Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2017

Supporting Information

The development of cell-initiated degradable hydrogel basing on methacrylated alginate applicable to multiple microfabrication technologies

Jing Sun, Dan Wei, Ke Yang, You Yang, Xiaolu Liu, Hongsong Fan*, Xingdong Zhang

National Engineering Research Center for Biomaterials, Sichuan University, 610064, Chengdu, P. R. China.

*Corresponding author: hsfan@scu.edu.cn; Tel: 86-28-85410703; Fax: 86-28-85410246

Hydrogel preparation

Methacrylated alginate (ALG) was first synthesized basing on the esterification of sodium alginate and methacrylic anhydride,[1] and the methacrylation percentage was about 50%. ALG was dissolved in a triethanolamine-buffered saline (TEOA buffer: 0.2 M TEOA, 0.3 M total osmolarity, pH 8.0) containing I2959 (0.5 wt %). Basing on the Michael-type addition chemistry, RGD peptide dissolved in TEOA buffer was first added to ALG solution at a concentration consuming 10% acrylate groups on ALG and reacted at 37℃ for 1 h. MMP-degradable peptide corresponding to 25% acrylate consumption was then added. The resultant homogenous hybrid solution was subsequently poured into molds and then exposed to 7.9 W/cm^2 UV light (360-480 nm, Omnicure S1500) for 20 seconds to carry out free radical polymerization of ALG chain by photocrosslinking.

Gene expression

Cell–laden hydrogels were harvested and gene expressions were analyzed after culturing for 1, 2, and 3 weeks. Samples were frozen in liquid nitrogen and pulverized immediately. Trizol reagent (Invitrogen) was employed to isolate the total RNA, which was then converted into complementary DNA using RT-PCR Kit (Toyobo, Osaka, Japan) following the kit protocol. Quantitative RT-PCR was performed using FTC-2000 Real-Time Fluorescence Quantitative Thermocycler (FungLyn Biotech Corp. Ltd, Shanghai, China) to determine the gene expression of Cbfa1 (core-binding factor α1), BMP-2 (bone morphogenetic protein-2), COL-I (collagen I) and OCN (osteocalcin) relative to the expression of GAPDH

(glyceraldehyde-3-phosphate dehydrogenase). Primers used for amplification are listed in Table 1.

Table1. Primers sequences for target genes.

Symbol	Primers
GAPDH	5′-GCCAAGGCTGTGGGCAAGGT-3′
	5′-AGGTGGAGGAGTGGGTGTCG-3′
Cbfa1 (core-binding factor α1)	5′-CTCTACTATGGCACTTCGTCAG-3′
	5′-GCTTCCATCAGCGTCAACAC-3′
BMP-2 (bone morphogenetic protein-2)	5′-TTACTGCCACGGAGAATGCC-3′
	5′-CCCACAACCCTCCACAACCA-3′
COL-I (collagen I)	5′-CACACGTCTCGGTCATGGTA-3′
	5′AAGAGGAAGGCCAAGTCGAG3′
OCN (osteocalcin)	5′-GAGGGCAGCGAGGTAGTGAA-3′
	5′-CCTCCTGAAAGCCGATGTGGT-3′

^{1}H NMR characterization

The degree of methacrylation (methacrylation percentage) was confirmed using 1H-NMR analysis with D_2O as the solvent. The relative integrations of the methacrylate proton peaks (methylene, δ=6.2 and 5.77 ppm and the methyl peak, δ=1.99 ppm) to carbohydrate protons (δ=3.7~4.2) were used to determine molar percent of methacrylation. A 50% modified MAA was synthesized and used for all subsequent studies.

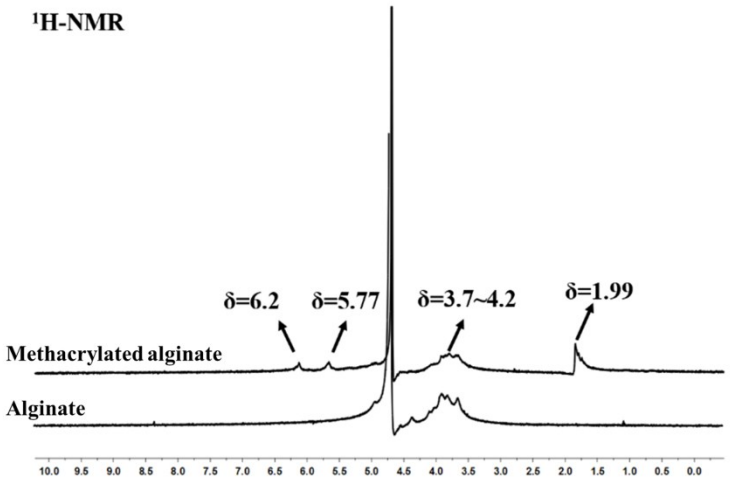

Fig. S1 ^{1}H-NMR spectra of methacrylated alginate.

