Fabrication and Perfusion Culture of Anatomically Shaped Artificial Bone

Dajiang Du[a,b], Katsuko S. Furukawa[b], Yasuhito Anbai[c], Teruo Asaoka[c], Takashi Ushida[d].
a. Harbin Medical University, China; b. University of Tokyo, Japan; c. Tokyo Denki University, Japan

Statement of Purpose: As the bone defects of the patients were usually various and complicated in shape, it would be favorable to produce a custom-made engineered bone graft according to the anatomical information of a target defect from a patient. In this work, we aimed to investigate the feasibility of fabrication and perfusion culture of customized artificial bone with 3D osteogenic activity by oscillatory flow. We used a segment of rabbit femur as bone defect model for reconstruction in this research, and fabricated a custom-made ceramic scaffold according to the CT data by sterolithography successfully. Furthermore, the customized scaffold was cultured with rabbit MSCs by a novel oscillatory perfusion system.

Methods: The 3D information of rabbit femur was extracted by a microCT. After the 2D images were reconstructed by Mimics 10.0 into a 3D image, the lower segment of the femur was selected as a study model for rebuilding. Subsequently, the modified 2D images were reconstructed again as an external contour model for prototyping experiment by Magics 10.0. The customized internal channels were added.

A stereolithography system was applied for negative mold fabrication. The cured resin negative mold for customized scaffold and the internal interconnection was further confirmed by microCT. After the TCP slurry was filled into the negative resin mold, the whole sample was sintered at 1050℃. Then the custom-made porous TCP scaffold was evaluated by microCT again.

The silicon cassettes were fabricated by the internal negative resin mold prototyped according to the contour data of the scaffold. After the TCP scaffolds were assembled into a novel oscillatory perfusion system, the whole system was sterilized. MSCs from rabbit bone marrow were seeded and cultured by either static and perfusion method. After 5 days, the scaffolds was taken out and stained by Calcein-AM/PI for the study of cell viability. The celluary and ALP activity were also evaluated

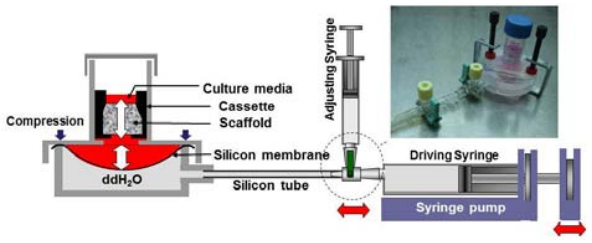

Figure 1. An illustration of the operation of the oscillatory perfusion system.

Results: We successfully fabricated customized ceramic scaffolds by stereolithography, which had not only anatomic external shape according to the CT data of the proximal segment of a rabbit femur, but also the interconnecting internal network designed for perfusion culture. Furthermore, the scaffolds were cultured with rabbit bone marrow stromal cells by a novel oscillatory perfusion system, the cassettes of which were fabricated fitting well to the scaffolds by stereolithography. After 5 days of 3D culture by oscillatory flow, the cells grew homogeniously throughout the scaffold, but the cells within the scaffolds culture in a static condition died after the prolonged in vitro culture. The DNA content and ALP activity was also significantly higher in perfusion group than static group.

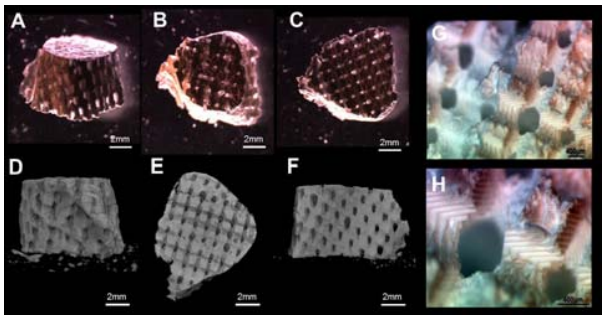

Fig.2. The sintered ceramic scaffolds with the anatomically shape of the target segment of rabbit femur.

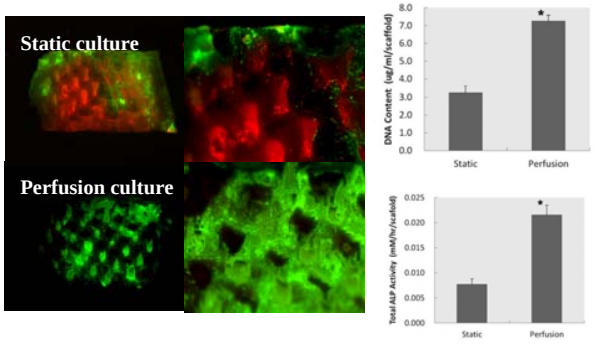

Fig.3. Left: Calcein-AM/PI staining of customized artificial bone cultured (green, living cells; red, dead cells) Right: DNA content and total ALP activity after culture.

Conclusions: The anatomically shaped artificial bone with 3D cellular osteogenic activity could be constructed successfully using stereolithography and oscillatory culture technology, and could be useful for bone tissue engineering.

References: Du D, Furukawa KS, Ushida T, Biotechnol. and Bioeng., 102(6): 1670-1678 (2009)