Angiogenesis-Osteogenesis Coupling and Immunomodulatory CGRP@Nano MOF-Loaded CMCS/GelMA Hydrogel for Bone Regeneration

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Preparation and characterization of GelMA

The synthesis of GelMA was conducted following established protocols. Briefly, 5 g of gelatin was dissolved in 50 mL of phosphate-buffered saline (PBS). At 50 °C, 4 mL of methacrylic anhydride was slowly added to the gelatin solution while stirring magnetically for 3 hours. The reaction was subsequently terminated by adding 200 mL of pre-warmed PBS. The resulting solution underwent continuous dialysis at 40 °C for 7 days, after which the product was freeze-dried to obtain GelMA. Characterization of GelMA and gelatin was performed using a Bruker Avance 600 MHz nuclear magnetic resonance (NMR) spectrometer to obtain the ^{1}H-NMR spectra. The degree of functionalization (DOF) of GelMA was quantified using a ninhydrin colorimetric assay. A series of

standard glycine solutions of known concentrations were prepared and reacted with the ninhydrin working solution. The optical density (OD) at 570 nm was measured using a microplate reader to create a standard curve correlating amino group concentration with absorbance.

Subsequently, gradients of gelatin and GelMA solutions were prepared. Following the same procedure, the OD values were measured, and the amino group concentrations of gelatin (C1) and GelMA (C2) were calculated based on the standard curve. The degree of functionalization of GelMA was then calculated with the equation:

$$DOF = \left(1 - \frac{C2}{C1}\right) \times 100\%$$

Preparation of nanoparticles ZIF-8 and CGRP@MOF

To synthesize ZIF-8, 0.4 g of $Zn(NO_3)\cdot 6H_2O$ and 2.76 g of 2-methylimidazole were each dissolved in 10 mL of ddH_2O to prepare the respective $Zn(NO_3)\cdot 6H_2O$ and 2-methylimidazole solutions. The two solutions were then mixed rapidly and stirred magnetically for 3 minutes. Following this, the mixture was centrifuged at 14,000 rpm for 20 min to collect the precipitate. The resulting precipitate was thoroughly washed and dried to yield ZIF-8.

For the synthesis of CGRP@MOF, 10 mg of CGRP and 1.38 g of 2-methylimidazole were dissolved in 10 mL of ddH2O. Concurrently, 0.2 g of $Zn(NO_3)\cdot 6H_2O$ was dissolved in another 10 mL of ddH2O. The two solutions were then mixed to facilitate a complete reaction, followed by centrifugation of the resultant mixture. The precipitated product was subsequently dried to yield CGRP@MOF.

Table S1. Gene primer sequences for MC3T3-E1 cells

Name		Sequences (5' to 3')
ALP	F	CCAACTCTTTTGTGCCAGAGA
	R	GGCTACATTGGTGTTGAGCTTTT
Runx2	F	GACTGTGGTTACCGTCATGGC
	R	ACTTGGTTTTTCATAACAGCGGA
BMP2	F	CGCCTCACAAACAACCACAG
	R	AATGACTCGGTTGGTCTCGG
OCN	F	TGAGAGCCCTCACACTCCTC
	R	CGCCTGGGTCTCTTCACTAC
COL1A1	F	GAGAGCATGACCGATGGATT
	R	CCTTCTTGAGGTTGCCAGTC
GAPDH	F	AGGTCGGTGTGAACGGATTTG
	R	TGTAGACCATGTAGTTGAGGTCA

Table S2. Gene primer sequences for RAW264.7 cells

Name		Sequences (5’ to 3’)
iNOS	F	AGCGCTCTAGTGAAGCAAAG
	R	CATACTGTGGACGGGTCGATG
Arg1	F	TTGTGAAGAACCCACGGTCT
	R	AGATGCTTCCAACTGCCAGA
CD206	F	ACCTGGGGACCTGGTTGTAT
	R	ATGGCACTTAGAGCGTCCAC
GAPDH	F	AGGTCGGTGTGAACGGATTTG
	R	TGTAGACCATGTAGTTGAGGTCA

