Supplementary materials

1.BMMSCs preparation and identification

The BMMSCs were prepared as previously described [1,2]. Briefly, BMMSCs were obtained from the long bone shafts of the newborn rabbits after the muscles and tissues were trimmed. Bone marrow was flushed and centrifuged on a 1.073 g/ml Percoll density gradient (EPPendorf Ltd., Shanghai., China). Subsequently, the cells were washed with PBS (HYclone Biological Technology, Ltd., USA), seeded into 25-cm^2 cell culture flasks, and cultivated in L-DMEM (Sigma) supplemented with 10% FBS (Sigma) and 20 mg penicillin-streptomycin/ml (Sigma) in a humidified 5% CO_2 atmosphere at 37°C. The medium was changed every 3 days. When the cells became subconfluent, the cells were released from the culture substratum using trypsin/EDTA (0.25% trypsin and 0.02% EDTA) (Sigma). The cell surface molecules, CD44 and CD34, were analyzed on 3 cultures by flow cytometry (FACSCalibur; Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

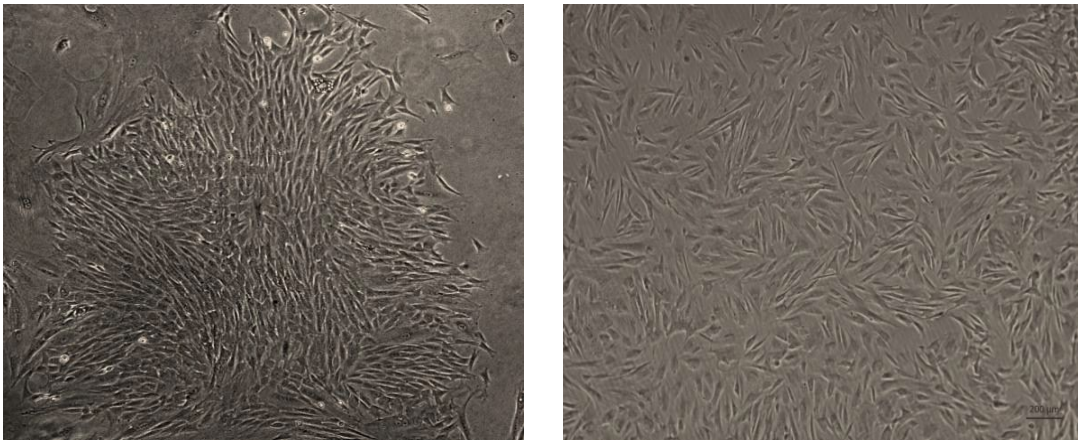
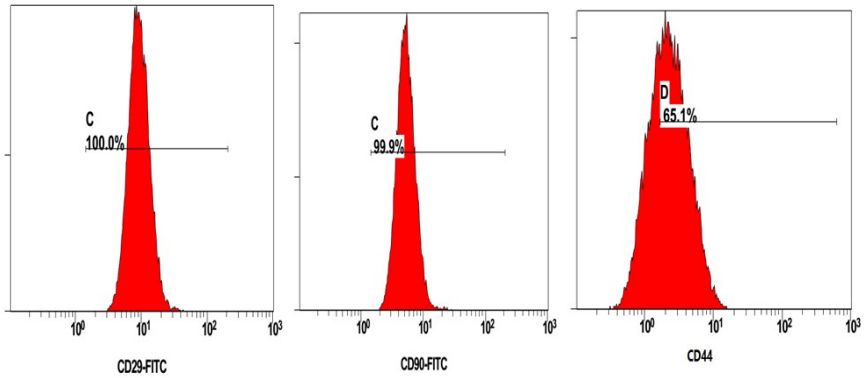


Figure 1. Primary cell at seventh day (left) and the third passage BMMSCs (right).



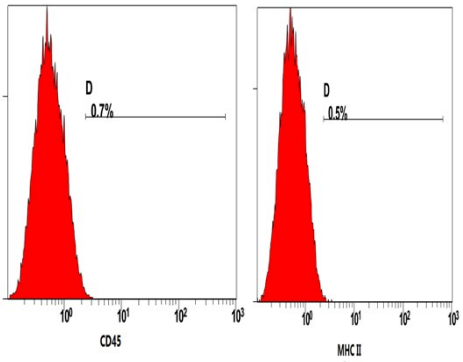


Figure 2. Identification results of the third passage BMMSCs. BMMSC Surface marker antigen: CD29、CD44 and CD90 were positive. While white blood cell surface marker antigen: CD45 and MHC II were negative.

