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Notes

Tissue Engineering Using a Cyclic Strain Bioreactor and Gelatin/PLCL Scaffolds

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Introduction

Tissue engineering with cells and three-dimensional polymeric scaffolds is a potential means of growing new tissues to replace lost or malfunctioning organs and tissue.[1] In order to create a successful tissue engineering construct, it is required to provide appropriate signals for cells to assemble and integrate into biologically functional tissue. Soluble molecules such as growth factors and cytokines, and non-soluble factors including extracellular matrix (ECM) components have been identified to stimulate desirable cellular responses. In addition to these chemical signals, there is growing evidence that mechanical stimulus applied to cell/ECM composites regulates numerous cellular activities such as cell proliferation, migration, and differentiation.

Skin is complex tissue composed of multiple layers including dermis and epidermis. Each layer has different cell types that are exposed to diverse mechanical environments. Keratinocytes and melanocytes are dominant cell types in epidermis experiencing direct stress and strain while fibroblasts are a major cellular component in dermis under a less-mechanically stimulated condition. Several investigators have reported that cyclic mechanical stretch increases ECM production in cultured fibroblasts on flexible membranes.[2-6]

In this study, we prepared an elastic scaffold using an electrospinning method. In our laboratory, we have previously reported the mechanical property and biocompatibility of poly(l-lactide-*co*-ε-caprolactone) (PLCL). PLCL is flexible but rubber-like elastic to maintain a complete recovery even under the cyclic loading in culture media for up to 2 weeks.[7] In order to improve cellular interactions with the underlying fibrous scaffold, small amount of gelatin was blended to PLCL. By using a home-made bioreactor that is able to provide a cyclic strain to cell/scaffold composites, we investigated the effect of mechanical stimulus on cellular response. We examined whether the cyclic mechanical strain controls the proliferation of human dermal fibroblasts (HDFs) in tissue engineered scaffolds, and the amount of ECM proteins produced in the HDFs was analyzed.

Experimental

Preparation of a Gelatin/Poly(l-lactide-*co*-ε-caprolactone) (PLCL) Fibrous Scaffold. The elastic fibrous scaffold was fabricated from the blend of gelatin and PLCL using an electrospinning method. Both gelatin and PLCL were dissolved in 2,2,2-trifluoroethanol at 10 %(w/w) and then mixed together at the ratio of 3:7 (gelatin: PLCL by weight). The scaffold was prepared by using a custom-designed electrospinning equipment. The gelatin/PLCL blend solution was placed within a glass syringe with a stainless-steel blunt ended needle (20G) and electrospun directly onto the aluminum foil-covered collector (infused at the rate of 20 μL/min at 18-19 kV). The resulting scaffold was cross-linked by 0.35% EDC. The tensile properties of the prepared scaffolds were measured using Instron (Model 5567, Canton, MA).

Cell Culture. Normal human dermal fibroblasts (HDFs) were generously donated by the Department of Dermatology (Dongguk University, Korea), and cultured in DMEM supplemented with 10% FBS and 1% antibiotics (penicillin and streptomycin) at the standard culture condition (37 °C, 95% humidity, and 5% CO_2 concentration). The HDFs were seeded onto the gelatin/PLCL scaffold (40 × 10 × 0.5 mm) (5.0×10^6 cells/cm^2) and maintained for two days with the media changed every day, and then subjected to the cyclic stretch using a custom-made cyclic stretching unit (a frequency of 0.25 Hz (1 cycle per second) and an amplitude of 5% of the initial scaffold length).

Assays. In order to examine the proliferation of HDFs cultured on the scaffold, samples at 1, 5, 10, and 14 days were rinsed with PBS and frozen at -80 °C. On the day of an assay, samples were thawed, cut into smaller pieces, suspended, and vortexed repeatedly in 4 mL of 10 mM EDTA (pH 7.3) to purify DNA. The samples were then reacted with Hoechst dye 33258 (Polyscience Inc., Niles, IL, USA) for 30 min and their fluorescent intensity was recorded. To assess the expression of collagen type IV, northern blot analysis was performed on RNA isolated from tissues suspended in 10 mL Trizol (Life Technologies), homogenized

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with a Polytron (Brinkmann Instruments, Westbury, NY), and processed as manufacturer's instructions. The amount of fibronectin in the engineered tissue was quantified using western blot analysis. The same amounts of protein samples were mixed with Laemmli sample buffer, which were loaded and separated by 10% sodium dodecyl sulfate-poly (acrylamide) gel. Luminescence was recorded onto X-ray film, and band intensity was analyzed by an imaging densitometer, and quantified by using Scion Image (NIH Image). The total amount of collagen and fibronectin in the cell-scaffold composites was determined by using assay kits.

