established textile manufacturing strategies as an efficient and cost-effective approach to engineering 3D anisotropic, elastomeric PCU scaffolds that can serve as a cardiac patch.

1. Introduction

Cardiovascular diseases remain one of the major health problems in the United States. According to the 2012 statistical update of the American Heart Association, 7,900,000 Americans have had a myocardial infarct (MI). Annually, an estimated 610,000 Americans will have a new coronary attack, 325,000 will have a recurrent MI and 195,000 will experience a silent first MI [1]. MI-associated ischemia leads to regional cell death followed by replacement of the injured myocardial tissue by fibrous scar tissue, which is comprised mainly of collagenous extracellular matrix (ECM) and fibroblasts, and results in discontinuous propagation of the electrical signal and impaired cardiac function [2]. In repairing or replacing the damaged myocardium, a major goal is to engineer

Abbreviations: Bionate, Bionate® thermoplastic polycarbonate urethane (PCU); PLGA, poly(lactic-co-glycolic) acid; CT, cotton fabric template; PE, polyester fabric template; TG, target plate template; PLGA_CT, PLGA fibers collected on cotton fabric; PLGA_PE, PLGA fibers collected on polyester fabric; PLGA_TG, PLGA fibers collected on flat target; Bionate_CT, Bionate fibers collected on cotton fabric; Bionate_PE, Bionate fibers collected on polyester fabric; Bionate_TG, Bionate fibers collected on target plate.

* Corresponding author. Temple University, Dept. Bioengineering, College of Engineering, Engineering Building Room 811, 1947 N. 12th Street, Philadelphia, PA 19122, USA. Tel.: +1 215 204 3307; fax: +1 215 204 3326.

E-mail addresses: hgs24@drexel.edu (H.G. Şenel Ayaz), Anatperets@gmail.com (A. Perets), hasan.ayaz@drexel.edu (H. Ayaz), kyle.gilroy@temple.edu (K.D. Gilroy), govindarajm@philau.edu (M. Govindaraj), yarnboy@aol.com (D. Brookstein), pilelkes@temple.edu, lelkes123@gmail.com (P.I. Lelkes).

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biomimetic 3-D cardiac scaffolds, which emulate the structural organization and mechanical properties of the healthy native tissue, especially its elasticity and its anisotropy.

The mammalian heart is composed of collagen-based ECM with well-aligned myocytes and fibroblasts and capillary endothelial cells, leading to structural and mechanical anisotropy of the contractile ventricular myocardium [3,4]. One of the central aims of functional cardiac tissue engineering is to generate anisotropic scaffolds that will emulate the intrinsic anisotropy of the myocardium and guide the alignment of cells growing on/in these scaffolds. The desired orientation and elongation of cultured cells can be induced by mechanical and electrical stimulation [4–7] and also by tissue scaffolds engineered to provide "contact guidance" [8], i.e. a permissive environment in which the cells are "guided" to align according the surface topography of the scaffolds. Photolithography [8,9], soft lithography [10], micro-grooving [11], molecular self-assembly [12], micro-fluidics [10], micro-abrasion [13], micro-contact printing [14], micro-ablation [15,16] are some of the techniques used for manufacturing anisotropic scaffolds. For example, Gonnermann and colleagues described a series of geometrically anisotropic collagen-GAG (CG) scaffolds with aligned tracks of ellipsoidal pores, fabricated via directional solidification and freeze-drying technique [17]. Engelmayr and colleagues used micro-ablation of polyglycerol sebacate (PGS) to manufacture accordion-like honeycomb scaffolds, which matched the anisotropy and mechanical properties of native myocardium and guided the alignment of cultured neonatal rat heart cells and C2C12 myoblasts without any external stimuli [15]. However, while elegant, these techniques are tedious, time-consuming and costly in terms of manufacturing [18] and entail potential thermal degradation of bioresorbable polymers and biomaterials [19]. Therefore, one of the goals of this study was to develop a simple, efficient and cost-effective approach to engineering complex 3-D cardiac scaffolds, which emulate the mechanical properties of the native tissue, specifically its elasticity and anisotropy.

Textile engineering and tissue engineering are two distinct disciplines that are rapidly becoming intertwined in providing life-saving solutions to debilitating biomedical problems [20]. Currently, there are a number of textile-based biomedical devices on the market, such as vascular grafts made of Dacron®; (polyethylene terephthalate) and Goretex® (expanded polytetrafluoroethylene) to replace blocked large and medium-sized blood vessels [21], or silk-fibroin based surgical meshes, such as SeriACL™ and SeriFascia, for ACL repair and abdominal surgery, respectively. For cardiac applications, knitted textile structures have been implemented because of their high elasticity, porosity, and micro-scale patterns that promote anisotropy. For example, the Acorn CorCap™ passive cardiac support device is a knitted poly(ethylene terephthalate) (polyester, or PET) mesh that is wrapped around the dilated heart to provide mechanical support [22]. Knitted fabrics are also being used as scaffolds for cartilage and heart tissue engineering [23–26].