2. The optimal concentration of rhEPO on cell culture.

Prepared the DMEM medium containing different concentration of rhEPO including 0 U/ml,5 U/ml,10 U/ml, 20 U/ml, 30 U/ml and 50 U/ml, and were used for BMMSCs culture. MTT was used to test the cells viability at 3,5,7,10,14 day (results as the figure 3 shows).

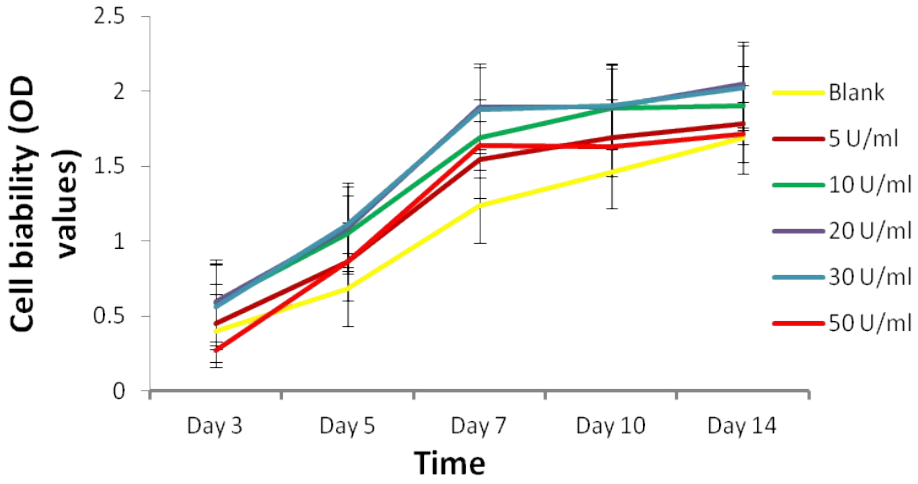


Figure 3. BMMSCs proliferation curve with different rhEPO concentration.

According to the figure3, the 20U/ml and 30U/ml of rhEPO achieved better OD valueds than other concentration. So, we chose 20U/ml as our optimal minimum concentration.

3. Identification of HA and Li-HA

Fig.4a indicated the FTIR spectra of HA and **Fig.4b** indicated the FTIR spectra of 1.5% Li-HA. The two samples in **Fig.4a,b** demonstrated similar characteristic peaks. It revealed an important information that low-content lithium had no effect on the FTIR spectra of scaffolds. In addition, the XRD data of the LiHA particles before sintering (**Fig.4c**) and after sintering (**Fig.4d**) are shown. The diffraction peaks of LiHA were nearly same as HA diffraction peaks. In addition, because of the small size of lithium and its doping in small quantity, the XRD pattern of LiHA could match HA standard card (JCPDS No. 09-0432), which explained that the incorporation of low-dose Li into HA did not change the crystal structure of HA. XPS of HA (**Fig.4e**) and 1.5%Li-HA (**Fig.4f**) was show in **Fig.4** too, we found that the HA and 1.5%Li-HA had similar element diffraction peak, and the **Fig.4f** presented a small typical Li diffraction peak.