Table S3. Gene primer sequences for HUVECs

Name		Sequences (5’ to 3’)
VEGF	F	TGCGGATCAAACCTCACCA
	R	CAGGGATTTTTCTTGTCTTGCT
HIF-1α	F	CCATGTGACCATGAGGAAAT
	R	CGGCTAGTTAGGGTACACTT
NOTCH1	F	GACTGCTCCCTCAACTTCAAT
	R	GTGGTCCTGCAGTACTGGT
GAPDH	F	GATTTGGTCGTATTGGGCG
	R	CTGGAAGATGGTGATGG

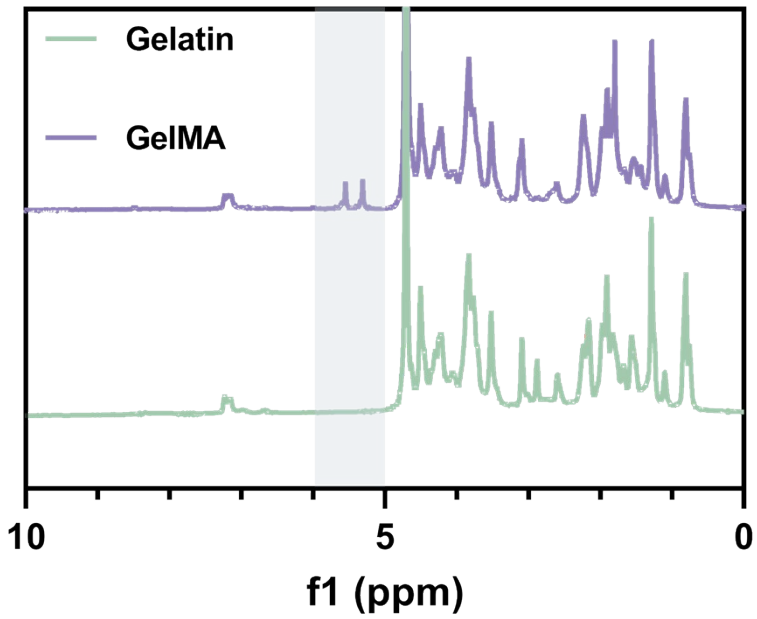

Figure S1: ^{1}H-NMR spectral of GelMA and gelatin.

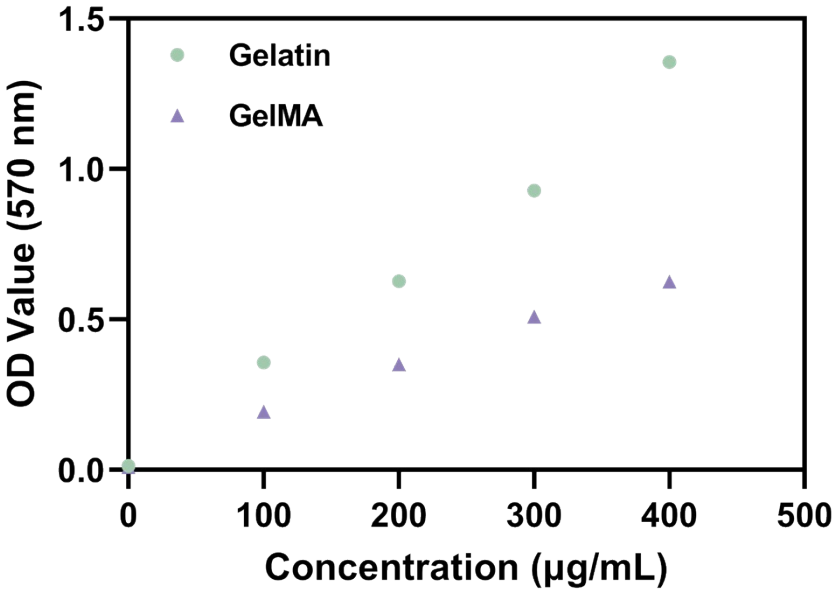

Figure S2: Assessment of the degree of functionalization for GelMA.

