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Aligned multilayered electrospun scaffolds for rotator cuff tendon tissue engineering

Steven B. Orr [a], Abby Chainani [a,b], Kirk J. Hippensteel [a], Alysha Kishan [a,b], Christopher Gilchrist [a], N. William Garrigues [a,b], David S. Ruch [a], Farshid Guilak [a,b], Dianne Little [a,*]

[a] Department of Orthopaedic Surgery, Duke University Medical Center, Durham, NC 27710, USA
[b] Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA

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ABSTRACT

The rotator cuff consists of several tendons and muscles that provide stability and force transmission in the shoulder joint. Whereas most rotator cuff tears are amenable to suture repair, the overall success rate of repair is low, and massive tears are prone to re-tear. Extracellular matrix (ECM) patches are used to augment suture repair, but they have limitations. Tissue-engineered approaches provide a promising solution for massive rotator cuff tears. Previous studies have shown that, compared to nonaligned scaffolds, aligned electrospun polymer scaffolds exhibit greater anisotropy and exert a greater tenogenic effect. Nevertheless, achieving rapid cell infiltration through the full thickness of the scaffold is challenging, and scaling to a translationally relevant size may be difficult. Our goal was to evaluate whether a novel method of alignment, combining a multilayered electrospinning technique with a hybrid of several electrospinning alignment techniques, would permit cell infiltration and collagen deposition through the thickness of poly(ε-caprolactone) scaffolds following seeding with human adipose-derived stem cells. Furthermore, we evaluated whether multilayered aligned scaffolds enhanced collagen alignment, tendon-related gene expression, and mechanical properties compared to multilayered nonaligned scaffolds. Both aligned and nonaligned multilayered scaffolds demonstrated cell infiltration and ECM deposition through the full thickness of the scaffold after only 28 days of culture. Aligned scaffolds displayed significantly increased expression of tenomodulin compared to nonaligned scaffolds and exhibited aligned collagen fibrils throughout the full thickness, the presence of which may account for the increased yield stress and Young's modulus of cell-seeded aligned scaffolds along the axis of fiber alignment.

Statement of Significance

Rotator cuff tears are an important clinical problem in the shoulder, with over 300,000 surgical repairs performed annually. Re-tear rates may be high, and current methods used to augment surgical repair have limited evidence to support their clinical use due to inadequate initial mechanical properties and slow cellular infiltration. Tissue engineering approaches such as electrospinning have shown similar challenges in previous studies. In this study, a novel technique to align electrospun fibers while using a multilayered approach demonstrated increased mechanical properties and development of aligned collagen through the full thickness of the scaffolds compared to nonaligned multilayered scaffolds, and both types of scaffolds demonstrated rapid cell infiltration through the full thickness of the scaffold.

1. Introduction

The prevalence of rotator cuff tears increases with age to >50% in individuals over the age of 60 [1,2]. Currently, over 300,000 surgeries are performed annually in the United States to repair rotator cuff tears [3], and this number is likely to rise with the projected increase in elderly populations [4]. Re-tear rates are high, especially with increasing tear size [5,6], and massive rotator cuff tears may not be amenable to traditional suture repair [7]. In this regard, tissue engineering approaches to enhance or augment traditional suture rotator cuff repair could have significant clinical impact. Extracellular matrix (ECM) patches have been used to augment repair but generally have inadequate mechanical properties

* Corresponding author at: Orthopaedic Research Laboratories, DUMC Box 3093, 375 MSRB1, Durham, NC 27710, USA. Tel.: +1 (919) 681 6867; fax: +1 (919) 681 8490.
E-mail address: dianne.little@duke.edu (D. Little).

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[8], and slow cell infiltration prevents rapid integration of many commercially available ECM patches [9,10].

Therefore, there is a need for tissue-engineered approaches that both stimulate rapid tendon healing and provide adequate mechanical augmentation for the rotator cuff [11]. Electrospun scaffolds have shown significant potential in this regard [12–15], but do not yet provide adequate mechanical properties. A further challenge has been achieving cell infiltration through the full thickness of the scaffold [16,17]. Various methods to improve porosity of the electrospun scaffold have been evaluated [16,18–22]. To address this need specifically for rotator cuff tendon tissue engineering, we have recently modified a multilayered electrospinning technique [22] to achieve rapid infiltration of human adipose-derived stem cell (hASC) and tenogenic ECM synthesis through the full thickness of randomly multilayered electrospun scaffolds [23]. However, several recent studies indicate that, compared with nonaligned or randomly oriented fibers, aligned nanofibers can enhance tenogenesis [12,24,25]. Furthermore, such fiber alignment creates mechanical anisotropy that more closely mimics tendon mechanical properties. Electrospun fiber alignment can be achieved through the use of a rotating disk [26–28], rotating mandrel [29–31], patterned electrodes [32,33], air-gap techniques [34,35], patterned insulators [36], or ceramic magnets [37–39]. However, as with nonaligned scaffolds, achieving cell infiltration can be problematic when using rotating mandrel techniques, unless sacrificial fibers are simultaneously co-spun [16,40]. Air-gap techniques are typically limited by short lengths of fiber alignment (~1 cm) [41] or by decreasing alignment with increasing duration of electrospinning [35]. Multilayered aligned scaffolds (produced by stacking aligned layers on top of each other) across short lengths of fiber alignment have previously been reported to control the hierarchical structure within the scaffold [36,42], and thus may be advantageous for the development of scaffolds for rotator cuff tendon tissue engineering [24,25]. The objectives of this study were to (1) to develop a novel multilayered electrospinning technique that allows for prescribed alignment of each layer in a clinically relevant patch size, and (2) to evaluate the ability of these aligned scaffolds to induce complete cellular infiltration, tenogenic ECM formation, and development of tensile mechanical properties by hASCs compared to nonaligned multilayered scaffolds.

