Functional Ligament Tissue Engineering with Crimp Fibrous Scaffolds

Wen-Ling Lily Liao and Pen-Hsiu Grace Chao

Department of Biomedical Engineering, National Taiwan University, Taipei, Taiwan

Introduction

- Aligned wavy fibrous scaffolds recapitulate the structure function relationship of native ligaments and tendons [1-3]. The wavy structure is also cell instructive, promoting ligamentogenic expression profiles and improving mechanotransduction [4]. However, inhomogeneous construct development from poor cell infiltration and heterogeneous cell morphology is a significant issue in the wavy scaffolds.
- We previously demonstrated that collagen coating and sequential growth factor supplementation enhanced homogeneous construct development in aligned straight fibrous materials [5,6]. In this study, by initially straightening the wavy fibers with static loading to stimulate cell infiltration, we successfully promoted homogeneous and increased collagen deposition in wavy scaffolds in long-term culture.

Methods

- **Scaffold:** PLLA (8.5% in HFP) and PEO (10% in ethanol) were electrospun at 1 kV/cm and collected on a rotating mandrel at 1000 rpm. PEO was removed by ddH2O washes to generate porous and straight (S) fibrous materials. Wavy (W) materials were made by heating the straight fibers at 85°C for 15 minutes [4]. Scaffolds were treated with 2 mg/mL dopamine hydrochloride (pH=8.5) and incubated in 88 μg/mL type I collagen overnight at 37 °C.
- **Tissue Culture:** $8x10^5$ primary porcine ACL fibroblasts were seeded on the scaffolds and cultured in DMEM with 50 μM ascorbate and 10% FBS. In the Stretch-Release (SR) group, cells were seeded on wavy fibers that are statically stretched at 6% strain, and released after one week of culture. bFGF-2 (100 ng/ml) was supplemented during the second week of culture, followed by TGF-β1 (5 ng/ml) for the rest of the experiment.
- **Imaging and Analysis:** Actin cytoskeleton was labeled with phalloidin, cell nuclei were labeled with DAPI, and collagen was tagged with antibody. Cross sections of the samples were imaged with fluorescence microscopy. Cell infiltration ratio was defined as the distance of the nuclei from the nearest scaffold edge normalized by scaffold thickness.
- **Mechanical Testing:** A Bose® ElectroForce® 5500 system with a 5 kg load cell was used to measure the equilibrium stress and strain relationships at the applied strain rate of 0.01/s.
- **Biochemical Assay:** DNA and collagen content were measured by PicoGreen and orthohydroxyproline (OHP) assays, respectively.
- **Statistical Analysis:** One-way ANOVA was performed with Origin.

Conclusions

- By applying a 6% static strain, the wavy fibers were straightened, similar to the straight fibers (SR, Fig 1AB). This stretch did not change the mechanical properties of the scaffold and improved cell infiltration to levels comparable with the straight fibers (Fig 1C), indicating that the wavy topography are indeed an impediment to cell migration.
- Aligned cells with strong stress fibers were found on the straight scaffold, while cells on wavy fibers were more spread and disorganized. Cells in the SR group were elongated and followed the wavy fibers (Fig.2A). Cross-sectional views revealed more homogeneous cell distribution in the S and SR groups compared to the W groups at the end of one week (quantified by cell infiltration ratio, Fig 2C), as well as after long-term culture (Fig 3A).
- The SR groups exhibited significantly higher collagen deposition after 5 weeks, which is further enhanced with growth factor supplementation (Fig 3B). Our results demonstrate that the initial static straightening period promoted cell migration and the subsequent release-induced wavy morphology enhanced matrix deposition. Moreover, the wavy scaffold promoted collagen synthesis and acted synergistically with growth factor supplementation.
- Current studies are characterizing detailed cell and nuclear structural differences in the W and SR groups as well as the effects of dynamic stretch on construct development.