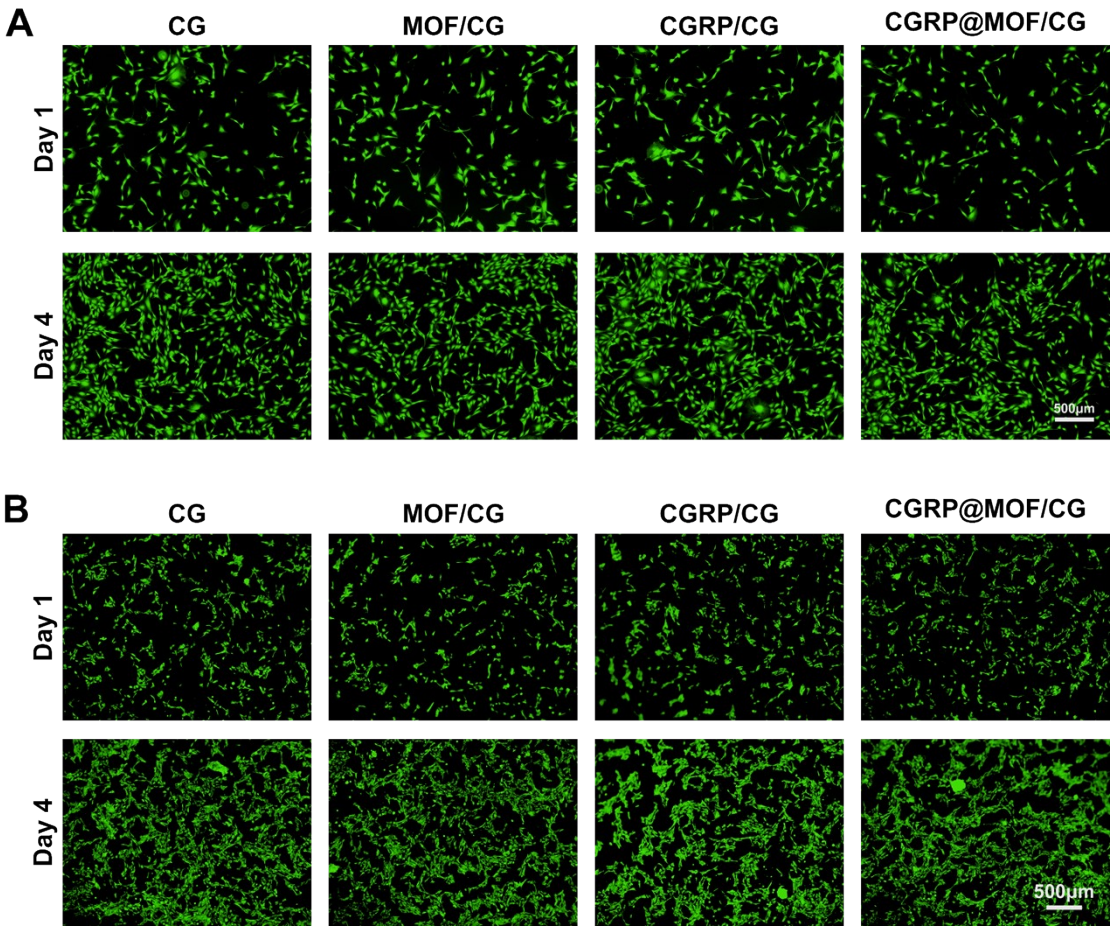

Figure S3: (A) Calcein-AM/PI staining of MC3T3-E1 cells co-cultured with different hydrogels in a non-contact manner for 1 and 4 days. (B) Calcein-AM/PI staining of MC3T3-E1 cells co-cultured with different hydrogels in a contact manner for 1 and 4 days.