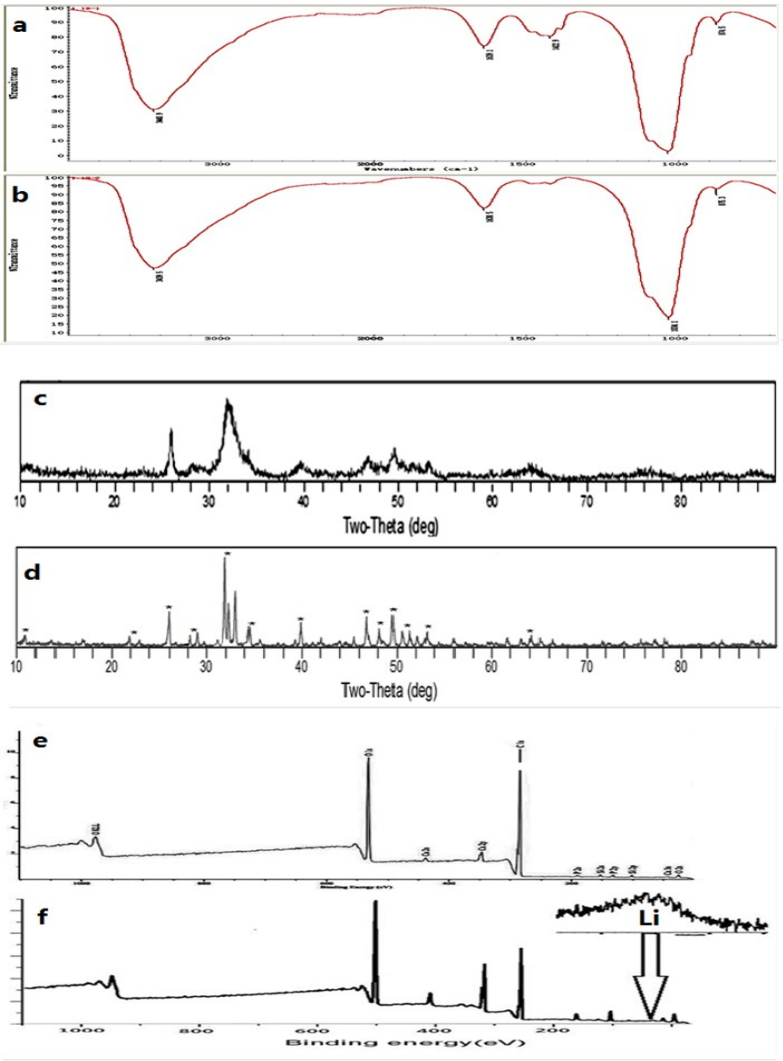


Figure. 4. FTIR, XRD and XPS.

4. The optimal concentration of Li in nHA

In our primary experiment, we have prepared different concentration of Li-nHA including 0.5%Li-nHA, 1.0%Li-nHA, 1.5%Li-nHA, 3%Li-nHA and studied their Physical and chemical properties and biological activities. The results were presented below.

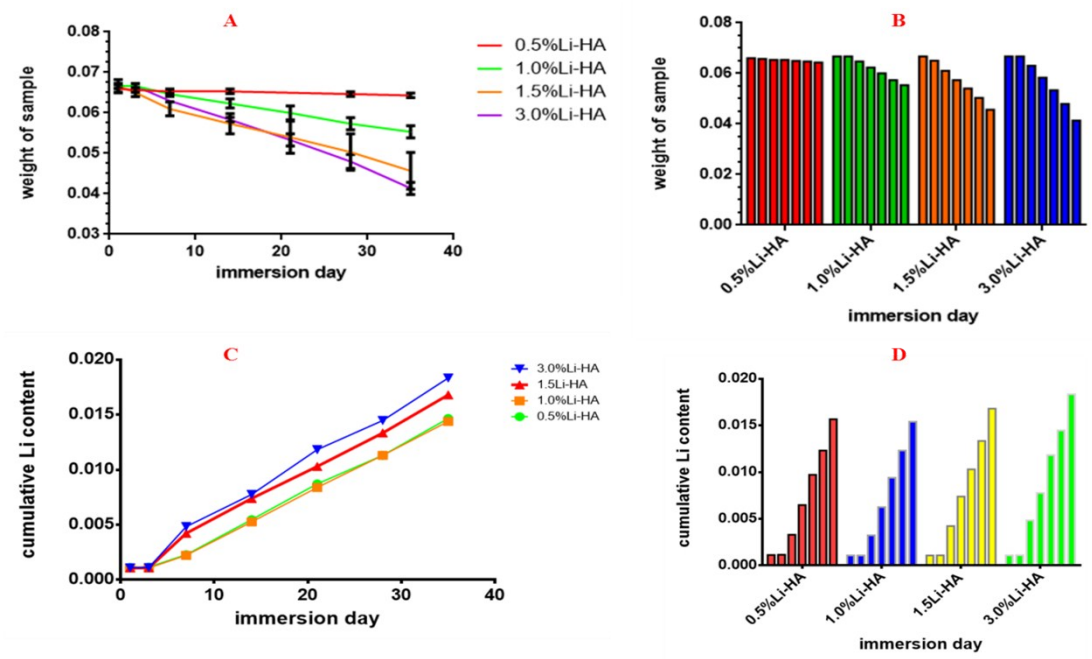


Figure 5. Solution-mediated degradation of different concentration of Li-nHA. A,B: weight lose curve and cartogram; C,D: Li^+ release curve and cartogram.

