Regeneration of the osteochondral defect by a wollastonite and macro-porous fibrin biphasic scaffold

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Abstract: Osteochondral defect refers to the damage of cartilage as well as subchondral bone. Cartilage tissue engineering focusing on the regeneration of cartilage and disregarding the subchondral bone always leads to partial regeneration of the damage, resulting in poor mechanical and physiological properties. A scaffold suitable for *in situ* inductive regeneration of both types of tissues is urgently needed. In this study, a biphasic scaffold integrated by macro-porous fibrin and 3D-printed wollastonite (containing 8% $MgSiO_3$ (CS-Mg8)) scaffolds, either pre-loaded with rabbit bone marrow mesenchymal stem cells (BMSCs) or not, were fabricated and were used to repair osteochondral defects *in vivo* (full thickness osteochondral defects in rabbits, 4 mm in diameter and 4 mm in depth with bone marrow blood effusion). The fibrin scaffold had a pore size of 100~200 μm, and was degraded gradually and reached weight loss over 80% at 28 days. The presence of BMSCs could accelerate the degradation rate. BMSCs could well proliferate in the fibrin scaffold along with time prolongation. The CS-Mg8 scaffold possessed a regular structure of cross stacked CS-Mg8 rods, and was degraded rather slowly with a mass loss of 8.5% at 28 days. BMSCs adhered and showed well spreading on the CS-Mg8 scaffold, without apparent proliferation *in vitro*. *In vivo* transplantation of the biphasic scaffolds, either pre-loaded with BMSCs or not, could induce the regeneration of both cartilage and subchondral bone to a great extent. Loading of BMSCs enabled better regeneration of cartilage layer, leading to smoother macroscopic appearance, good integrity with surrounding tissue and tide mark formation. However, no significant difference in bone formation and in gene expression was found with and without BMSCs loading.

Introduction: Osteochondral defect refers to the damage of cartilage as well as subchondral bone. Cartilage tissue engineering focusing on the regeneration of cartilage regardless of the subchondral bone always lead to partial regeneration of the damage, resulting in poor mechanical and physiological properties. Therefore, a proper scaffold suitable for *in situ* inductive regeneration of both tissues is of urgent need. Wollastonite and macro-porous fibrin biphasic scaffolds loaded with rabbit bone marrow mesenchymal stem cells were fabricated and were used to induce osteochondral regeneration.

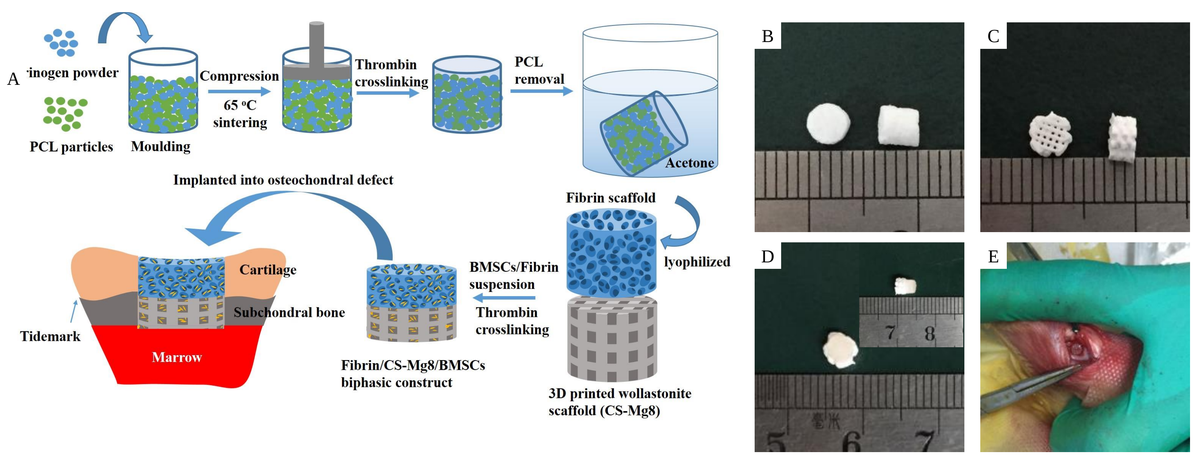


Fig. 1. (A) Schematic illustration of the fabrication process of the cell-seeded biphasic scaffold and its application in osteochondral regeneration in rabbit in vivo.. (B, C, D, E) Grow view of scaffolds and surgical procedure in rabbit knee joint.

