(CT) analysis was performed to determine the microstructure and porosity of the materials. The rat bone marrow-derived mesenchymal stem cells (MSCs) were seeded onto the scaffolds, the cell proliferation was determined using Alamar Blue cell viability assay. Surface of the materials were scanned by scanning electron microscope (SEM) before and after cell seeding. Segmental bone defects (3 mm*3 mm*2 mm) were made on the right femora of Sprague-Dawley rats under anesthesia and filled with β-TCP (n = 6), natural coral (n = 6), hydroxyapatite nanoparticle-coated coral (n = 6), or hydrothermal treated coral (n = 6), respectively. Samples were harvested for micro-CT and then decalcified for histology analysis after 8 weeks.
Results: Results of Micro-CT showed all the scaffolds exhibited a homogenous structure with interconnected open pores. The internal porosity of the four materials were 40–60%. Cell proliferation was significantly increased when MSCs were seeded onto the surface of hydrothermal treated coral scaffolds compared to other materials after 3 days. Results of SEM showed that a large number of MSCs attached well onto the surface of hydrothermal treated coral rather than other materials. In the animal study, there was no significant difference in the volume of mineralized tissue within the defect area in the four groups. The histological results showed significantly more bone formation in the femoral defect region in the group transplanted with hydrothermal treated coral scaffolds, indicating that bone formation was enhanced by the hydrothermal treated coral scaffolds.
Conclusion: Hydrothermal converted coral scaffolds showed superior repair effect in rat critical-sized femoral defect models, when compared with either β-TCP, natural coral, or hydroxyapatite nanopartical-coated coral. This may be a new biomaterial to be explored as bone substitute.
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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF STRONTIUM/MAGNESIUM-CO-SUBSTITUTED HYDROXYAPATITE

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The present study aims to investigate the contribution of two biologically important cations, Mg^{2+} and Sr^{2+}, when co-substituted into the structure of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA). The substituted samples were synthesized by a hydrothermal method that involved the addition of Mg^{2+} and Sr^{2+} containing precursors to partially replace Ca^{2+} in the apatite structure. Four co-substituted HA samples with different concentrations of Mg^{2+} and Sr^{2+} ((Mg+Sr)/(Mg+Sr+Ca) = 30%) were investigated, and they were compared with pure HA. Experimental results showed that only a limited amount of Mg (Mg/(Mg+Ca+Sr) < 14%) could successfully substitute for Ca in HA. In addition, Mg substitution resulted in reduced crystallinity, thermal stability, and lattice parameters of HA. In contrast, Sr could fully substitute for Ca. Furthermore, the addition of Sr increased the lattice parameters of HA. Here, we obtained the cation leach liquor (CLL) by immersing the prepared samples in a culture medium for cell experiments. The *in vitro* study showed that 10Mg20Sr promoted better MG63 cell attachment, proliferation, and differentiation than HA. Thus, the presence of an appropriate proportion of Mg and Sr could play a significant role in the increased biocompatibility of HA.
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3D PRINTING OF OSTEOPROMOTIVE POLY(TRIMETHYLENE CARBONATE)-HYDROXYAPATITE IMPLANTS FOR BONE REGENERATION.

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Introduction: Manufacturing polymer scaffolds with controlled internal structure and degradation using stereolithography, and with incorporation of osteoinductive ceramic has seldom been achieved. Poly(trimethylene carbonate) (PTMC) based resin loaded with nano-hydroxyapatite (nHA) were recently produced to create implants using stereolithography (SLA)[1]. In this study, 3D macroporous scaffolds were fabricated and their osteopromotive effect was characterized under *in vitro* and *in vivo* conditions.
Subjects and Methods: PTMC-methacrylate resin mixed with nHA at 0, 20 and 40% w/w were prepared and scaffolds with 500 μm pores were fabricated by SLA. Human bone marrow stromal cells (hMSCs) were seeded at 150 × 10^3 cells/scaffold and cultivated for 4 weeks in osteogenic media. At the end of the cultivation, cell proliferation and viability was assessed using DNA quantification and Live-and-Dead staining and collagen deposition was evaluated histologically using Safranin O Fast Green. Subsequently, *in vivo* experiment were conducted by creating 4 calvarial defects of 6 mm Ø on 8 rabbits (agreement 19A/2015) using Codman perforator device (DePuySynthes). After cleaning and washing, the defects were either left empty (control group) or PTMC and PTMC/nHA at 20 and 40% w/w scaffolds (Ø 6 mm × H 3.5 mm) were inserted in the cavities. Following 6 weeks of implantation, osseointegration was assessed by X-ray scan and by histology (Giemsa–Eosin staining).
Results: PTMC scaffolds without and with 20 and 40% of nHA were successfully fabricated using SLA (Figure 1A). *In vitro* study showed that hMSCs were able to proliferate similarly in all scaffolds (Figures 1B and 1C) and deposit collagen-rich matrix. Following implantation, microCT analyses revealed that the incorporation of 40% w/w of nHA in PTMC significantly increased the amount of bone formation in the porosity of the biomaterials (Figure 1, 6 weeks post-implantation), which was also confirmed histologically. Importantly, post-mortem injection of black India ink staining permitted to appreciate the intense vascularization occurring in the porous network of the scaffolds, which is a critical requirement for bone tissue engineering.

