In Vivo Evaluation of Bilayered Silk/Silk-Nano CaP Scaffolds for Osteochondral Regeneration

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Statement of Purpose: Osteochondral defects (OCD) are common problems in orthopedics. The simultaneous regeneration of injured cartilage and subchondral bone is critical and a big challenge for the healing of osteochondral (OC) tissue. Our group recently developed high strength silk/silk-nano CaP (Silk/Silk-NanoCaP) scaffolds for OC regeneration. This study aimed to evaluate the in vivo biocompatiblity and OC repair performance of the bilayered scaffolds in rabbit model.

Methods: The bilayered scaffolds were prepared by using 16 wt.% aqueous silk solution and salt-leaching approach as mentioned in our previous studies [1-3]. The sterilized scaffolds were implanted subcutaneously in the back and also implanted in the critical size OCD of New Zealand White rabbits for 4 weeks. The subcutaneous explants were observed by H&E staining. The OC explants were scanned by micro-CT (SkyScan). Histological staining (Masson's trichrome and Safranin O) and immunohistochemical staining (Col II and SNA-lectin) were performed to evaluate the regeneration of neocartilage tissue and subchondral bone.

Results: Regarding the subcutaneous explants, the bilayered scaffolds were still stable and maintained original shape after 4 weeks of implantation. A thin layer of connective tissue surrounded the implants, and no infection or acute inflammation was observed. The H&E staining showed that some tissues filtrated into the interior region of the scaffolds. A few vessels and some macrophages were observed in the inner domain of the scaffolds. The micro-CT scanning of the OC explants demonstrated that the bilayered scaffold group exhibited less void space and more regular morphology compared with the defect control. Additionally, both the ingrowths of subchondral bone in the bottom region and the formation of neocartilage in the top surface of the implant were observed. The quantitative porosity data showed that the scaffold group displayed less than 20% porosity in the region accessed. The scaffold group exhibited abundant CaP content in the Silk-NanoCaP layer. In terms of the histological staining of the OC explants, the Masson's trichrome staining indicated that there was no acute inflammation in all the implants. The scaffolds presented integrated structure and firmly bound to the host tissues. In the top silk layer, the neocartilage formed and spread from the border to the centre of the defects. In the bottom, new bone growth into the Silk-NanoCaP layer was observed and only limited to this layer. High magnification staining images showed that the chondrocytes grew inside the silk layer presenting normal phenotype. In the Silk-NanoCaP layer, the formation of new vessel was also observed. The production of glycosaminoglycan (GAG) in the explants was examined by Safranin O staining. The staining results showed that the scaffolds supported the formation of chondrocyte containing GAG tissue in the edge and into the interior region of the top silk layer. The immunohistochemical staining result showed that the neocartilage tissue formed inside the top silk layer was obviously stained for Col II (Figure 1). The defect control did not show positive staining of Col II. Another immunohistochemical staining of an angiogenic marker (SNA-lectin) was performed. It was observed a substantial invasion of the Silk-NanoCaP layer by the endothelial cells. The enlarged staining image showed that abundant endothelial cells colonized in the interior region of the Silk-NanoCaP layer, and the formation of new bone and blood vessel was also identified. The defect control did not present new bone formation and displayed much lower endothelial cells density as compared with the defect with implant.

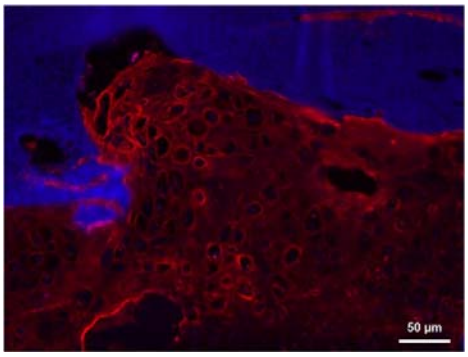

Figure 1. Col II immunohistochemical staining for the neocartilage (red) formed in the silk layer (blue).

Conclusions: **N**ovel porous bilayered scaffolds built up by well integrated a SF layer and a Silk-NanoCaP layer were generated for OCD regeneration. These scaffolds induced very weak foreign body reaction when subcutaneously implanted in rabbit. In the rabbit critical size OCD model, the bilayered scaffolds integrated well with the host tissues. Furthermore, these scaffolds supported the cartilage regeneration in the top silk layer, favored abundant subchondral bone ingrowths and angiogenesis in the bottom Silk-NanoCaP layer. Even though the long-term in vivo analysis and big animal model are still necessary, these preliminary in vivo results of the bilayered scaffolds along with their other promising performance verify that the Silk/Silk-NanoCaP bilayered scaffolds are suitable candidates for OCD regeneration.

References: [1] Yan LP, …Reis RL. Acta Biomater. 2012;8:289-301. [2] Yan LP, …Reis RL. Nanomedicine (Lond). 2013;8:359-378. [3] Yan LP, …Reis RL. Acta Biomater. 2014;DOI:10.1016/j.actbio.2014.10.021.