SUPPORTING INFORMATION

A pH-triggered, self-assembled and bioprintable hybrid hydrogel scaffold for mesenchymal stem cell-based bone tissue engineering

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Table S1. List of TqPCR Primers			
Genes	qPCR Primer Sequences		Accession Number
	Forward	Reverse	
mouse Runx2	CCGGTCTCCTTCCAGGAT	GGGAACTGCTGTGGCTTC	NM_001146038
mouse Pparγ	TTTTCAAGGGTGCCAGTTTC	AATCCTTGGCCCTCTGAGAT	NM_011146
mouse Osx	GAAGTCCAATGGGGATCTGA	AGAATCCCTTTCCCTCTCCA	NM_130458
mouse Alp	CCCCATGTGATGGCGTAT	CGGTAGGGAGAGCACAGC	NM_007431
mouse Ocn	CCTTCATGTCCAAGCAGGA	GGCGGTCTTCAAGCCATAC	NM_001032298
mouse Opn	CCTCCCGGTGAAAGTGAC	CTGTGGCGCAAGGAGATT	NM_001204203
mouse Sox9	GCAAGCAAAGGAGACCAAAA	CGCTGGTATTCAGGGAGGTA	NM_011448
mouse Gapdh	GCCTCGTCCCGTAGACAAAA	TTCCCATTCTCGGCCTTGAC	NM_008084