Results and Discussion

In this study, we investigated an effect of cyclic strain on proliferation and ECM protein production of HDFs cultured on the gelatin/PLCL nanofibrous matrix. Gelatin/PLCL (blend ratio: 3:7) was electrospun using a collector with a high surface speed to prepare fibrous scaffold as shown in Figure 1(c). The detailed characteristics of the scaffolds (diameter, mechanical properties) were previously reported.[8] The resulting scaffold is biodegradable and biocompatible, and most importantly possesses an elastic mechanical property, which is a prerequisite to maintain cell culture without major deformation or breakage under a cyclic mechanical strain.

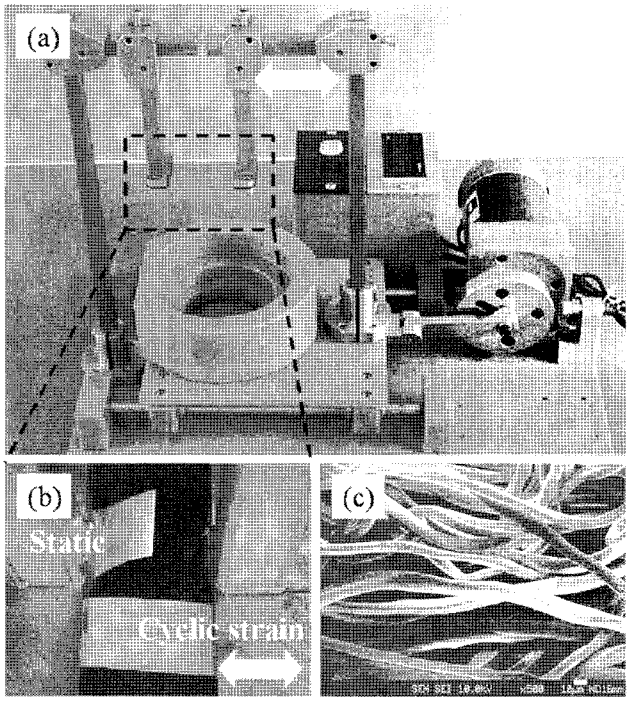

Figure 1. Bioreactor design, morphology of the gelatin/PLCL nanofiber scaffold, and its mechanical property; (a) the scaffold was clamped and then immersed in the culture media, (b) the cyclic strain was applied to the cell/scaffold composite, (c) scanning electron micrograph of the fibrous gelatin/PLCL scaffold (scale bar: 10 μm).

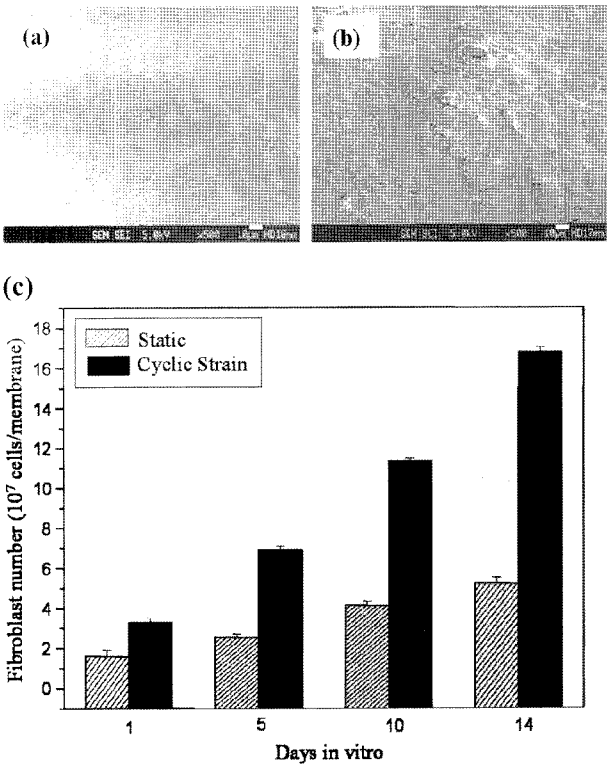

Figure 2. (a) SEM micrographs of fibroblasts cultured on the gelatin/PLCL fiber scaffolds without cyclic strain and (b) under cyclic strain (scale bar: 10 μm). The number of fibroblasts cultured in cell/scaffold composites as a function of culture time.

Cyclic mechanical strain has been shown to influence many cellular activities including proliferation, gene expression, and synthesis of matrix proteins. Therefore, we first analyzed the proliferation of HDFs. As shown in Figure 2, the number of fibroblasts cultured under the cyclic mechanical strain significantly increased as compared to that cultured under the static condition. In addition to the increase in the proliferation, the mechanical loading contributed to the migration and distribution of HDFs on the scaffold. SEM images showed that HDFs were highly elongated and well-distributed on the scaffolds. HDFs and matrix on the scaffold cultured under the cyclic mechanical strain were more homogeneously distributed throughout the internal structure of the scaffold while in statically cultured samples the majority of cells were observed in the circumference of the polymer scaffold (date not shown).

