

Supplement Information for

Hierarchically Porous Hydroxyapatite Hybrid Scaffold Incorporated with Reduced Graphene Oxide for Rapid Bone Ingrowth and Repair

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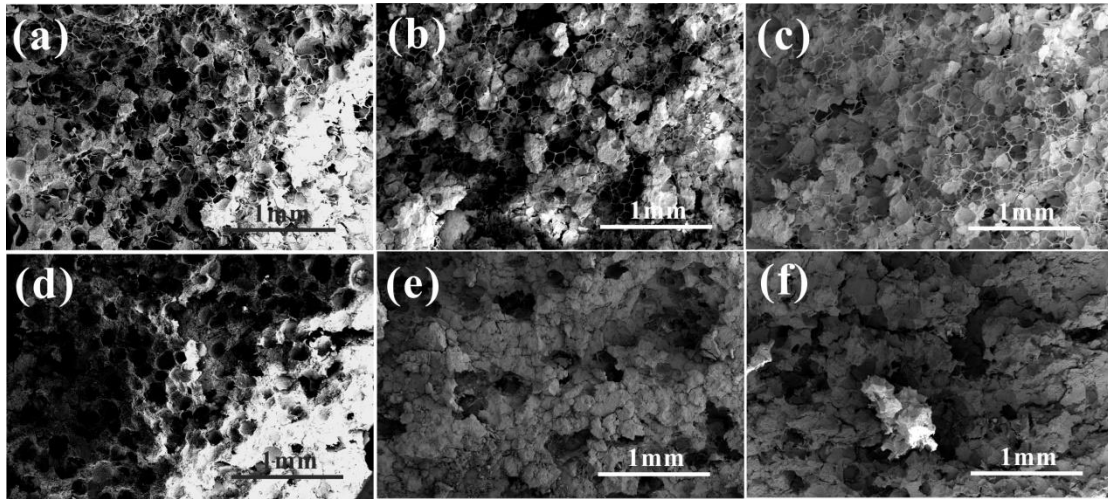


Figure S1. SEM images of before sintering (a) HA-6 (b) HA-6-CS and (c) HA-6-D;
SEM images of (d) HA-6 (e) HA-6-CS and (f) HA-6-D

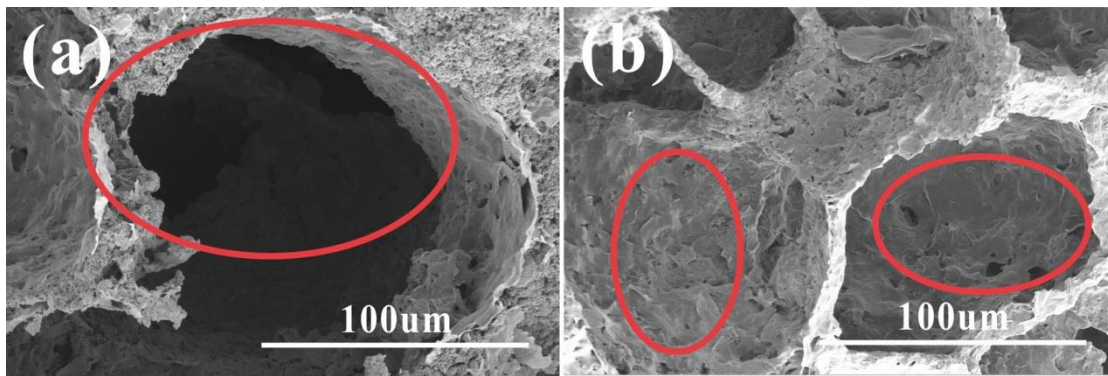


Figure S2. SEM images of (a) H/G-6/0.3% and (b)H/G-6/0.9%.

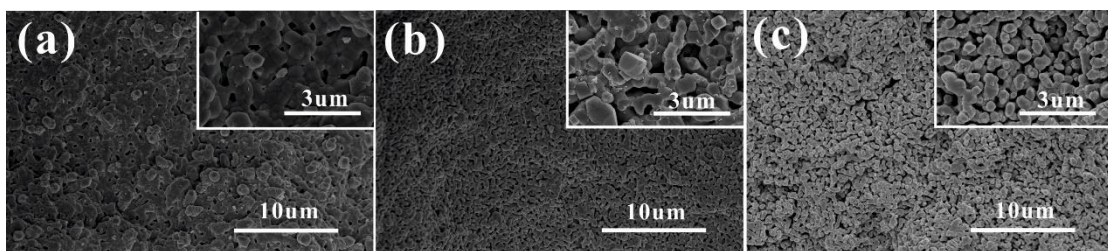


Figure S3. SEM images of (a) H/G-6/0.3% (b) HA-6-D (c) HA-6-CS.