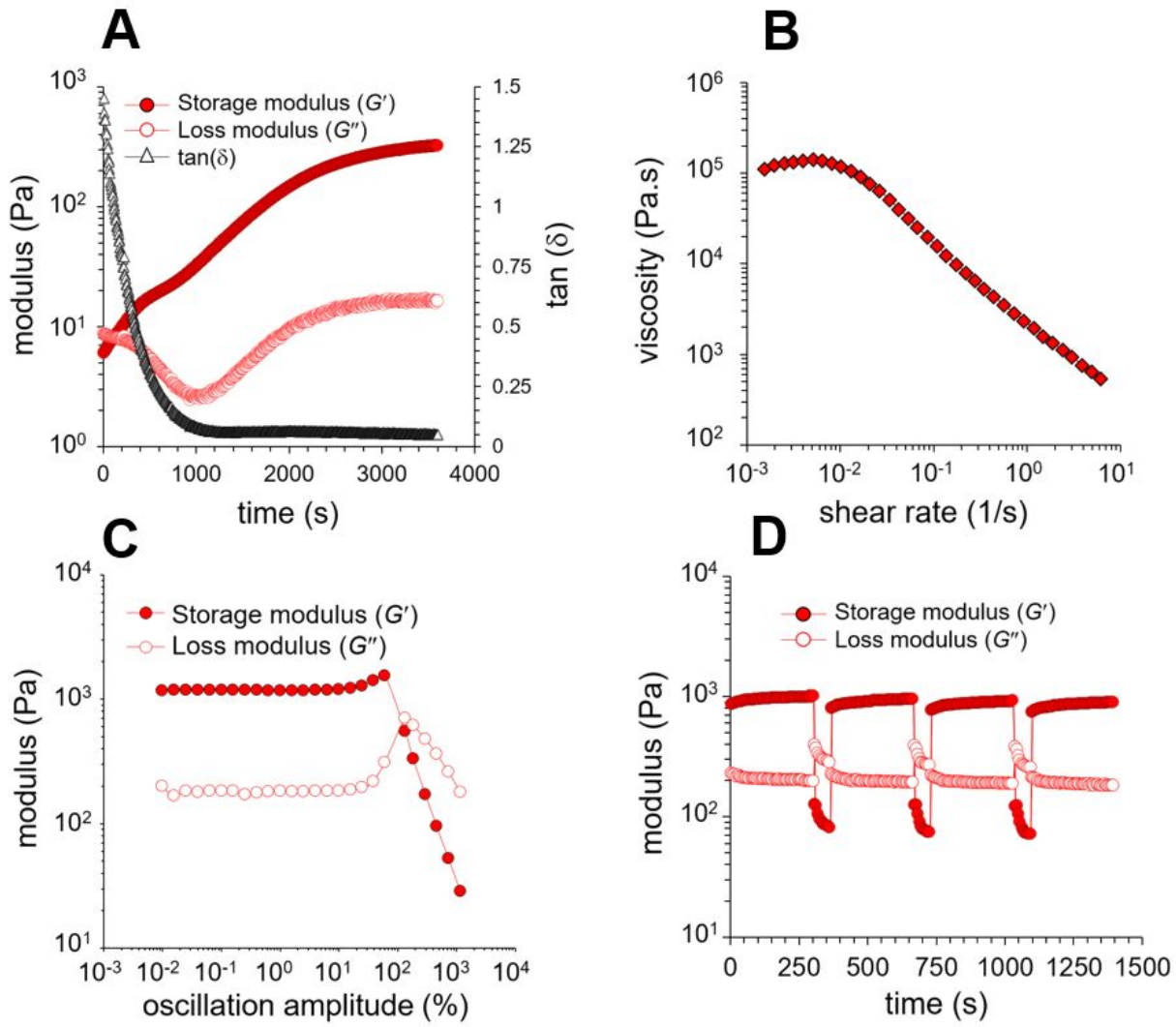

Figure S1. Rheological measurements of pH-induced self-assembly, shear-thinning behavior, and self-healing characteristics of CMCh-ACP hybrid hydrogels. (**A**) Evolution of storage (G') and loss (G'') moduli and tan(δ) after addition of GDL to a 2.5 wt% dispersion of CMCh-ACP. (**B**) The change of viscosity of CMCh-ACP gel (10 wt%, pH 7.5) in shear rate sweep measurement. (**C**) The variation of G' and G'' in oscillatory strain sweep measurements (10 wt%, pH 7.5). (**D**) Evolution of G' and G'' as a function of time showing the structural recovery behavior of the CMCh-ACP gel (10 wt%, pH 7.5, strain 1%, 1 Hz) after multiple destruction cycles by a 1000% oscillatory shear strain for 1 min at 1 Hz.