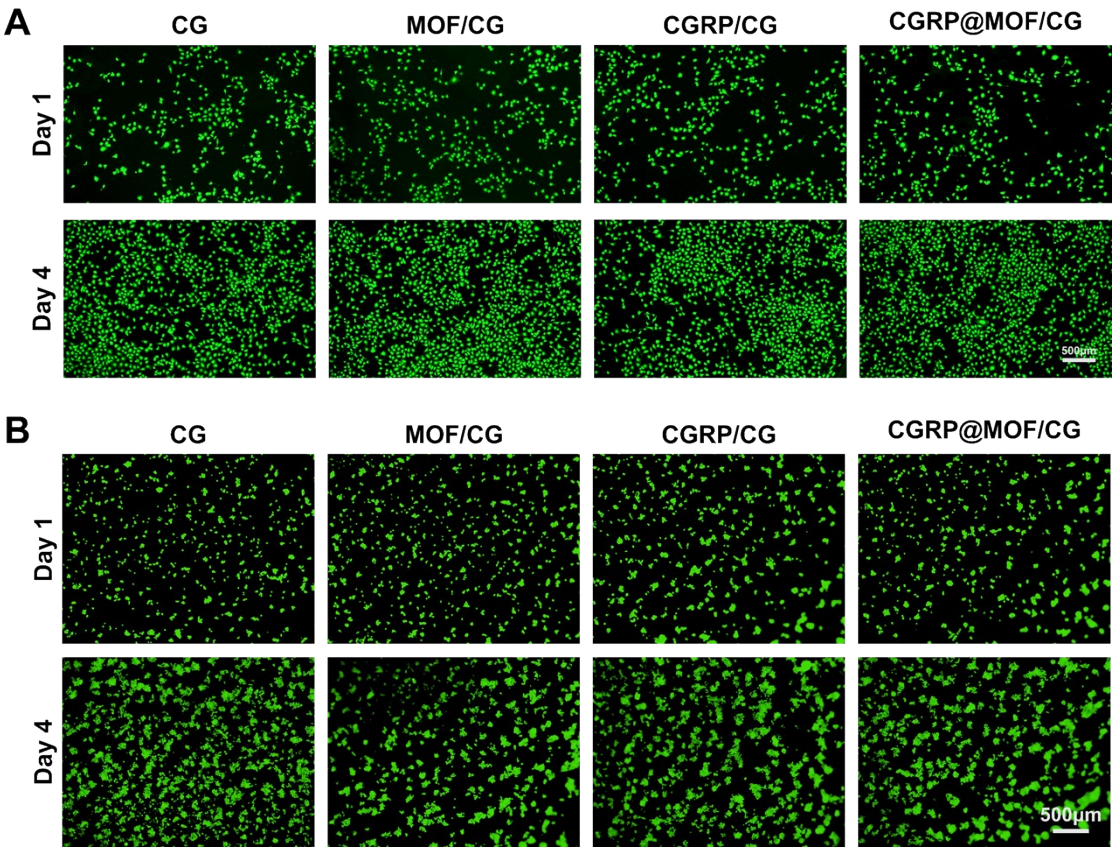

Figure S4: (A) Calcein-AM/PI staining of HUVECs co-cultured with different hydrogels in a non-contact manner for 1 and 4 days. (B) Calcein-AM/PI staining of HUVECs co-cultured with different hydrogels in a contact manner for 1 and 4 days.

