# Fabrication of a TGF-$\beta_3$ Encapsulated PLCL Scaffold by Supercritical $CO_2$-HFIP Co-Solvent System

Su Hee Kim[1,2], Soo Hyun Kim,[1,2*] , Youngmee Jung[1*]
[1]Center for Biomaterials, Korea Institute of Science and Technology, Seoul, Korea
[2]NBIT, KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul, Korea

**Introduction:** Mimicking microenvironment of native tissue is a critical issue for effective tissue regeneration. Particularly, in the cases of load-bearing tissues such as articular cartilage or bone, mechanical cues and sustained biological cues are important factors. Various carriers including hydrogels and nanoparticles have been investigated to achieve the sustained release of protein drugs. However, it is difficult to apply these carriers alone as a scaffold for cartilage regeneration because of their weak mechanical properties. So, it was needed to combine to other biomaterials which have the proper mechanical strength. In this study, we developed the multifunctional scaffold which has similar mechanical properties to native cartilage and encapsulates TGF-β3 for chondrogenesis.

**Materials and Methods:** In our previous work, we confirmed that PLCL was not foamed by supercritical CO2 below 45℃. Here, we used supercritical carbon dioxide (scCO2)-1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) co-solvent system to facilitate processing under mild condition for application of the growth factor. This processing made it possible to fabricate an elastic porous PLCL (Poly (lactide-co-caprolacton)) scaffold encapsulated TGF-β3 at 37℃. We investigated tissue regeneration of TGF-β3 encapsulated PLCL scaffold with human adipose-derived stem cells in vitro and in vivo. (Groups; 1) PLCL scaffold+Fibrin gel+TGF-β3, 2) TGF- β3 encapsulated PLCL scaffold+Fibrin gel, 3) TGF-β3 encapsulated PLCL scaffold). In detail, we evaluated the chondrogenic abilities of the scaffolds at 4, 8, and 12 weeks after immune-deficient mice subcutaneous implantation of the constructs.

**Results and Discussion:** We used The scCO2 - HFIP co-solvent system to could facilitate the supercritical fluid processing under mild condition (<40°C) for the application of proteins like the TGF-β3. This processing made it possible to fabricate an elastic porous PLCL scaffold encapsulated TGF-β3 at 37℃. We confirmed that TGF-β3 molecules were released out from the encapsulated scaffold over 8 weeks and still remained in the polymer matrix. In vivo studies exhibited distinct improvements in the compressive E-modulus and the deposition of extracellular matrix with the TGF-β3 encapsulated PLCL scaffold. Furthermore, long term delivery of TGF-β3 would be enable affected to form hyaline cartilage-specific lacunae structure and to prevented hypertrophy of the differentiated chondrocytes.

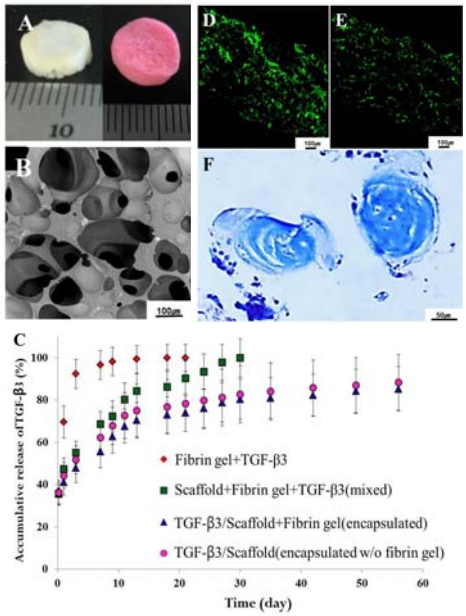


Fig. 1. (A) PLCL scaffold fabricated by scCO2-HFIP foaming system and biomolecules encapsulated PLCL scaffold (model compound; rhodamine B) (B) SEM image of the PLCL scaffold (C) TGF-β3 release profile. (D) Immunofluorescence staining of encapsulated TGF-β3 in PLCL scaffolds before release tests. (E) 8weeks after beginning of release tests. (F) Masson's Trichrome staining of the TGF-β3 encapsulated PLCL scaffold explanted at 12 weeks.

**Conclusions:** We developed a TGF-$\beta_3$ encapsulated PLCL scaffold by the scCO2 - HFIP co-solvent system, which could deliver the chemical and mechanical signals for a certain period of time for differentiation.mimics microenvironment of native cartilage tissue. From the results, consquently, it was considered that the TGF-β3 encapsulated PLCL scaffold by the scCO2 - HFIP system would be useful as a functional scaffold for cartilage tissue engineering.

**References:**

1. S.H. Kim, et. al., Tissue Eng. Part C. 2013;19: 181-188.
2. M. Kalani, et. al., Int. J. Nanomedicine. 2011;6:1429-1442.