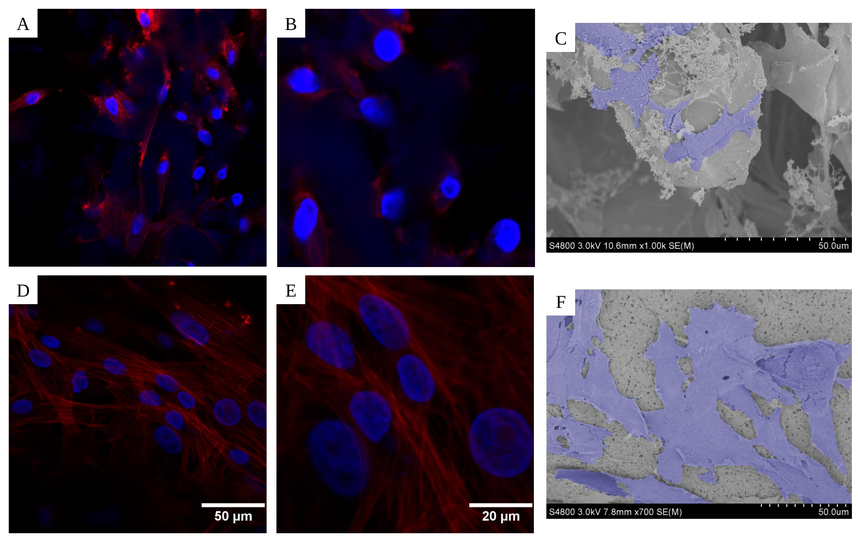


Fig. 3 CLSM images (A, B, D, E) and SEM images (C, F) of BMSCs in (A-C) fibrin scaffolds, and (D-F) CS-Mg8 scaffolds. For CLSM observation, the cell nucleus was stained by DAPI (blue), and the cytoskeleton was stained by rhodamine-conjugated phalloidin (red). The cells in the SEM images are distinguished by an artificial blue color..

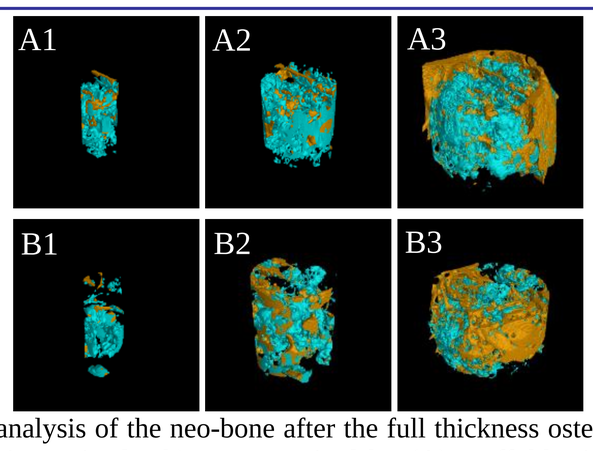
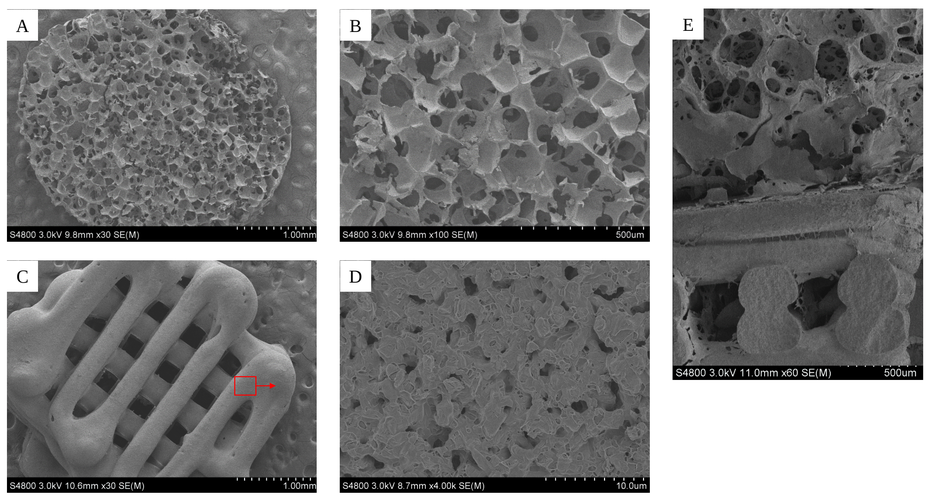