According to the figure 5, we found that the degradation rate of 1.5% and 3% were higher than the 0.5% and 1%. In order to establish a continuous release system and obtained effective concentration of Li, we think the 1.5% or 3% may be more proper.

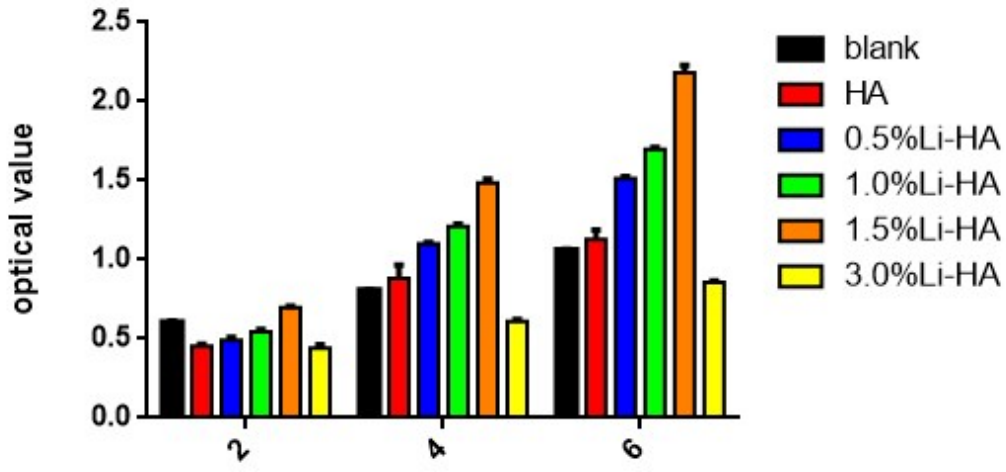


Figure 6. Different concentration of Li-nHA cultured with BMMSCs.

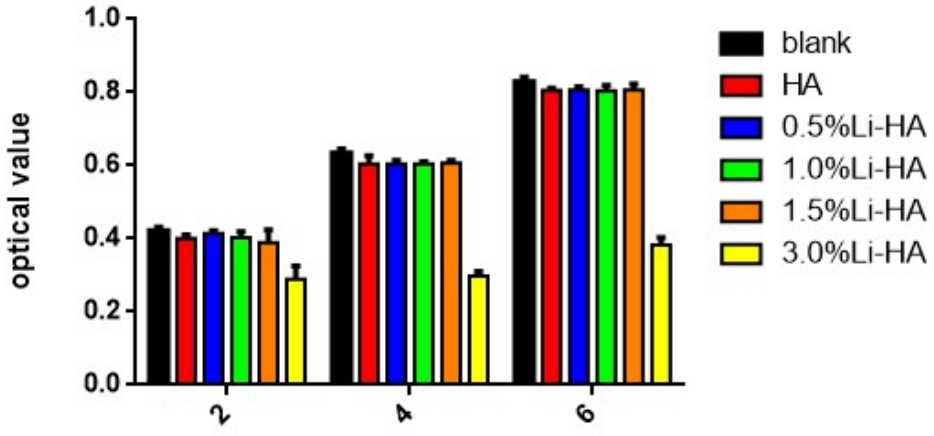


Figure 7. Different concentration of Li-nHA cultured with HUVEC.

From the figure 6 and figure 7, 1.5% Li-nHA was most active on BMMSCs proliferation, while 3% Li-nHA showed the inhibition cell proliferation. When cultured with HUVEC, 0.5%-1.5% showed similar cell viability while 3% Li-nHA showed the inhibition the HUVEC proliferation.

In conclusion, 1.5% Li-nHA was regarded as the optimal concentration in this study.