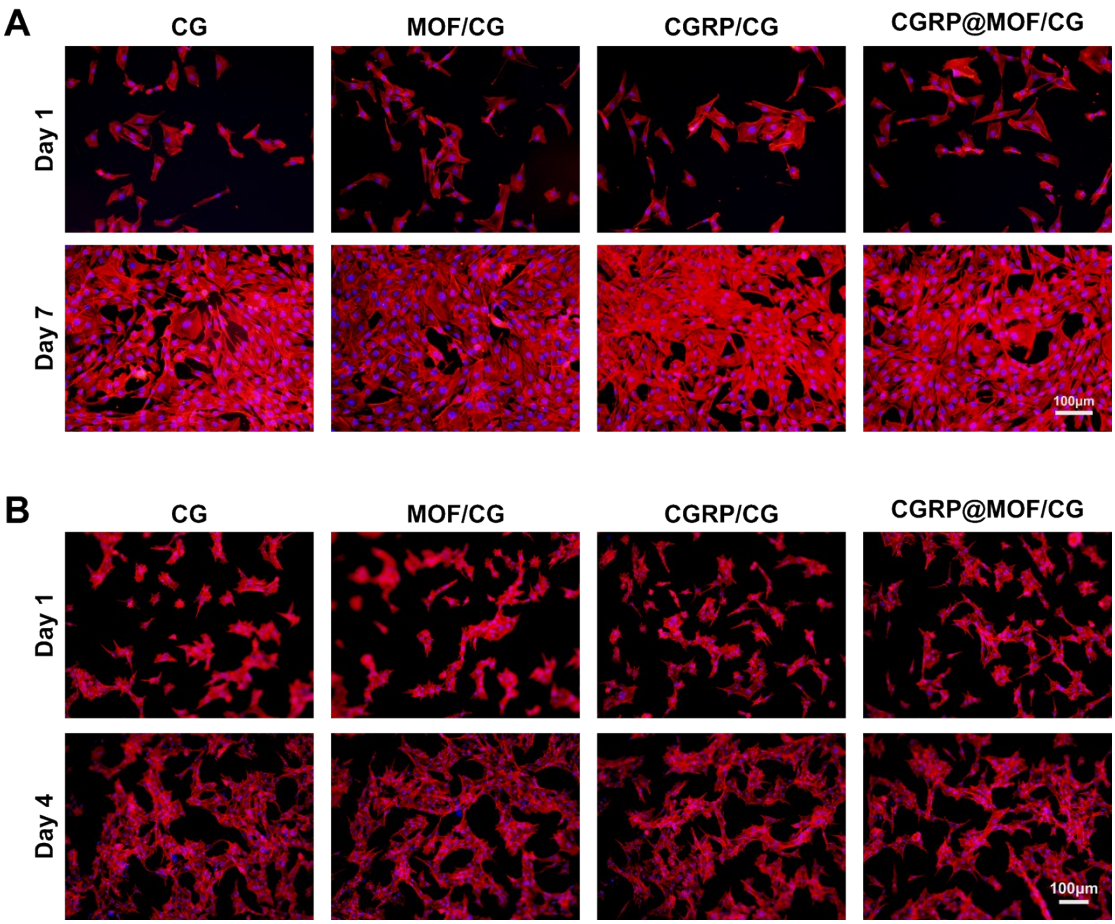

Figure S5: (A) Cytoskeletal staining of MC3T3-E1 cells co-cultured with different hydrogels in a non-contact manner for 1 and 7 days. (B) Cytoskeletal staining of MC3T3-E1 cells co-cultured with different hydrogels in a contact manner for 1 and 4 days.

