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Evaluation of Nanofiber-based Engineered Cartilage and its Integration with Native Cartilage

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1 Introduction

Integration between tissue-engineered cartilage and native cartilage poses a challenge for clinical applications. An in vitro integration model can potentially provide insights into cartilage regeneration and integration, for translation into in vivo applications. In this study, we fabricated a chondrocyte-nanofiber composite-based engineered cartilage, implanted a native cartilage in the engineered cartilage, and cultured the grafted tissue in a bioreactor to evaluate the regeneration of the novel engineered cartilage and the integration between the engineered and native cartilage.

2 Materials and Methods

Chondrocytes were mixed with electrospun poly(L-lactic acid) nanofibers and co-pelleted by centrifugation to form a homogeneous cell-nanofiber aggregate. The cell-nanofiber aggregate was then maintained in a horizontal axis rotary bioreactor as dynamic cultures, or in a conical tube as static cultures.

A circular explant was harvested from native calf cartilage and press-fitted into a circular defect pre-created in an engineered cartilage obtained from Day-42 dynamic or static cultures. The composites were further maintained as dynamic or static cultures for additional 42 days.

Magnetic resonance imaging was performed on the samples. T_2-weighted images, magnetization transfer (MT) weighted images, and T_1-weighted images were obtained sequentially. T_2, pseudo-first order rate constant (k), and gadolinium-enhanced T_1 maps were generated from the T_2-weighted, MT-weighted, and T_1-weighted images, respectively.

Hematoxylin & eosin and alcian blue staining were performed to examine morphology and matrix structure of the cultures.

Interface strength of the engineered and native cartilage of the end time point samples was measured with a push-out test.

3 Results

Macroscopically, the engineered construct appeared as a white, cartilaginous tissue, suggesting the production of newly synthesized extracellular matrix, and resembled the native cartilage. The composite cartilage for integration evaluation was composed of the circular, nanofiber-based, tissue-engineered cartilage disc with 20-mm in diameter and 3-mm in thickness, and the 8-mm core native cartilage (**Fig. 1**).

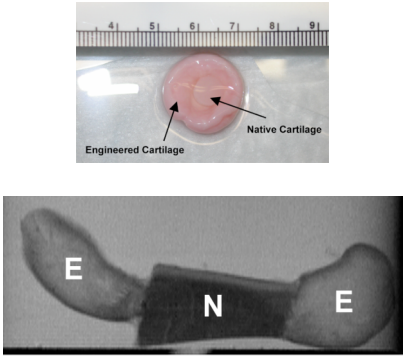

Figure 1 : Macroscopic appearance of the engineered/native cartilage composite cultured for 42 days. NMR macrograph of the sample cultured for 42 days. E: engineered cartilage, N: native cartilage.

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NMR results showed small T_2 values, which relate to restricted water mobility, were observed on the outer edges of the engineered cartilage and the native cartilage. In contrast, the inner portion of the engineered cartilage had larger values of T_2, which suggest less restricted water mobility. The pseudo-first order rate constant generated from the MT-weighted images suggest a large presence of collagen on the surface of the engineered cartilage comparable to what was observed on the surface of native articular cartilage, but approximately a third of what was present in the deeper regions of the native cartilage. The Gd-enhanced T_1-maps showed large value of T_1, which suggest a large presence of GAG, within the native cartilage. In contrast, the engineered cartilage in both the outer and inner portions of the sample produced T_1 values only slightly larger than what was observed in the surrounding fluid.

Histological analysis showed that better integration was present in the sample cultured in the same environment before and after the native cartilage was incorporated. Less integration was found in composites that had been switched between static and dynamic culture conditions. Notably, cells present in the integration region in dynamic cultures showed more chondrocyte-like morphology than those found in static cultures. The dynamic bioreactor was therefore chosen to analyze the integration of engineered and native cartilage.

The push out analysis showed an interface strength of 123 ± 40 kPa between the engineered and native cartilage, suggesting a fair level of tissue integration was established.

4 Discussion

Cartilage regeneration and integration between native and nanofiber-based engineered cartilage are influenced by the culture environment. Our results show that maintenance in dynamic culture improves interface integration. In addition, NMR analysis serves as a useful, macroscopic and physiological tool to evaluate tissue integration. Our results suggest that nanofiber-based engineered cartilage may be a suitable integration-competent tissue construct for cartilage repair applications in vivo.