Swelling exprement of lyophilized scaffolds

The photocrosslinked hydrogel disks were lyophilized and initial dry weights (W_i) were measured. The dried samples were immersed in PBS at 37℃, and the swelling ratio (S_d) was calculated by the following formula: S_d =(W_s-W_i)/W_i×100% (W_s: wet weight of the swollen samples after incubation; W_i: initial dry weight of lyophilized scaffolds).

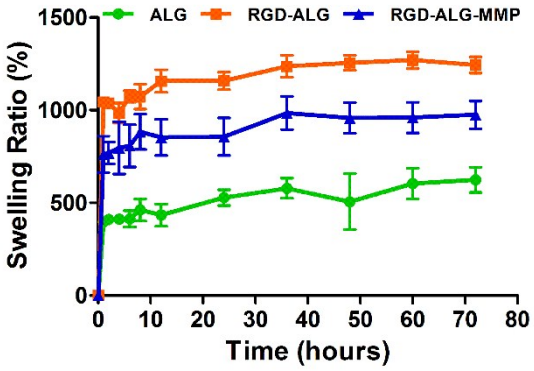

Fig. S2 Swelling properties of lyophilized methacrylated alginate-based scaffolds.

As shown in Fig. S2, swelling properties of hydrogels after lyophilisation exhibited increased tendency in the order of ALG, RGD-ALG-MMP and RGD-ALG due to the increase of hydrophilicity. While for wet alginate hydrogel, it is already in a state of relative swelling equilibrium. Moreover, the stronger the hydrophilicity of the molecule, the closer its gel is to the swollen equilibrium state, and its absorbent capacity is relatively weaker after immersing it in PBS. Thus, the swelling ratio of the wet gel RGD-ALG and RGD-ALG-MMP was lower than ALG.

Cell proliferation

The MTT assay was implemented to quantitatively investigate the viability of MG-63 cells encapsulated in photocrosslinked alginate based hydrogels. Cells encapsulated in samples gelating with 50 μL prepolymer solutions were incubated with 0.5 mg/ml MTT for 4 h at 37℃. The solution was then removed and purple formazan salts dissolved with dimethyl sulphoxide, and the absorbance of the resulting solution was measured at 490 nm using a multidetection microplate reader (Bio-Rad 550).

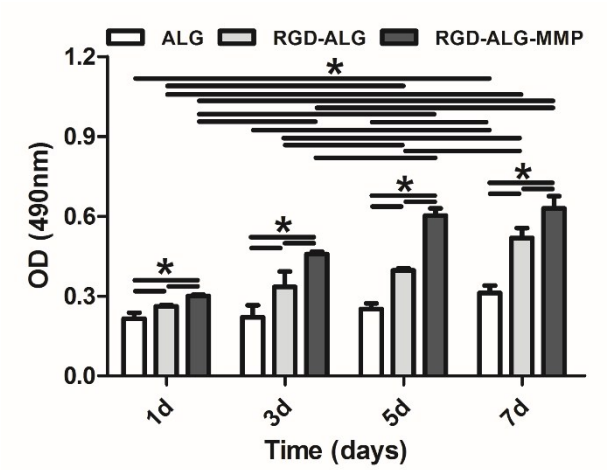

Fig. S3 MTT analysis of the MG-63 cells encapsulated in the methacrylated alginate-based hydrogels.

The construction of patterned tissue-specific microstructures

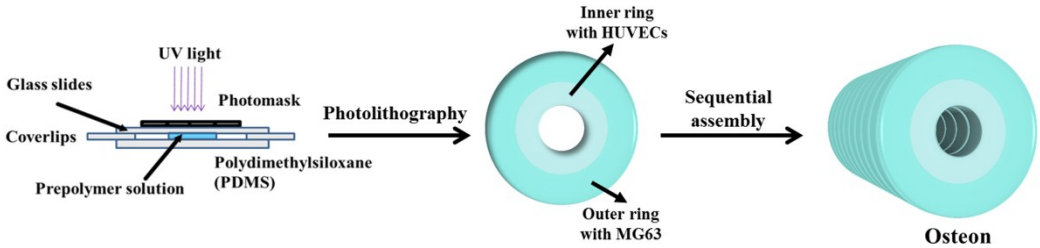

Fig. S4 Scheme for the construction of osteon-like structure by photomask-based stereolithography.