5. GMs/rhEPO Release study and cytotoxicity experiment

50 mg GMs/rhEPOs were placed in a dialysis bag, which was hung in 10 mL PBS containing 0.2 g/L emulsion and 0.2 g/L sodium azide. The PBS solution was put in a water bath containing a rotating plate (50 rpm) at 37 ℃. Collect 50 uL PBS solution at 12 h, 24 h, 3 days, 7 days, 14 days, 21 days, 28 days (added 50 uL new PBS after collecting the solution) and test the concentration of rhEPO with the rhEPO ELISA kit. At last, the cumulative percentage was calculated.

To explore the cytotoxicity, study the effect of GMs/rhEPO delivery system on proliferation of BMMSCs. Briefly, sterilized GMs/rhEPO and GMs were immersed in culture mediums for 24 h at 37 °C and then transfer to 24 well-plate. After that, 500 μL DMEM growth medium suspensions of MG63 at 5 × 104 cells per ml were seeded onto the corresponding wells each sample and cultured for 3, 5, 7 days. The cell morphology was observed by light microscope (BX41, Olympus, Japan). At the specified time, 20 μl/well of MTT solution (5 mg/ml in phosphate buffered saline) was added and then the plate was incubated at 37 °C to form formazan crystals.

After 4 h, the MTT solution was removed and 200 µl of dimethyl sulfoxide (DMSO) was added into each well to dissolve the dark blue formazan crystals. Then the optical density (OD) at 492 nm of each well was measured by a microplate reader (Model550, Bio Rad Corp. USA).

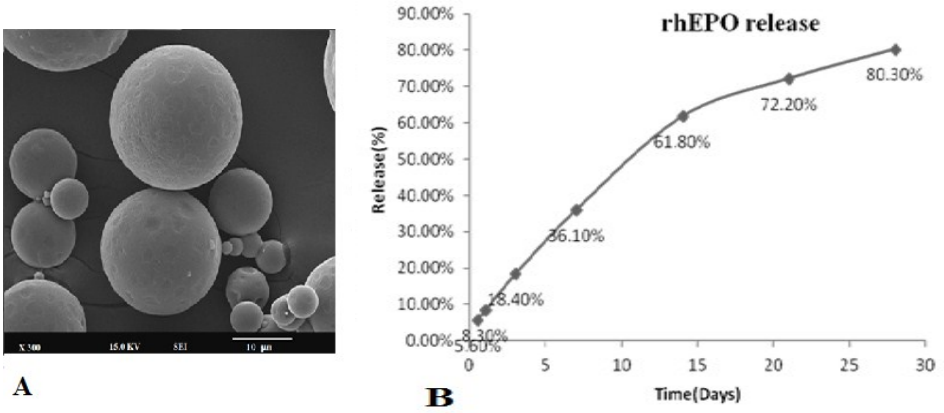


Figure 8. rhEPO Release curve from the GMs/rhEPO system.

GMs/rhEPO had a release pattern consisting of an initial burst in the first 14 days, followed by a sustained release that was slowly diminishing in time. At the 28th day, the releasing dose was 80.3 %. The average total rhEPO loaded content was 18 U/mg GMs.

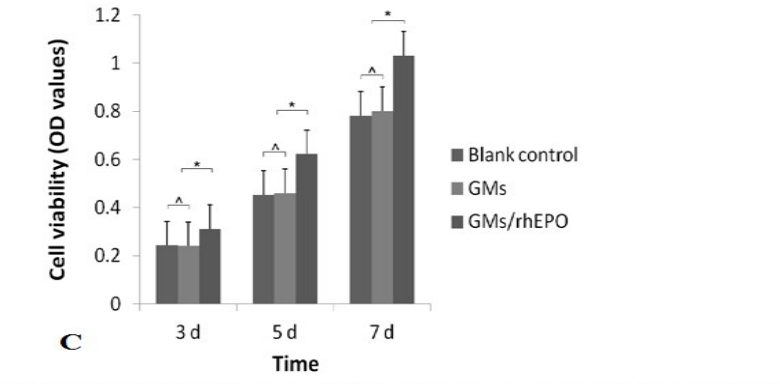


Figure.9 C: Cell viability of BMSCs cultured with Blank control, GMs and GMs/rhEPO at day3, day 5 and day 7. ^means $p>0.05$ between the two groups; * means $p<0.05$ when GMs/rhEPO group compared with other two groups. D-F: Light microscope shows MG63 cultured with GMs/rhEPO. D: 3 days; E:5 days; F:7 days.

