

An osteogenic, antibacterial, and anti-inflammatory nanocomposite hydrogel platform to accelerate bone reconstruction

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Materials and methods

Materials

Collagen was extracted from the tail of Sprague-Dawley rats (5-6 weeks old, Nanjing Sikerui Biological Technology Co. Ltd.). The inorganic salts including ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), strontium nitrate [$\text{Sr}(\text{NO}_3)_2$], and calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$], were purchased from National Medicine Chemical Reagent Company China. Diammonium hydrogen phosphate [$(\text{NH}_4)_2\text{HPO}_4$] was obtained from Regal Biotechnology, China. Ammonia solution (NH_4OH , 28-30%) was purchased from VWR Chemicals, and Irgacure 2959 was acquired from J&K Scientific Ltd., China. The methacrylic anhydride (MA) was received from Sigma-Aldrich. Hydrochloric acid, acetic acid, and nitric acid were bought from Shijiazhuang Asa Technology Co., Ltd., China. Ultrapure deionized distilled water (ddH_2O) was used in all experiments. Alpha minimum essential medium (α MEM), phosphate buffer saline (PBS), fetal bovine serum (FBS), antibiotics (penicillin/streptomycin), and supplementary cell culture reagents were purchased from Life Technologies and HyClone™.

Synthesis of strontium/iron co-substituted hydroxyapatite nanomaterials

The strontium and/or iron substituted hydroxyapatite (Sr:HAp, Fe:HAp, and Sr/Fe:HAp) nanomaterials were synthesized following the early reported protocol ^{1,2} with some modifications via hydrothermal treatment, using calcium nitrate tetrahydrate, strontium nitrate, ferric chloride hexahydrate, diammonium hydrogen phosphate, and ammonia solution. Initially, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ salt was dissolved in deionized distilled water together with the desired concentrations of $\text{Sr}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and/or $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. 1.0 M cationic mixture was gently added to the 0.6 M of ammonium hydroxide (HN_4OH , pH ~9) solution. Thereafter, to enhance the bioactivity, improve organic-inorganic phase compatibility, and increase drug loading capacity, we added 0.05 wt% of collagen to the mixture, which was then stirred at room temperature for 24 hours. The final product was washed with deionized distilled water and freeze-dried for 72 h and kept the nanomaterials at $-20\text{ }^\circ\text{C}$ till further use.

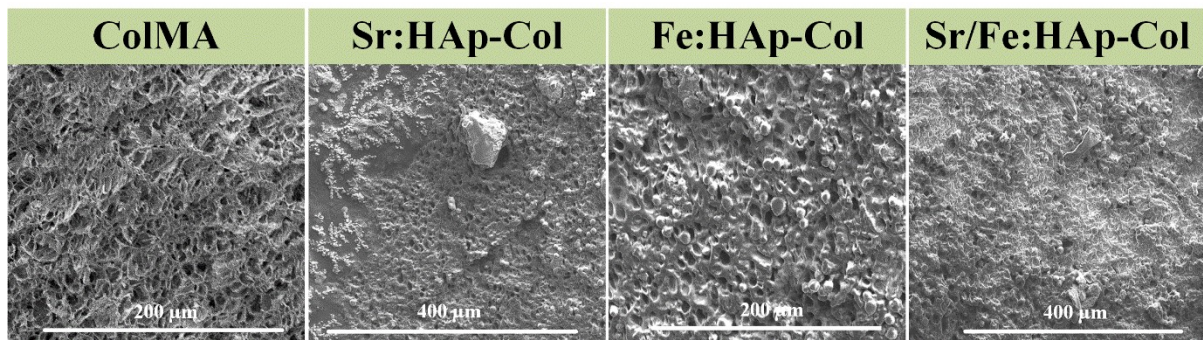


Figure S1. Cross-sectional SEM images of ColMA and nanocomposite hydrogels, showing the even distribution of nanocomposites within the hydrogel matrix. All the samples have rough and semi-porous network structures.

Table S1. Chemical reagents were used for the preparation of 1 X SBF/L, ion concentration of human blood plasma, and SBF.

Chemical	Quantity	Ion concentration	Blood plasma (mM)	SBF (mM)
NaCl	6.5456 g	Na ⁺	142	142
NaHCO ₃	2.2682 g	K ⁺	5	5
KCl	0.373 g	Mg ²⁺	1.5	1.5
Na ₂ HPO ₄	0.1419 g	Ca ²⁺	2.5	2.5
MgCl ₂ .6H ₂ O	0.3049 g	HPO ₄ ²⁻	1	1
1 M HCl	9 mL	HCO ₃ ⁻	27	4.2
CaCl ₂	0.3675 g	Cl ⁻	103	147.8
Na ₂ SO ₄	0.071 g	SO ₄ ²⁻	0.5	0.5
Tris {(CH ₂ OH) ₃ CNH ₂ }	6.057 g	Buffering agent	-	Tris

Table S2. The primer sequences data for qPCR analysis.

Target	Primer sequence	Length	Product size (bp)
RUNX2	F-5'-CATCCATCCACTCCACCACG-3'	20nt	172
NM_001271631.1	R-5'-AAAGGGCCCAGTTCTGAAGC-3'	20nt	
OPN	F-5'- TCTGATGAGACCGTCACTGC -3'	21nt	170
NM_001204203.1	R-5'- AGGTCCTCATCTGTGGCATC -3'	21nt	
OCN	F-5'- GACCCTCTCTCTGCTCACTC-3'	20nt	123
NM_001032298.3	R-5'- ACCTTATTGCCCTCCTGCTT-3'	20nt	
GAPDH	F-5'-GAAGGTGGTGAAGCAGGCATC-3'	21nt	109
NM_001289726.1	R-5'-CGAAGGTGGAAGAGTGGGAGTTG-3'	23nt	

Table S3. DLS average particle cluster size of as-synthesized nanomaterials and drug-laden nanomaterials.

Sample	Average Particle cluster size (nm)	Polydispersity index
Sr:HAp	400 ± 25	0.234 ± 0.015
Fe:HAp	540 ± 17	0.420 ± 0.01
Sr/Fe:HAp	362 ± 26	0.289 ± 0.009
D-Sr:HAp	315 ± 21	0.295 ± 0.01
D-Fe:HAp	200 ± 25	0.149 ± 0.008
D-Sr/Fe:HAp	297 ± 20	0.217 ± 0.011

Table S4. The rat blood serum ions data was measured via ICP-AES after treatment with Sr/Fe:HAp-Col at different times.

Elements (mg/dl)	Time		
	0 week	3 weeks	6 weeks
Ca	8.923±0.642	10.382±0.524	9.853±0.705
P	4.813±0.370	6.481±0.413	5.914±0.392
Fe	0.311±0.039	0.379±0.051	0.342±0.041
Sr	ND*	0.117±0.038	0.085±0.021

ND*: not detectable

Reference

- 1 I. Ullah, W. Li, S. Lei, Y. Zhang, W. Zhang, U. Farooq, S. Ullah, M. W. Ullah and X. Zhang, *Ceram. Int.*, 2018, **44**, 21338–21348.
- 2 I. Ullah, Z. Hussain, Y. Zhang, X. Liu, S. Ullah, Y. Zhang, P. Zheng, T. Gao, Y. Liu, Z. Zhang, Y. Cao, Z. Wang, M. Mansoorianfar, M. Xu and R. Pei, *Appl. Mater. Today*, 2022, **28**, 101532.