Electrospun Osteoconductive and Osteoinductive Bicomponent Scaffolds: Controlled Release of rhBMP-2 and Enhanced Biological Performance of Scaffolds

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Introduction: It is highly desirable that scaffolds for bone tissue engineering possess both osteoconductivity and osteoinductivity. However, scaffolds made of many biopolymers lack both. Using the composite approach, bioactive calcium phosphate (Ca-P) bioceramics can be coated on or incorporated into polymer scaffolds, rendering them osteoconductive (Wang M, *Biomaterials*, 2003; 24:2133-2151). If appropriate methods are employed, a bone morphogenetic protein (BMP) such as BMP-2 can be adsorbed or encapsulated in polymer scaffolds, providing osteoconductivity when BMP molecules are released (Duan B, Wang M, *MRS Bulletin*, 2011, 36:998-1005). Combining these two strategies, osteoconductive and osteoinductive scaffolds can be achieved. Electrospinning is widely used for scaffold fabrication due to advantages of the technique itself and the nanofibrous structures it produces. In this investigation, novel scaffolds for bone tissue engineering were made via dual-source dual-powder electrospinning (DSDP-ES) and were subsequently assessed.

Methods: Ca-P nanoparticles were produced in-house. PDLLA, PLGA (LA:GA=50:50), rhBMP-2 and other chemicals were provided by reputable manufacturers or suppliers. Using DSDP-ES (Duan B, et al., *J Biomed. Mater. Res A*, 2007, 83A:868-878), bicomponent scaffolds consisting of osteoconductive Ca-P/PLGA nanocomposite fibers and osteoinductive rhBMP-2/PDLLA nanofibers were constructed. In DSDP-ES, rhBMP-2/PDLLA fibers were made via emulsion electrospinning (Wang C, Wang M, *Adv. Mater. Res*, 2012, 410:98-101) and Ca-P/PLGA fibers were electrospun via an established route (Tong HW, et al., *Biomed. Mater.*, 2010, 5:054111.). Scaffolds were made as F1, F2, F3, F4 and F5 scaffolds when the ratio between rhBMP-2/PDLLA fibers and Ca-P/PLGA fibers in the scaffold was set at 100:0, 0:100, 66:33, 50:50 and 33:66, respectively. The morphological and structural properties of scaffolds were examined using SEM, TEM and fluorescence microscopy. The *in vitro* release behaviour of rhBMP-2 and Ca^{2+} ions were evaluated using ELISA kit assay and calcium bioassay. For biological evaluation, MC3T3-E1 cells were seeded on various scaffolds and cultured for up to 14 days. Live and dead assay was used to assess cell viability/cytotoxicity after 4 day culture. MTT assay was used to study cell proliferation. ALP activity assay and alizarin red S staining were conducted to evaluate cell differentiation and mineralization. Confocal laser scanning microscopy was also used.

Results: Beadless rhBMP-2/PDLLA and Ca-P/PLGA nanofibers were produced in all scaffolds and through DSDP-ES, both types of nanofibers were evenly distributed in bicomponent scaffolds. In the 28 day release test period, for mono- or bicomponent scaffolds, rhBMP-2 exhibited a quick release in the initial 24 hr and a sustained release subsequently (Fig.1a). rhBMP-2 released from bicomponent scaffolds with different component ratios exhibited similar release profiles but different release amounts. Similarly, similar release profiles were found for Ca^{2+} ions from Ca-P/PLGA scaffolds and bicomponent scaffolds (Fig.1b). High cell viability for all scaffolds was revealed via the live and dead assay. Compared with electrospun PDLLA or PLGA polymer scaffolds without the incorporation of rhBMP-2 or Ca-P nanoparticles, both mono- and bicomponent scaffolds exhibited significantly enhanced cell proliferation, with bicomponent scaffolds performing better than monocomponent scaffolds (Fig.2). It was observed that after 14 day culture, cells on mono- and bicomponent scaffolds expressed much higher ALP activity than pure polymer scaffolds and that bicomponent scaffolds caused significantly higher ALP activity than monocomponent scaffolds (Fig.2). After 14 day culture, the amount of mineral deposit produced by cells on different scaffolds also varied. While pure polymer scaffolds did not induce cells for calcium nodule deposition, cells cultured with bicomponent scaffolds produced much larger amounts of calcium nodule deposition than cells with monocomponent scaffolds.

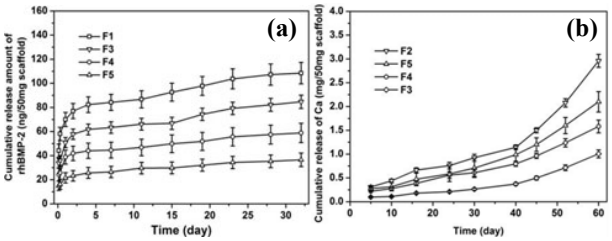

Fig.1. *In vitro* release profiles for bicomponent scaffolds: (a) rhBMP-2 release, (b) Ca^{2+} ion release.

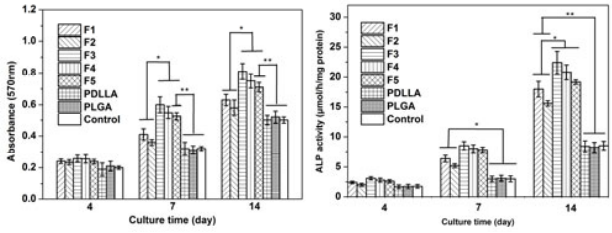

Fig.2. Proliferation (left) and ALP activity (right) of MC3T3-E1 cells after 4, 7, and 14 day culture.

Conclusions: Bicomponent scaffolds incorporated with rhBMP-2 and Ca-P nanoparticles were successfully made using DSDP-ES and the component ratio could be varied. Controlled release of rhBMP-2 and Ca^{2+} ions could be achieved and the released amount could be modulated. Compared with pure polymer scaffolds, mono- and bicomponent scaffolds exhibited much enhanced *in vitro* biological performances. Furthermore, bicomponent scaffolds performed better than monocomponent scaffolds due to combined osteoconductivity and osteoinductivity.