As Fig.9D-F showed, cells kept good cell morphology and their number was rising as time goes on. In addition, cells seemed to grow around the GMs/rhEPO. Fig.9C presented the cell viability and it indicated that GMs groups were without significant difference when compared with Blank control groups ($p>0.05$), while the GMs/rhEPO groups showed better proliferation effect that the other two groups ($p<0.05$).

6. Degradation and mechanical properties

Methods: The solution-mediated degradation properties of porous gelatin/Li-nHA/GMs/rhEPO scaffolds were evaluated in simulated body fluid (SBF) which was used as the release medium. Porous gelatin/Li-nHA/GMs/rhEPO scaffolds and Li-nHA scaffolds were placed in clean vial bottles respectively (n = 4, per group), and completely immersed in 4 ml of SBF solution. The bottles were then sealed and put into a water-bathing constant temperature vibrator with a temperature at 37 °C. The release medium (SBF) was refreshed every 3 days. An obsolete release medium was gathered for measuring rhEPO, ionized calcium/lithium/phosphate concentrations at the 3, 6, 9, 12,15,18,21,24,27 and 30 day. The gathered supernatants were diluted by distilled water and the concentrations of rhEPO was measured using elisa test kits (ab30545), the ion Ca2+, Li+ and phosphate were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, IRIS,Thermo Elemental, USA). The cumulative release amount of Ca^{2+}, Li+and phosphate were calculated at the predetermined times as mentioned above.

The compressive strengths of the dried scaffolds were measured by an universal testing machine (Model 4881, Instron, Norwood, MA) at a crosshead speed of 0.1 mm/min. For each group, five samples were measured, and then the mean value and standard deviation were determined.

Results: The scaffolds had porosity of 72.8%±4.6% according to our detection. Every scaffold loaded 5±0.6mg GMs, and containing of 90.6 U±8.4U rhEPO. After the scaffolds were surface modified with gelatin and composited with GMs/rhEPO, their degradation and mechanical properties had some difference but didn't change too much. Briefly, most of rhEPO were released at the first 30 days (**Fig.10a**) and there was more weight lose in Li-nHA/GMs/rhEPO scaffolds (**Fig.10b**). Besides, the gelatin modified scaffolds presented similar degradation rate and Li+, Ca2+, PO43- ion released compared with Li-nHA (**Fig.10c,d,e**).

The maximum compressive of the two kinds of scaffold can come to 3 MPa, which can met the strength requirements of cancellous bone (2–12 MPa). The Li-nHA/GMs/rhEPO scaffolds didn't significantly change the compressive strengths when compared with Li-nHA. (**Fig.10f**).