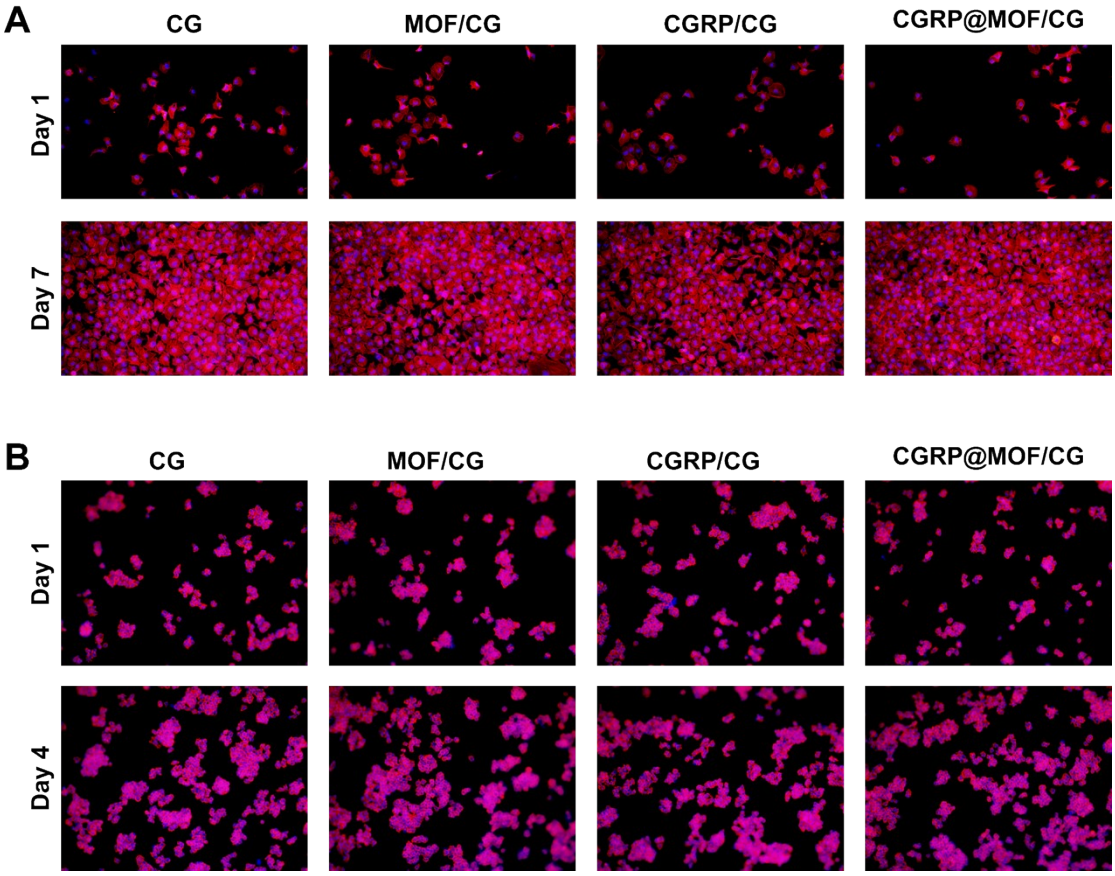

Figure S6: (A) Cytoskeletal staining of HUVECs co-cultured with different hydrogels in a non-contact manner for 1 and 7 days. (B) Cytoskeletal staining of HUVECs co-cultured with different hydrogels in a contact manner for 1 and 4 days.

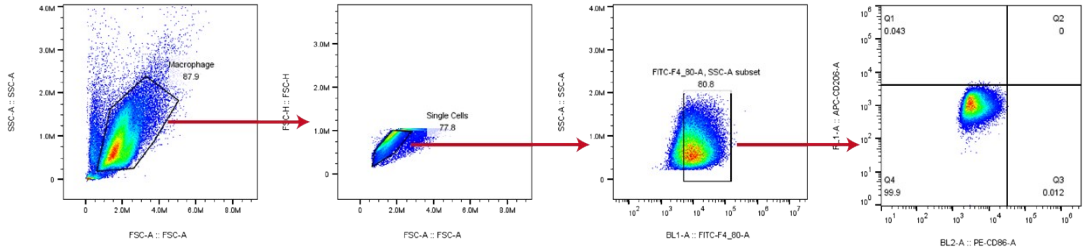

Figure S7: Flow cytometry gating strategy.

