Reconstruction of Ligament to Bone Interface: The Stratified Complex of Ligament and Calcified Fibrocartilage Incorporating BMP-2 Loaded Heparin-Based Hydrogel

Jongman Lee[1], Won-Il Choi[1], Hanna Lee[1], Vincenzo Guarino[2], Luigi Ambrosio[2], Giyoong Tae[1], Young Ha Kim[1]
[1]Dept. of Materials Science and Engineering, Gwangju Institute of Science and Technology, Gwangju, Republic of Korea
[2]Institute of Composite and Biomedical Materials-National Research Council Piazzale Tecchio 80, 80125 Naples, Italy

Statement of Purpose: The ligament-to-bone (LTB) junction has been emphasized for the effective transmission of mechanical force and the reduction of stress concentration between soft ligament and hard bone. The aim of this study was to regenerate the integrated LTB interface by inoculating the LTB-relevant cells separately in each designated region on a single porous rod-type scaffold. Furthermore, the bone morphogenetic protein-2 (BMP-2) was loaded in heparin-based hydrogel carrier and released in a controlled rate by using the binding affinity of BMP-2 to heparin.

Methods: Rod-type PLCL scaffolds were divided into three parts: fibrocartilage (FC) regions at both ends and ligament (LIG) region in the center. The LTB-relevant cell sources (fibrochondrocytes, fibroblasts, 1×10^6 cells / section) were seeded at each section in combination with heparin hydrogel and BMP-2. After cultivating the *in vitro* stratified FC-LIG tissues and *in vivo* FC tissues implanted to the back of athymic mice, the LTB-specific ECM secretion and mechanical properties were evaluated.

Results: The *in vitro* biochemical analysis demonstrated that all the FC samples possessed the significantly higher calcium and GAG content than all the LIG ones in the order of EXP (cells, hydrogel, and BMP-2 loaded) > CON II (cells and hydrogel loaded) > CON I (cell alone loaded) FC. However, the collagen production and cell proliferation exhibited the LTB tissue-dependant pattern: the more enhanced collagen and cell proliferation for LIG tissue was attributed to the ability of fibroblast to secrete collagen actively and the rapid doubling time. These up-regulated minerals and GAG of the *in vitro* FC samples resulted in the higher compressive stiffness, compared to the *in vitro* LIG samples.

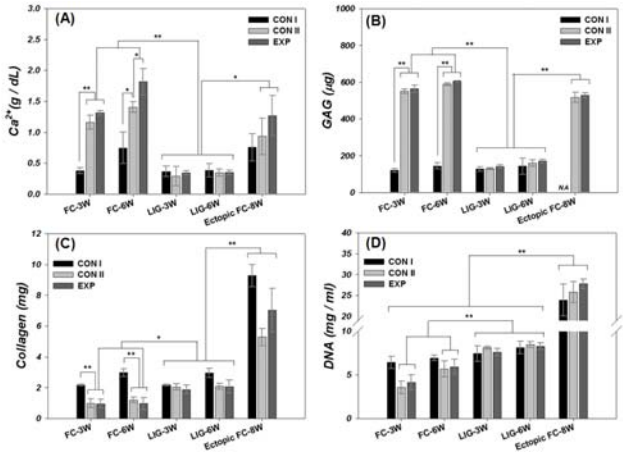

*Fig. 1. Quantitative analysis of calcium (A), GAG (B), collagen (C), and DNA (D). *P < 0.05, **P < 0.01.*

The *in vivo* ectopic FC samples at 8 weeks, combined with hydrogel and / or BMP-2, revealed a little less calcium and GAG but larger collagen and DNA content. It was mainly caused by the surrounding neo-collagenous tissues formed during the host tissue infiltration under the subcutaneous circumstances. Interestingly, the *in vivo* ectopic FC samples provided more remarkable expression of both minerals and GAG with the homogeneous distribution than *in vitro* FC samples did as a result of von Kossa and alcian blue staining, and it consequently gave rise to the outstanding increase in the compressive stiffness compared to each corresponding *in vitro* FC one. Still, it was odd that we could not observe any positive staining of alcian blue only at the *in vivo* CON I-FC samples as coincided with the biochemical GAG assay.

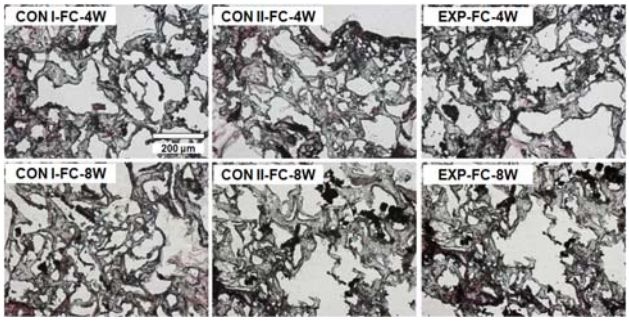

Fig. 2. Von kossa staining of in vivo CON I, CON II, EXP-FC samples, Original magnification ×100.

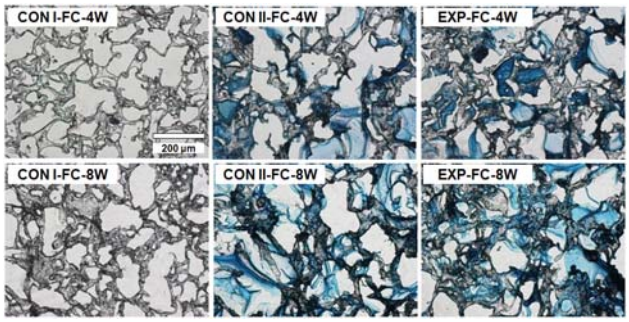

Fig. 3. Alcian blue staining of in vivo CON I, CON II, EXP-FC samples, Original magnification ×100.

Conclusions: The sustained release of BMP-2 would be confined to FC region mostly and could accelerate the maturation and differentiation of LTB-specific tissue by creating the unique stratification of calcified FC and LIG tissues. Therefore, it indicated a strong correlation between biochemical and biomechanical properties as reconstructing the LTB-mimicking tissues

Acknowledgement: This work was supported by the Italy-Korea Cooperation Research Program sponsored by KICOS, Korea and CNR, Italy.