Biomimetic Design of Calcium Phosphate- reinforced Collagen Hydrogels for Bone Tissue Engineering

Xuening Chen, Victor Lipnicki, Hongjun Wang
Department of Chemistry, Chemical Biology and Biomedical Engineering, Stevens Institute of Technology, Hoboken, NJ 07030

Statement of Purpose: Bone tissue engineering has proved to be a promising alternative to autografts in repairing and regenerating large bone defects. Previous studies suggested that type I collagen (COL-I) hydrogel could be a suitable matrix for bone tissue engineering, because of its ability to maintain the osteoblast phenotype and form mineralized matrix [1]. However, its relatively rapid degradation, large contraction rate and lack of biomechancial property determined that COL hydrogel itself cannot serve for the long-lasting bone graft [2]. In our study, calcium phosphate (CaP)-reinforced COL hydrogel was prepared by biomimicking the organic and inorganic composition of nature bone. To further investigate the combination of collagen and CaP, three common CaP particles with different Ca/P ratio were tested, including hydroxyapatite (HA, Ca/P=1.67), Tricalcium Phosphate (TCP, Ca/P=1.5) and Biphasic Calcium Phosphate (BCP, HA/TCP=70/30, Ca/P=1.63).

Methods: Collagen solution (8 mg/ml) was prepared by mixing type I collagen from calf skin (EPC, USA) in 0.01 N HCl (80%) with 5 × DMEM (20%), and neutralized with 1 N NaOH. 1 ml of collagen solution was then mixed thoroughly with 20 mg of either BCP (gift from Sichuan Univ. China), HA or TCP particles (71.43 wt. %, Sigma, USA), and 2×10^6 osteoblast-like MC 3T3 cells. The solution was transferred to 96-well plates (200 µl per well). Gelation was completed after half hour incubation at 37°C. Then the cylinder-shaped gels were transferred to 6-well plates. Culture medium containing DMEM (Invitrogen, USA) supplemented with 10 % FBS and 1 % antibiotics was added and then the gels were dynamically cultured on a lab rotator (Barnstead / lab-line, USA) with the rotation speed set at 20rpm to provide a uniform horizontal circular movement of medium. The cultured gels were harvested at Day 3, 7 and 14, washed with PBS and fixed with 4% paraformaldehyde for 2 h at RT. The gel contraction was recorded by using a stereoscopic microscope (Nikon SMZ-1500). The fixed gels were cut into two pieces through the middle. Half of the gel was stained by methylene blue (Fisher Chemical, USA). The other half was dehydrated in a series of gradient ethanol, embedded and sectioned into thin sections. The sections were stained with hematoxylin and eosin (H&E, Richard-Allan Scientific, USA) for histological examination.

Results: The gel contraction results were summarized in Table1, indicating a continuous contraction of hydrogels with time. By Day 14, maximum contraction occurred in TCP/COL gel (78%), where collagen portion was mostly digested and clusters of TCP particles were surrounded by a multilayer of cells. Compared to pure COL gel, BCP/COL and HA/COL gels significantly reduced the contraction from 64% to 46% and 36%, respectively ($p<0.05$). Methylene blue staining showed that osteoblast-like cells homogeneously distributed in all gels at Day 3. Then cells started to migrate to the periphery of the gels and by Day 14, most of the cells aggregated in the surface of pure COL and TCP/COL hydrogels. In contrast, majority of the cells were found inside the BCP/COL and HA/COL hydrogels.

Table 1. Contraction Rate of Cultured Hydrogels

Sample	BCP/COL	HA/COL	TCP/COL	COL
Day 3	8.00%	8.65%	19.91%	6.06%
Day 7	21.64%	11.25%	47.62%	32.25%
Day 14	46.43%	36.25%	78.25%	64.07%

H&E staining results (Figure 1) showed that MC 3T3 cells exhibited osteoblast-like phenotype in CaP-reinforced COL gels similar to those in pure COL gel without loss of viability. Compared to polygonal cell morphology in COL gel, the cells in the CaP/COL gels gradually changed from elongated and spindle shape to flattened and enlarged shape with large nuclei. At Day 14, the peripheral regions of gels were covered by multiple cell layers with parallel alignment.

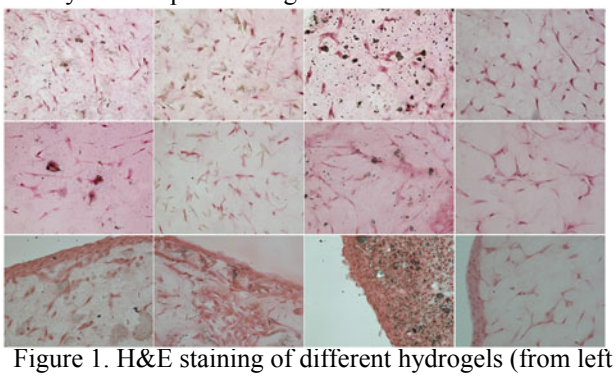

Figure 1. H&E staining of different hydrogels (from left to right: BCP/COL, HA/COL, TCP/COL and COL Gel) at Day 3 (top), Day 7 (middle) and Day 14 (bottom).

Conclusions: The results indicated that incorporation of BCP and HA particles in COL gel could significantly reduce gel contraction, maintain uniform 3D cell distribution and induce osteoblastic phenotype in the long-term culture compared to pure COL gel. The high bioactivity of BCP- and HA- reinforced COL gels could be due to their composition similarity to bone. Thus, Ca^{2+} and PO_4^{3-} released by BCP and HA particles together with COL fibrils might affect the osteoblasts and reduce their extracellular contractile forces for gel contraction [2]. However, the fast biodegradable TCP could accelerate the degradation of COL gel and led to structural instability. Our ongoing efforts focus on better understanding the bioactivity and biomechanical properties of these materials. In summary, BCP- and HA-reinforced COL hydrogels are promising in bone tissue engineering.

References: 1. Akhouayri O J Cell Biochem. 1999; 76:217-230. 2. Pohunkova H Biomaterials 1995; 18: 67-71. 3. Henemann S Acta Biomate. 2009;5: 1979-1990