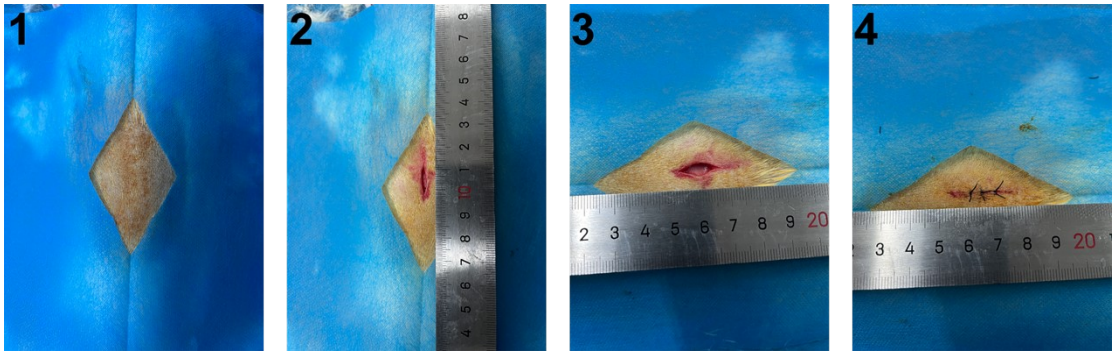

Figure S8: Surgical procedure for establishing a subcutaneous implantation model in rats.

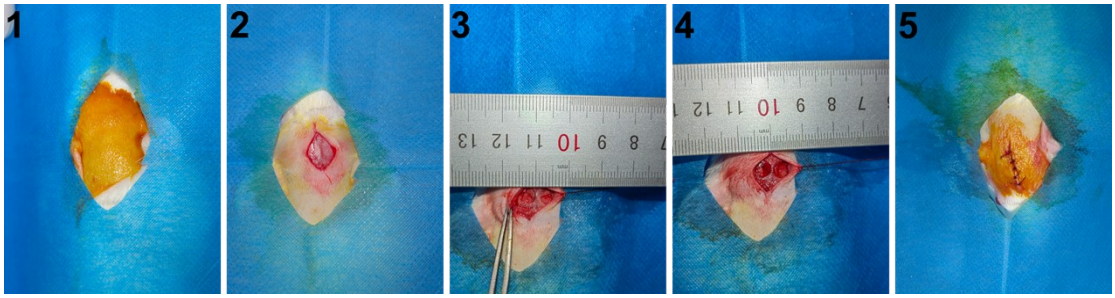

Figure S9: Surgical procedure for establishing a cranial defect model in rats.