Surgical Procedures

The surgical operation was performed under general anesthesia by abdominal injection of 10 % chloral hydrate (0.2 ml/100 g). Each rat was immobilized in a lateral position and its hind leg was shaved and swabbed twice with iodophor. Afterward, an outer thigh lateral approach with a 25 mm incision was performed and the deep fascia was divided. By blunt dissection of the interval of the quadriceps muscles, the femur was exposed and the periosteum was stripped carefully. The segmental defects were stabilized with the RatFix[®] (RISystem AG, Davos, Switzerland) osteosynthesis system.¹ A plate was first fixed by 4 screws at the mid-shaft femur where a 6 mm critical-size segmental diaphyseal bone defect was then created by fretsaw (Figure S1). According to the group, it is planned to implant the materials prepared in advance in the bone defect. The wound was irrigated and closed carefully. Following the surgeries, 400,000 units of penicillin were intraperitoneal injection for 3 days to prevent infection. After the operation, the animals were housed in ventilated rooms and allowed for full weight-bearing activities.

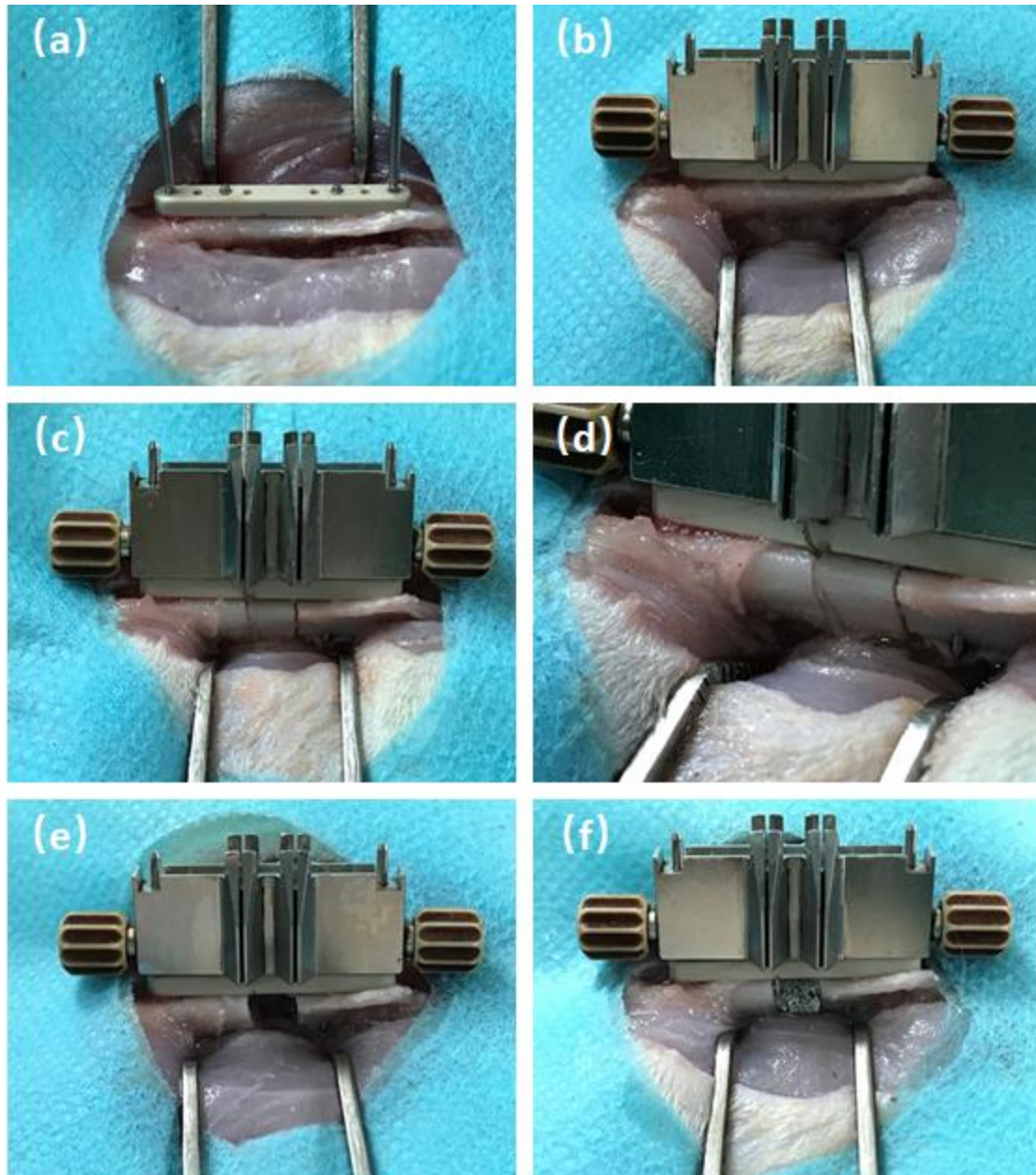