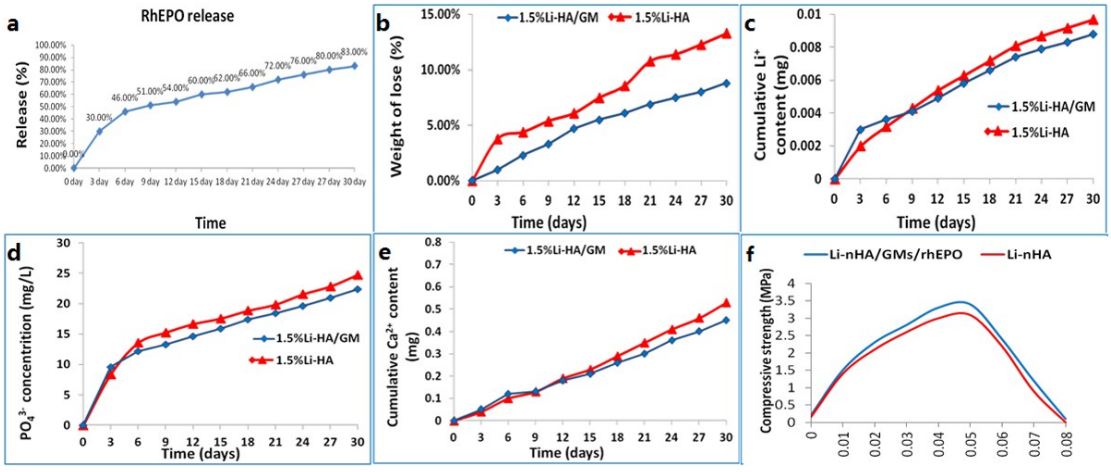


Figure 10. The release curve, degradation and mechanical cartograms

7. Established the osteonecrosis of femoral head model in rabbits

Fig.11a was the MRI picture of ON and it showed that there were a large area of low-signal intensity in bilateral femoral head, which suggested the ON was induced in this rabbit. **Fig.11b** was the HE staining of the section. We can find that the field of

view was covered with empty lacuna and the empty lacunae rate was come to 76.4%□6.8% (n=4). Consequently, the osteonecrosis of femoral head models were well established in this study.

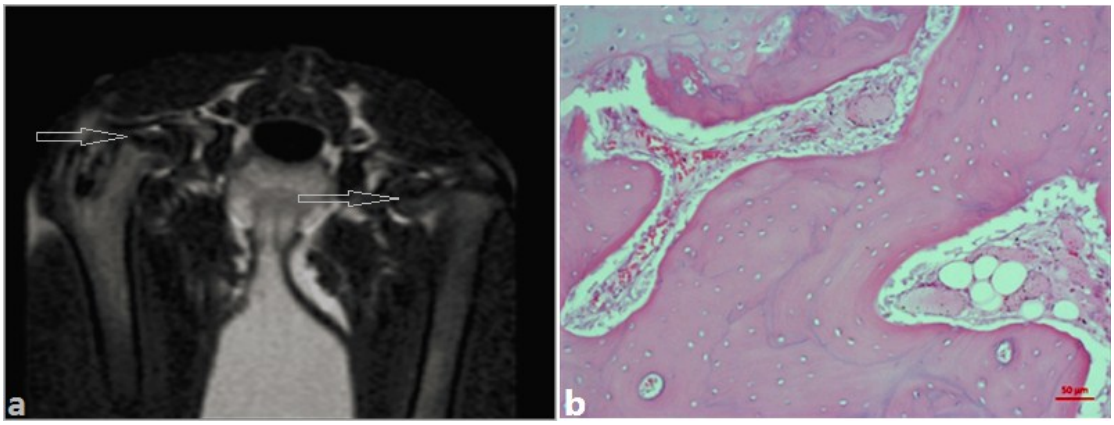


Figure.11 MRI and HE showed the established GIONF model.

8. The scaffold stereogram

The shape of scaffolds used in vitro studies and in vivo studies were presented in Fig.12.

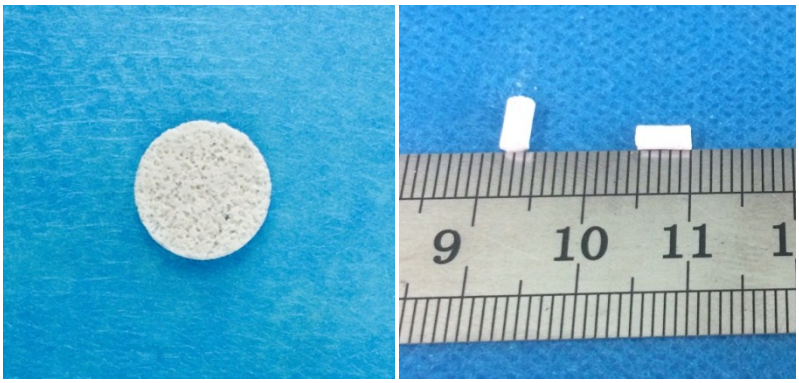


Figure. 12 The 1.5%Li-nHA/GMs/rhEPO scaffold stereogram. The right picture showing the scaffold with 1cm in diameter and imm in thickness, which was used for cell culture in vitro. The left picture showing the scaffold with 3mm in diameter and 5mm in height, which was used for in vivo study.