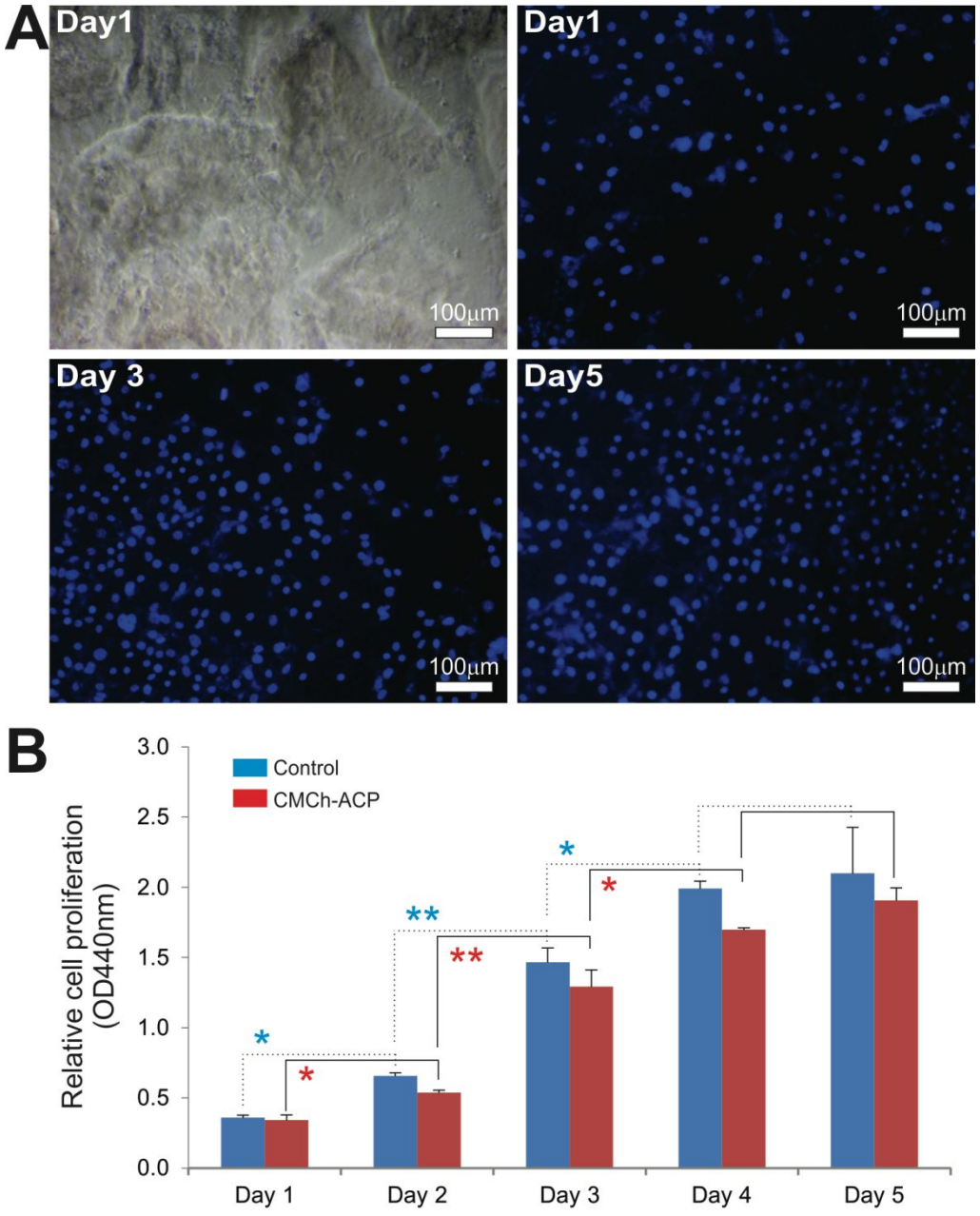

Figure S2. Cytotoxicity and cell proliferation of the iMADs cells entrapped in CMCh-ACP hybrid gel. (**A**) Hoechst staining assay. Exponentially growing iMADs cells were mixed with CMCh-ACP hybrid gel and seeded in 24-well plates. At the indicated time points, the culture medium in each well was removed, gently washed with PBS, fixed and stained with the Magic Solution containing Hoechst 33258. Apoptotic cells were examined and recorded under a fluorescence microscope. Each assay condition was done in triplicate. Representative results are shown. (**B**) WST-1 assay. Exponentially growing iMADs cells were mixed with CMCh-ACP hybrid gel (CMCh-ACP) or directly plated without the hybrid gel (Control) in 24-well plates. At 1, 2, 3, 4, and 5 days after plating, Premixed WST-1 Reagent was added to each well, followed by an incubation at 37°C for 60min and reading at 440nm using a microplate reader. Each assay condition was done in triplicate. The relative cell proliferation rates between control and CMCh-ACP groups were not statistically significant at the same time point, although the cell proliferation rates for either control or CMCh-ACP group were statistically significant from day 1 to day 4. "*" $p<0.05$; "**" $p<0.01$.

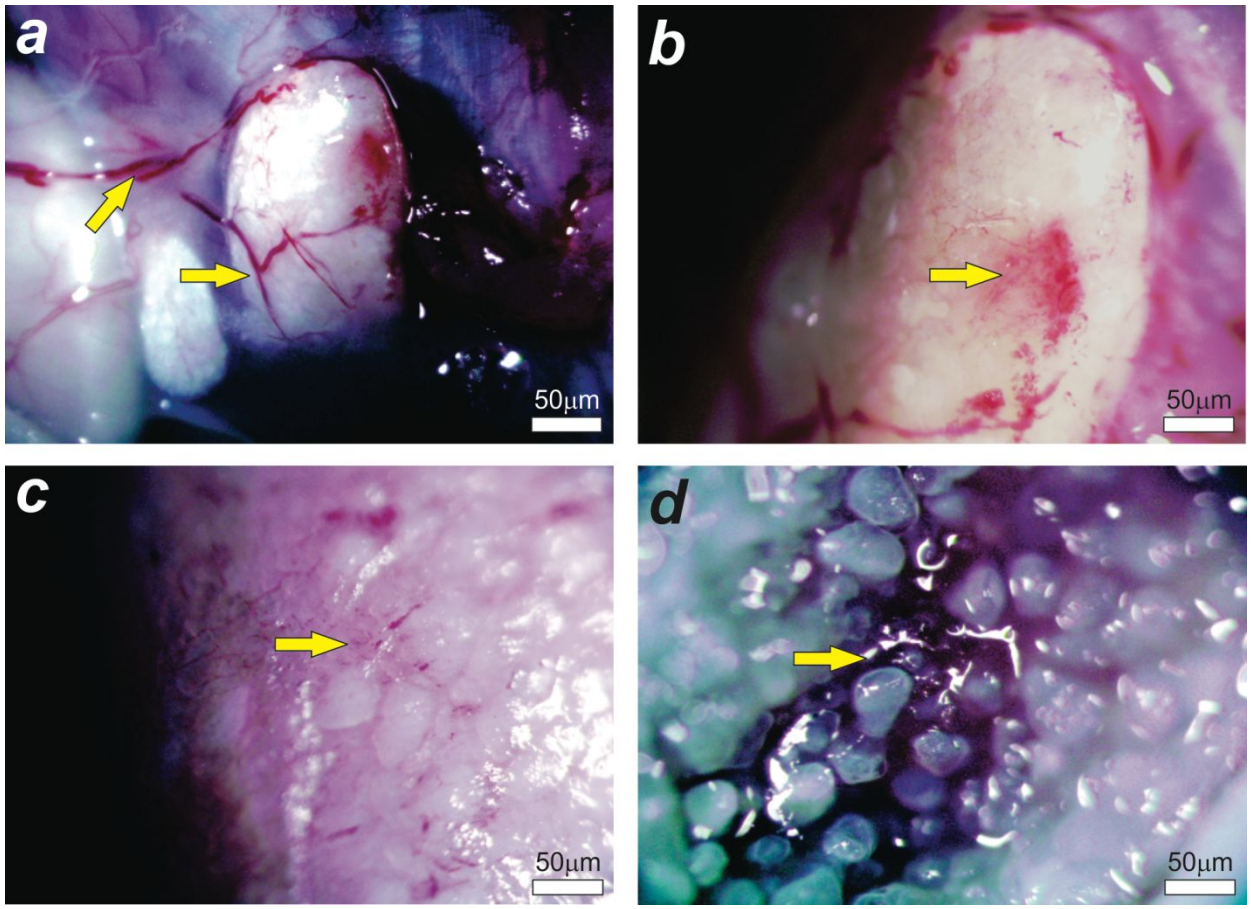

Figure S3. Vascularization feature of the ectopic bone masses retrieved from the BMP9 + CMCh-ACP group (6-week time point). Representative Gross images were taken by using the Olympus SZX16 stereomicroscope at lower (***a* & *b***) and higher (***c* & *d***) magnifications.