2. Materials and methods

2.1. Aligned multilayered electrospun scaffolds

Poly(ε-caprolactone) (PCL) (Mn = 80,000) (Sigma–Aldrich, St. Louis, MO) was dissolved at 100 mg/mL in 7:3 dichloromethane:ethanol for 24 h before use. Individual alignment methods (ceramic magnets, air-gap, patterned insulators, parallel copper electrodes) amenable to formation of multilayered square or rectangular patch scaffolds were first screened for their ability to induce aligned fiber formation over air-gaps of 5–8 cm, a size relevant for future clinical use. Each method of alignment was screened systematically using a range of polymer flow rates, voltages, needle sizes, needle-ground distance, and spinning times to most closely match fibers obtained using nonaligned techniques (see Section 2.2). As has been previously reported [32–34,36–39], each individual method was able to induce fiber alignment over a short (1–3 cm) air-gap, but as the size of the air-gap was increased, alignment was lost or was evident for progressively shorter periods of time before deposition of fibers occurred elsewhere (Fig. S1). However, when individual alignment methods were combined to include ceramic magnets and parallel copper electrodes outside of a rectangular rubber-coated reservoir containing distilled water (volume dependent on ambient temperature and humidity), robust aligned layers were obtained for up to 5 min of electrospinning across an air-gap of 10 cm. Therefore, the final electrospinning apparatus used (Fig. 1) was a rectangular, rubber-coated reservoir (10 cm wide × 15 cm long) containing distilled water, with grounded 6-cm wide parallel copper electrodes immediately outside the reservoir centered at the midpoint of the reservoir length and immediately surrounded by ceramic magnets (2.5 cm × 7 cm × 14.5 cm) oriented to attract each other. The following electrospinning parameters were used: 21 G needle fitted with a round wire mesh focusing cage (3 cm diameter, needle tip protruding 4 mm from bottom of cage), 5 mL/h, 16 kV, and a 13.5 cm needle-to-ground distance. Aligned layers were collected sequentially from the surface of the saline bath every 3 min onto a 5 cm × 7.5 cm glass slide, for a total of 140 layers (approximately 1 mm thick).

2.2. Nonaligned multilayered scaffolds

Nonaligned multilayered scaffolds were prepared by electrospinning into a grounded saline bath (1.25 g/L NaCl in distilled water) using the apparatus previously described (Fig. 1) [23]. PCL was electrospun using the following parameters: 25 G needle fitted with a round wire mesh focusing cage (3 cm diameter, needle tip protruding 4 mm from the bottom of the cage), 2.5 mL/h, 17 kV, and a 17 cm needle-to-ground distance. Nonaligned layers were collected sequentially from the surface of the saline bath every 2 min using a 5 cm × 7.5 cm glass slide, for a total of 70 layers (approximately 1 mm thick). Parameters were selected to obtain similar scaffold thickness and fiber diameters between aligned and nonaligned scaffolds (Section 3). For all scaffolds produced, relative humidity was 20–40%, and ambient temperature ranged from 18 °C to 25 °C. Each scaffold was allowed to dry at room temperature and then stored at room temperature protected from light until use.

2.3. Fiber diameter analysis

Three 0.5 cm × 1 cm strips were cut from each scaffold (center and two orthogonal edges), sterilized (see Section 2.4), critical point dried in CO_2, and then sputter coated with gold. Each sample was viewed with a Philips 501 scanning electron microscope. Three representative images were taken of each sample, and the diameter of 100–150 fibers for each type of scaffold was measured in ImageJ (NIH, USA).

2.4. Cell seeding and culture

Scaffolds were cut into individual 0.5 cm × 1 cm strips with long axis parallel to the expected direction of fiber alignment and sutured to a Teflon ring to maintain shape and suspension in media. Scaffolds to be used for mechanical testing were cut into dog-bone shapes in directions parallel and perpendicular to the direction of expected alignment, and similarly for nonaligned scaffolds. Each scaffold was rehydrated and sterilized in a graded series of ethanol baths to improve seeding before a final 30-min rinse in phosphate-buffered saline (PBS) at pH 7.4. Both sides of each scaffold were sterilized under ultraviolet light for 10 min and pre-wetted with PBS before cell seeding. We isolated hASCs by collagenase digestion of lipoaspirate surgical waste from five de-identified female donors (age 36–59, body mass index 19.6–33.1) with approval of the Duke University Institutional Review Board and used the cells at passage 4 [23,43]. Cells were seeded at a density of 1×10^6 hASCs/cm^2 for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and 0 or 0.5×10^6 hASCs/cm^2 for all other assays. Half of the cells were

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