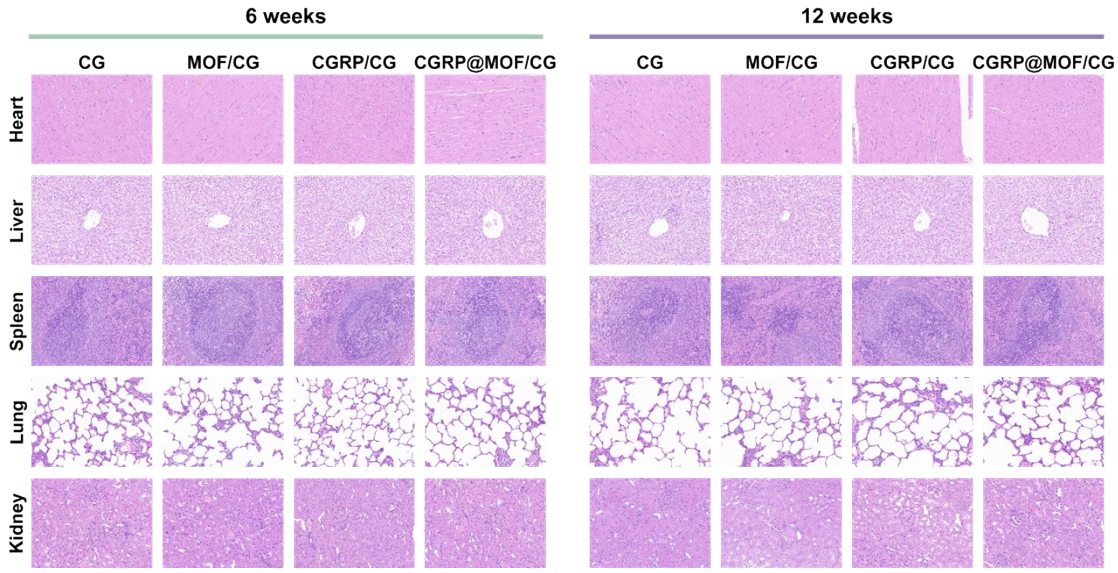

Figure S10: H&E staining of vital organs from rats treated with different hydrogels.

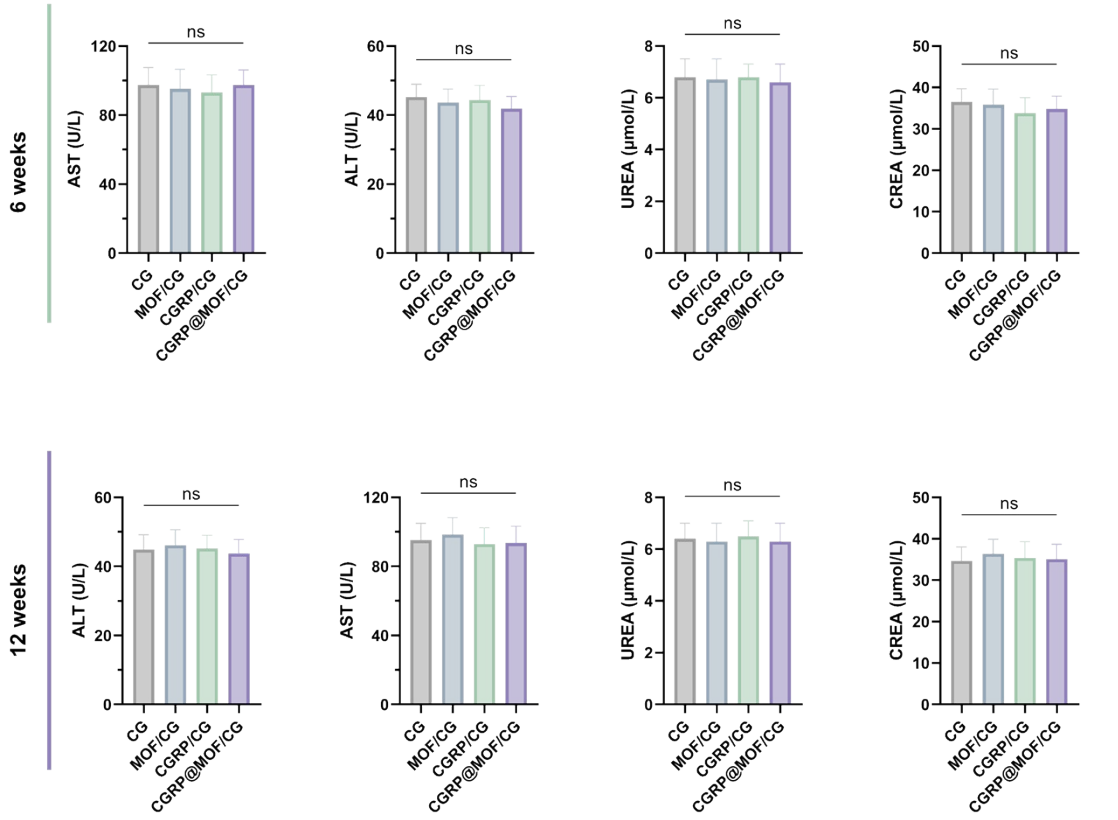

Figure S11: Biochemical results of blood samples of rats from different hydrogel groups at 6 and 12 weeks post-implantation.