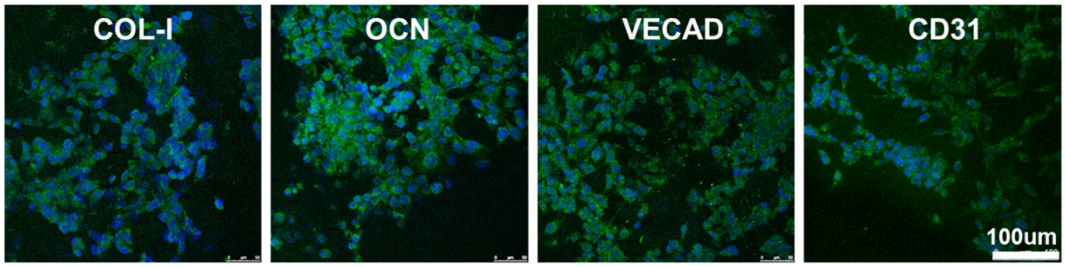

Fig. S5 Low magnification images of immunofluorescence staining of osetogenic (COL-I and OCN) and vasculogenic (VECAD and CD31) markers in the osteon-like structure.

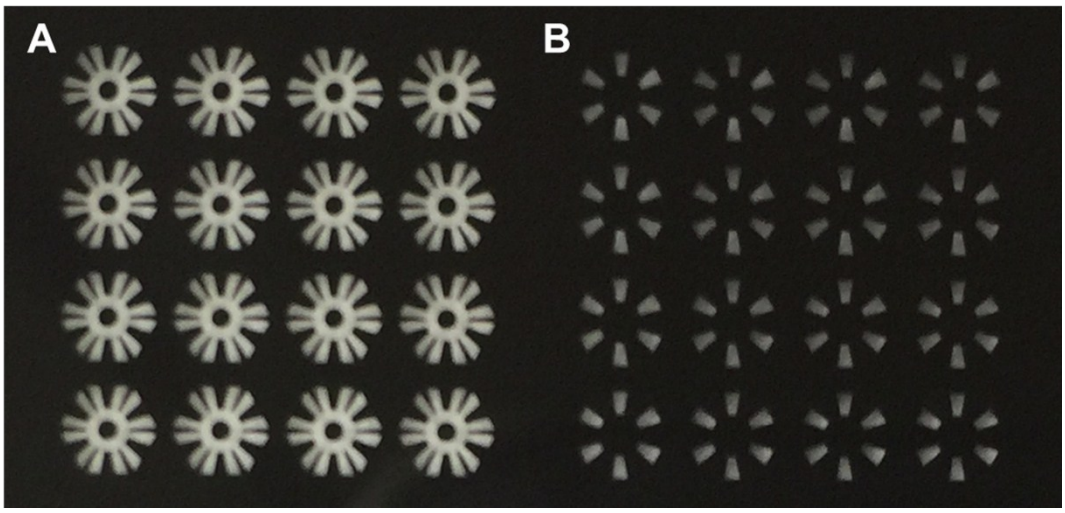

Fig. S6 The photomasks with (A) helical and (B) petaloid patterns for the simulation of hepatic lobular structure.

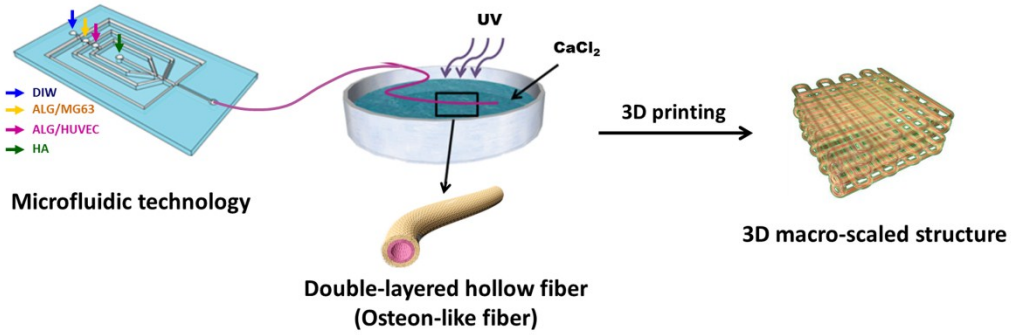

Fig. S7 Schematic illustration of continuous double-layered hollow microfibers fabricated by microfluidic technology to mimic the osteon like structure.

References:

1. J. Sun, W. Xiao, Y. Tang, K. Li and H. Fan, *Soft Matter*, 2012, **8**, 2398-2404.