Figure S4. Establishment of a rat femoral diaphyseal defect model with RatFix[®] osteosynthesis system. (a) Fully expose the femur, place the RISystem[®] plate, and fix it with screws; (b) Installation of osteotomy guide; (c), (d) Using wire saw to osteotomy; (e) Osteotomy completed; (f) Fill the material into the defect.

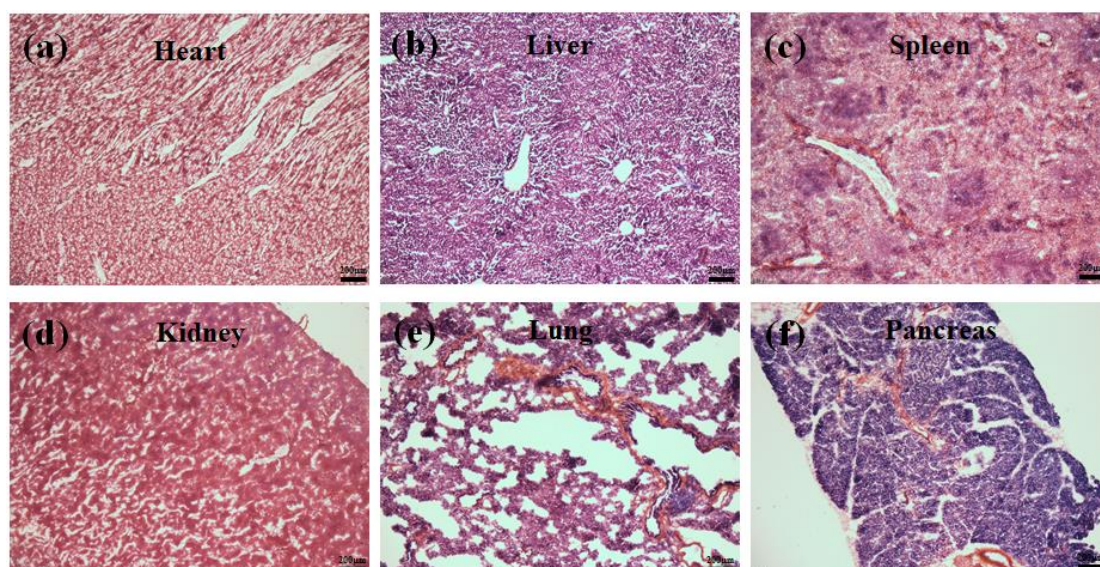


Figure S5. HE staining of rat heart, liver, spleen, kidney, lung and pancreas after implantation of HA/rGO-6/0.3 material for half an year. No obvious abnormalities in all observed organizations.

Table S1. Primer pairs used in real-time PCR analysis (F = forward, R = reverse).

Gene		Primers
β -actin	F	GTGACGTTGACATCCGTAAAGA
	R	GTAACAGTCCGCCTAGAAGCAC
ALP	F	TCGGGACTGGTACTCGGATAAC
	R	GTTCAGTGCGGTTCCAGACATAG
BMP-2	F	CGAATTTGAGTTGAGGCTGCTC
	R	GCCGTTTTCCCACTCATCTCT
OCN	F	TTTCTGCTCACTCTGCTGACC
	R	CAGCACAACCTCCTTCCTACCA

OPN	F	TTTCACTCCAATCGTCCCTACA
	R	CTGCCCTTTCCGTTGTTGTC
Runx2	F	AGCGGACGAGGCAAGAGTTT
	R	AGGCGGGACACCTACTCTCATA
COL-I	F	CATGTTCAACCCAGAGGAGCG
	R	GGTCCATGTAGGCTACGCTGTT

REFERENCE

- (1) Sebold, H. J.; Klenke, F. M.; Siegrist, M.; Albers, C. E.; Sebold, W.; Hofstetter, W. Inhibition of Endogenous Antagonists with an Engineered BMP-2 Variant Increases BMP-2 Efficacy in Rat Femoral Defect Healing. *Acta Biomater.* **2012**, *8*, 3816-3820.