Knitted fabrics like the Acorn CorCap™ provide global mechanical support to the heart, but have disadvantages for tissue engineering purposes, because of their macroscopic fiber structure and pore size. Cells seeded onto such macro-scale scaffolds need to deposit their own ECM in order to generate a suitable (nano-scale) microenvironment that promotes adhesion, proliferation and also functional tissue [25,27]. In the past hybrid scaffolds have been designed to provide an ECM-like environment, for example by forming collagen micro-sponges in the openings of a large-pore knitted silk mesh [28].

Textile engineering has provided a number of established platform technologies for biomedical applications, such as weaving, knitting or, more recently, electrospinning. Electrospinning, invented in 1930s [29], is a versatile textile engineering-based platform technology that is widely used for fabricating non-woven scaffolds with a nano-/micro-fibrous architecture for a variety of applications in tissue engineering, such as wound healing, drug delivery, biosensor, protective clothing, cosmetics and filtration [30]. The major advantages of electrospun scaffolds are high surface area to volume ratio and tunable porosity. The nano-structure of electrospun fibers mimics that of the ECM and facilitates cell adhesion, proliferation, and differentiation [30–32].

Contact guidance and anisotropy have also been investigated in the context of electrospun scaffolds. Several groups have reported the production of electrospun meshes with fibers aligned by post-stretching [33] or by using rotating cylinder collectors, auxiliary electric fields, rotating drums and counter electrodes, and electrostatic lenses [31,34–39]. The alignment of the fibers provided the necessary contact guidance for cellular alignment and anisotropy of the resulting constructs. However, with their densely packed fibers and reduced porosity, fully aligned electrospun scaffolds do not allow for ready cellular penetration throughout the depth of the scaffolds [40]. Previous studies suggested that the surface topography of nanofibrous mats electrospun onto patterned metallic collectors mimicked that of the targets [41]. Neves and colleagues determined that templated scaffolds demonstrate various tensile properties depending on their surface topography [41]. Since textile engineering provides an infinite number of designs and patterns of fabrics, we hypothesized that textile materials might serve as convenient and inexpensive templates for engineering electrospun tissue scaffolds with a wide variety of surface topographies, including anisotropic patterns.

In this study we used ordinary, commercially available knitted textiles as templates for electrospinning anisotropic scaffolds for engineering intrinsically anisotropic tissues, such as the myocardium. We hypothesized that by using knitted textiles as templates, the electrospun scaffold can emulate topographic properties of the knitted fabric, while its ECM-like nanofibrous structure can support cardiac cell adhesion, proliferation and the assembly of functional tissue-like, beating myocardial constructs.

2. Materials and methods

2.1. Materials

Poly(L-lactide-co-glycolide) (PLGA 80:20 PURASORB® 1.24 kg/l) was purchased from PURAC Biomaterials (Gorinchem, Groningen The Netherlands). Bionate® 80A UR Thermoplastic Polycarbonate Urethane was purchased from DSM Biomedical (Part Number: FP70063, Berkeley, California). Fabrics, made from polyester and cotton were knitted at Philadelphia University, Department of Engineering &Textiles, as previously described [42].

2.2. Electrospinning

Bionate scaffolds were electrospun using the NEU Complete Nanofiber Producing Unit (Nanospinner, Kato Tech, Japan) at Philadelphia University, Department of Engineering &Textiles. PLGA scaffolds were generated in a home-made electrospinning device, as previously described [42–46]. Briefly, PLGA and Bionate were dissolved at 5% (w/v) and 8% (w/v), respectively, in 1,1,1,3,3,3 Hexafluoro-2-Propanol (HFP, from Sigma) for 24 h. In the homemade device, polyester and cotton fabrics (8×8 cm^2) were mounted on a polycarbonate frame and stretched 50% in both the x and y directions. The frame was placed in front of a rectangular (10×10 cm^2) copper target. In the NEU Nanospinner unit a 20×30 cm^2 fabric was stretched to 50% in both directions on a stationary target. Optimized electrospinning parameters that yielded uniform bead-less PLGA fibers and a scaffold thickness of 20 μm in our homemade system were as follows: The distance between the textile and needle was 15 cm, the syringe pump flow rate was 0.5 ml/h, and the applied voltage was 20 kV. Parameters to obtain uniform bead-less Bionate fibers and a scaffold thickness of ~100 μm in the Nanospinner unit were as follows: The distance between the textile and needle was 18 cm, the syringe pump flow rate was 0.8 ml/h, and applied voltage was 20 kV. Electrospun PLGA and Bionate scaffolds were peeled off (separated) from the knitted textile templates and the flat target, respectively, immediately upon completion of the electrospinning process, and used within one week of manufacturing.