The next study was the investigation of the effect of the cyclic strain on the expression of collagen type IV and fibronectin, phenotypic markers of HDFs in a contractile condition (Figures 3 and 4). Densitometric examination indicated that the expression of collagen type IV and fibronectin was 4.0 and 3.5 times greater, respectively, in the cell/scaffold construct exposed to the cyclic stretching

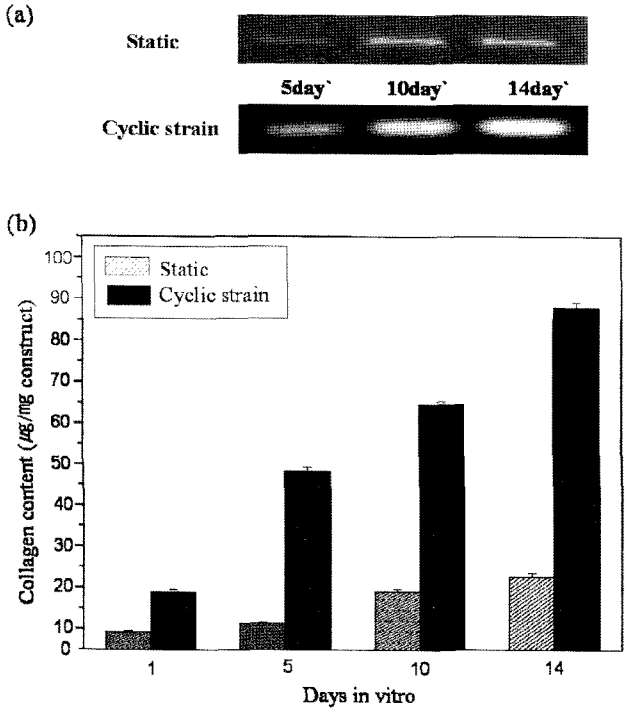

Figure 3. Expression of collagen in fibroblasts.

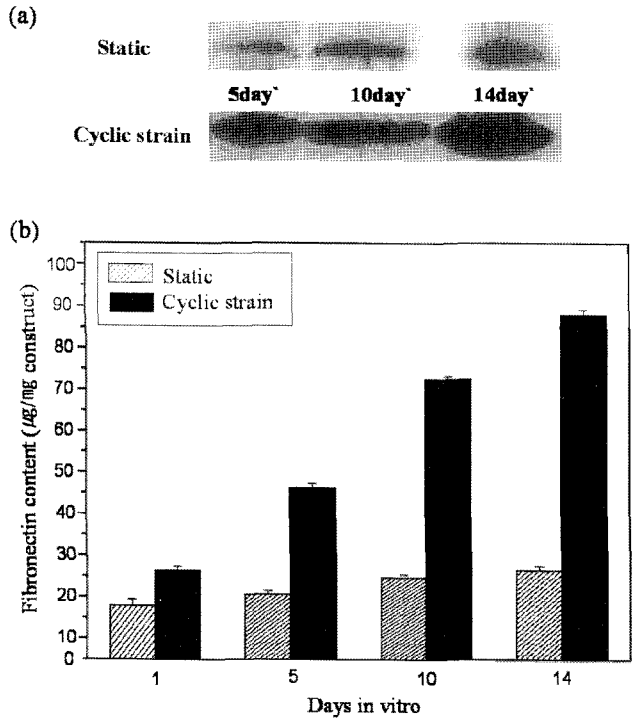

Figure 4. Expression of fibronectin in fibroblasts.

than in statically-cultured tissue. Northern blot analysis of the tissue-engineered composite indicates that cyclic mechanical strain had a positively stimulating effect on collagen type IV levels following 14 days of culture under the cyclic strain. The cyclic mechanical strain significantly increased collagen content within the nanofibrous scaffold as compared to the static culture. In addition to the increase in collagen expression, cyclic mechanical strain also significantly increased expression of fibronectin within the nanofibrous scaffold. These results suggest that cyclic mechanical stretching induces a differentiated, contractile phenotype of HDFs in the engineered tissue.

Conclusions

In this study, we demonstrate that cyclic strain increased the proliferation of HDFs and subsequent expression of ECM proteins. Specifically, HDFs engineered under the cyclic stretching exhibited higher proliferation and up-regulated expression of collagen type IV and fibronectin. These cellular features could allow the engineered HDF tissues to exhibit contractile functions. Future studies will examine dose-dependent responses of various skin cells cultured in nanofibrous scaffold under mechanical loading that vary in strain amplitude, rate, frequency, and duration.

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