9. Operation

Rabbits were tranquilized with acetylpromazine maleate (1 mg/kg) intramuscularly followed by anesthesia with Ethrane (enflurane), nitrous oxide, and oxygen. The rabbits were exposed the right hip beforehand and anterolateral approach via an aseptic technique was performed. The major vessels supplying the femoral head were preserved in this approach. The joint capsule was incised without dislocating the hip to expose the femoral head and neck. A 3-mm-diameter drill was used to

create a channel of bone defect from femoral neck and femoral head junction to osteonecrosis regions. Bone was removed until the subchondral bone and cartilage adjacent to the defect was 2 mm thick. After that, the defects were filled with autologous bone, nHA, Li-nHA, Li-nHA/GMs/rhEPO and nothing respectively (Fig.13). After the grafting procedures, the cartilage flap was replaced flush with the surrounding articular surface and held in place by two 6-0 sutures and by closing the capsule with two 6-0 sutures. Then the hip abductor was repaired. The autogenous cancellous bone was taken from the ilium through the same incision. After the operation, the animals were permitted to recover from general anesthesia and were allowed free activity. To prevent the wound infection, all animals were intramuscularly injected with gentamicin (4 mg/kg) one preoperative dose and two postoperative doses.

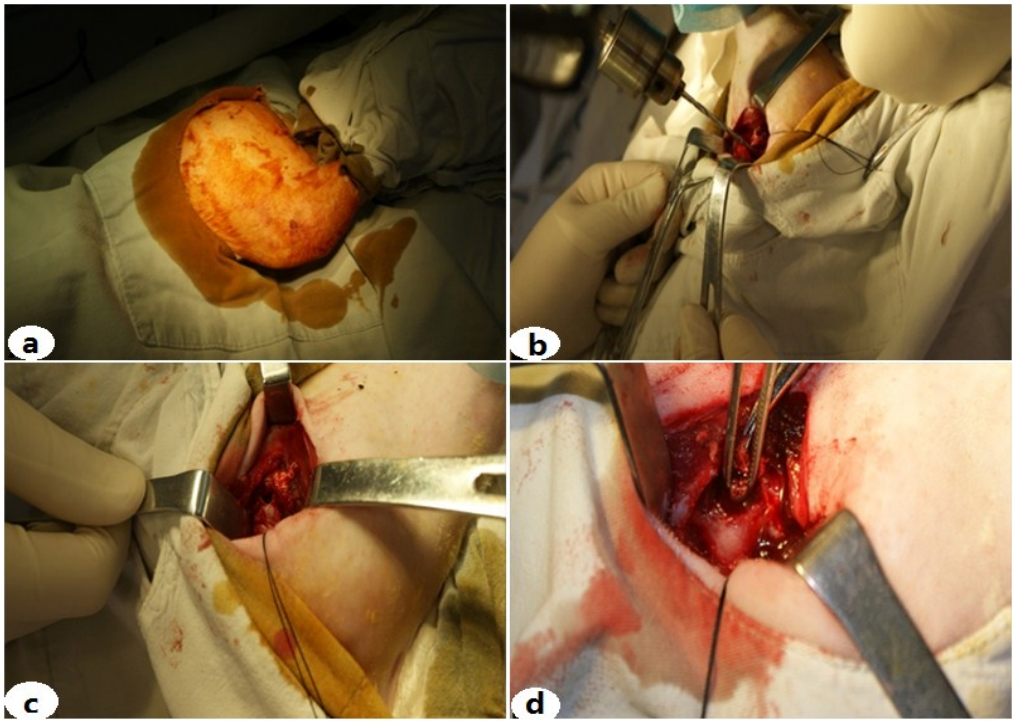


Figure.13 Operation pictures. a: exposure to operation area; b: drilling and core decompression; c: a defect channel was made for bone grafting; d: the scaffold was implanted into the defect.

Reference

[1]. Panepucci RA, Siufi JLC, Silva WA Jr, Proto-Siquiera R, Neder L, Orellana M, Rocha V, Covas DT and Zago MA: Comparison of gene expression of umbilical cord vein

and bone marrow-derived mesenchymal stem cells. Stem Cells. 2004; 22:1263–1278.

[2]. Yu Z, Fan L, Li J, Ge Z, Dang X, Wang K. Lithium chloride attenuates the abnormal osteogenic/adipogenic differentiation of bone marrow-derived mesenchymal stem cells obtained from rats with steroid-related osteonecrosis by activating the β-catenin pathway. Int J Mol Med. 2015;36(5):1264-72.