Acknowledgements

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Results

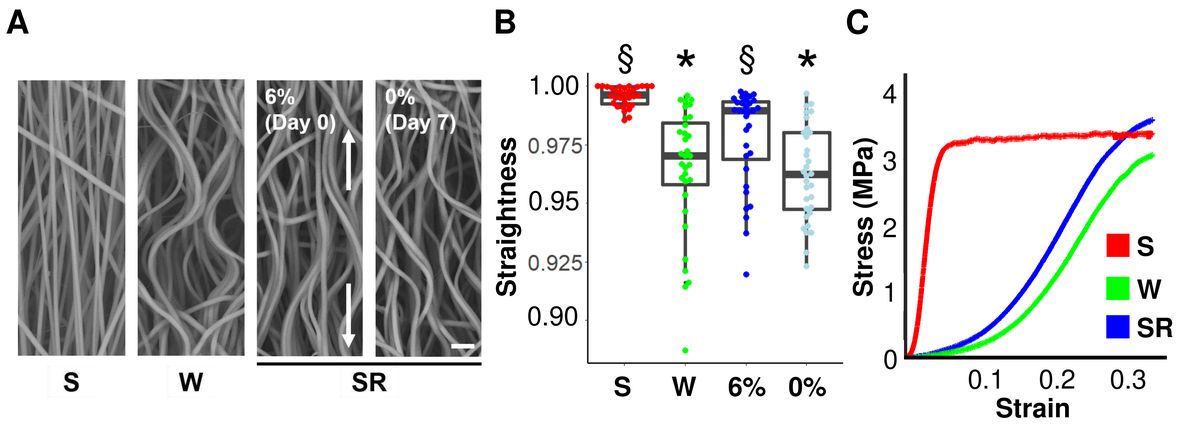

Figure 1. Structure and mechanics of the fibrous scaffolds (A) Representative SEM images (Scale bar=10μm) (B) Fiber straightness (*p<0.05 vs. S, §p<0.05 vs. W) (C) Representative stress-strain relationship of the fibrous scaffolds.

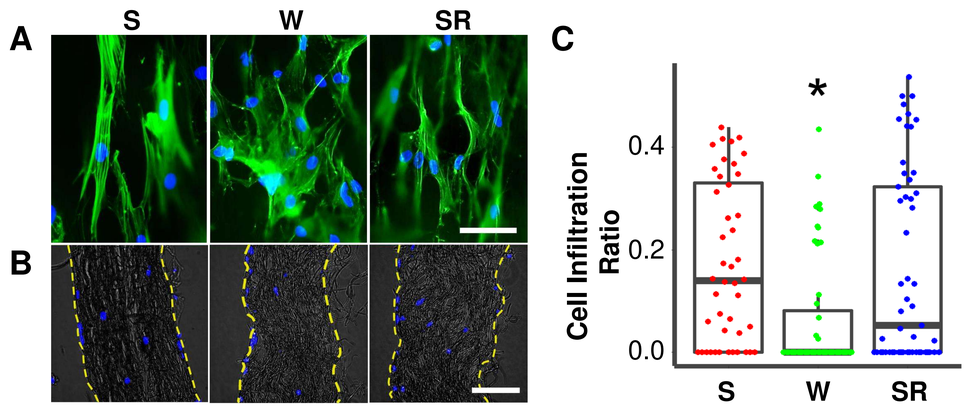

Figure 2. (A) Cell morphology (green-actin, blue-nuclei), and (B) cross-sectional view of cell infiltration (Scale bar = 50 μm in A and 100 μm in B). (C) Cell infiltration level in the fibrous scaffolds. (*p<0.05 vs. others)

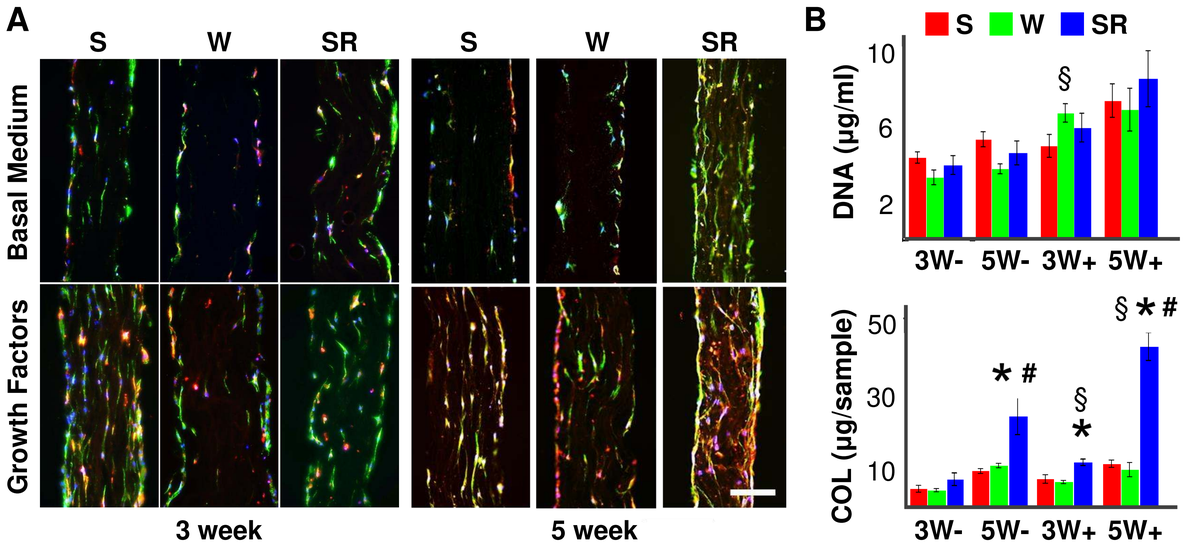

Figure 3. (A) Cross-section view of cell and collagen distribution (Nuclei labeled in blue, f-actin in green and collagen in red. Scale bar= 100 μm) (B) DNA and Collagen content. (3W:3 week, 5W: 5 week, -: basal medium (no growth factors), +: growth factor supplementation, *p<0.05 vs. S group, #p<0.05 vs. 3W same -/+, §p<0.05 vs. groups without GFs. n=3-8)

References

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