Fig. 4 Micro CT analysis of the neo-bone after the full thickness osteochondral defects (4 mm in diameter, and 4 mm in depth) were repaired by (A) scaffold with cells and (B) scaffold without cells for 18 w, blue is residual scaffold, yellow is neo-bone. Scan defect's diameter is 1 mm (A1, B1) , 2 mm (A2, B2) , 4 mm (A3, B3).



	F	G	H	I	J
Pore size (μm)	170 ± 109	325 ± 119	132 ± 50	307 ± 83	/
Porosity (%)	85.2	49.7	79.2	35.2	/

Fig.2 SEM images of (A, B) a macro-porous fibrin scaffold, (C, D) a CS-Mg8 scaffolds, and (E) a biphasic scaffold; μ-CT image of the scaffold, (F, G) fibrin scaffold and CS-Mg8 scaffold before loaded fibrin glue; (H, I) fibrin scaffold and CS-Mg8 scaffold after loaded fibrin glue; (J) whole image of biphasic scaffold..

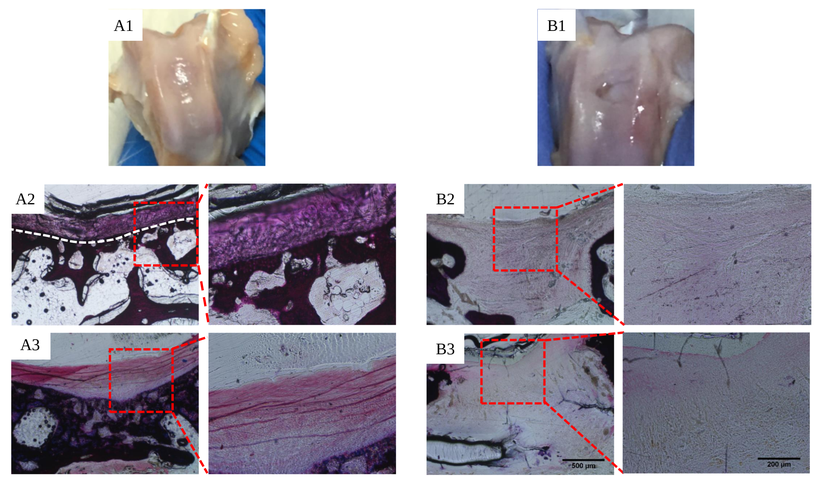


Fig. 6. Histological analysis of the neo-cartilages after the full thickness osteochondral defects (4 mm in diameter, and 4 mm in depth) were repaired by (A1-A3) cell-seeded composite scaffolds and (B1-B3) cell-free composite scaffolds for 18 w, respectively. (A1, B1) Gross view, (A2, B2) H&E staining, and (A3, B3) Masson trichrome staining of collagen fibers. White dot line outlines the tidemark.

Conclusions: A proper scaffold suitable for *in situ* inductive regeneration of both tissues was prepared. Wollastonite and macro-porous fibrin biphasic scaffolds loaded with rabbit bone marrow mesenchymal stem cells were fabricated. The wollastonite scaffolds contain 8% $MgSiO_3$, of which silicon ions can induce bone regeneration and magnesium is essential for skeletal metabolism, while bioactive macro-porous fibrin scaffold can induce cartilage regeneration. These two scaffolds were combined with fibrin gel. After transplantation of the composite scaffold which loaded cells in rabbit knee for 18 w, the cartilage defects and subchondral bone were both well regenerated. The neo-cartilage in the cell-seeded scaffold group integrated well with the surrounding tissues. Moreover, both of the cartilage and subchondral bone were better regenerated, and integrated well with each other.

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