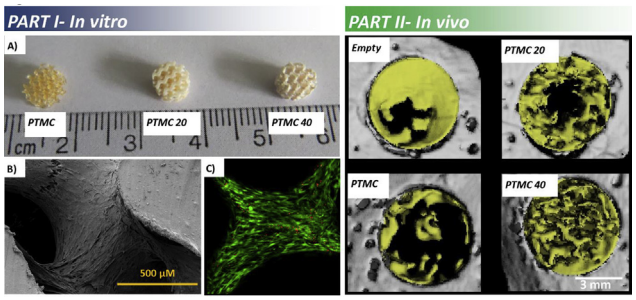

Figure 1 *In vitro and in vivo characterization of PTMC/nHA scaffolds* PART I-A) Macroscopic observation of the PTMC, PTMC 20 and PTMC 40% nHA scaffolds, and B) SEM and C) Live and Dead illustration of hBMSCs colonizing the scaffolds in vitro. PART II- MicroCT monitoring of neo-bone formation following 6 weeks of surgery for the empty defect (control group) compared to the different scaffolds (calvarial bone is coloured in grey and new bone tissue is coloured in yellow).

Discussion and Conclusion: For the first time, we reported the fabrication of PTMC/nHA-based SLA scaffolds for bone repair. We were able to endow PTMC biological activity by incorporating various amounts of nHA, allowing stimulation *in vivo* biomineralization.
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References
[1] Geven MA et al. Fabrication of patient specific composite orbital floor implants by stereolithography. Polymers for Advanced Technologies 2015;26:1433–8.
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THE EFFECTS OF FIBER DIAMETER OF ELECTROSPUN PLLA SCAFFOLDS ON ANNULUS FIBROUS-DERIVED STEM CELLS

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Background: Application of micro-/nanofibers as scaffold is a promising approach for annulus fibrous (AF) tissue engineering. However, it remains challenging because of heterogeneity of AF tissue in cellular, mechanical, and biochemical aspects. Previous studies have shown that the outer region of AF is rich in type I collagen and composed of larger fibers, while the inner region is rich in type II collagen and composed of smaller fibers. Therefore, mimicking the size of collagen fibers may be a feasible way for facilitating AF tissue reconstruction. In this study, we applied electrospinning technology to fabricate fibrous poly(L-lactic-acid) (PLLA) scaffolds of different fiber sizes and studied the effect of fiber diameter on the differentiation of annulus fibrous-derived stem cells (AFSCs).
Methods: PLLA fibrous scaffolds were fabricated using electrospinning technique and characterized through SEM, mechanical test and water contact angle measurement. After AFSCs were cultured on the scaffolds for 7 days, their morphology was examined using SEM and cytoskeleton staining. Expression of genes (Col-I, Col-II, Aggrecan) was quantified by RT-qPCR and the related proteins were analyzed by ELISA.
Results: PLLA fibrous scaffolds with three different fiber sizes were fabricated, of which the fiber diameter ranged from 3 μm to 8 μm. AFSCs proliferate well on all

the scaffolds. The cells were round or near round on the scaffolds of small fiber diameter, while became spindled on the scaffolds of large fiber diameter. After 7 days, the expression of collagen-II and aggrecan genes decreased with the fiber diameter, whereas the gene expression of collagen-I increased. The related proteins level was similar to the gene expression.
Discussion and Conclusion: The scaffolds of different fiber diameters may mimic the outer, middle, and inner fibrous structures of native AF tissue. The gene expression and protein production of AFSCs on the scaffolds with different fiber diameters was similar to that of AF tissue. Specifically, the gene expression of collagen-II and aggrecan genes decreased from small diameter scaffold to large diameter scaffold, whereas the gene expression of collagen-I was the opposite case. Therefore, this study provides solid basis for the use of biomimetic scaffolds which mimic different AF zones for AF tissue engineering.
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SYNERGISTIC EFFECT OF DECELLULARIZED ANNULUS FIBROUS MATRIX AND SUBSTRATE ELASTICITY ON ANNULUS FIBROUS-DERIVED STEM CELLS

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Background: Due to the similarity of biochemical composition and microstructure between decellularized matrix (DCM) and native extracellular matrix (ECM), DCM have been widely used in tissue engineering. Meanwhile, the effects of mechanical property (e.g. elasticity) of cell culture substrate on the proliferation and differentiation of cells have also been well documented. This study aims to explore the combined effect of decellularized annulus fibrous matrix (DAFM) from porcine and substrate elasticity on the behaviors of rabbit annulus fibrous-derived stem cells (AFSCs).
Methods: DAFM was coated onto polyacrylamide gels (PAG) with elastic modulus of 2.6 kPa (soft), 10.6 kPa (middle), and 34.9 kPa (rigid), respectively. Collagen-I coated PAGs were used as control. The cell proliferation, morphology, gene expression and protein production of AFSCs were examined in both groups with different substrate elasticity.
Results: After 7 days of culture on both DAFM and collagen-I coated PAGs, AFSCs proliferated well. The cells on soft PAGs exhibited the least expression of collagen-I gene, yet the greatest expression of collagen-II and aggrecan genes. In contrast, the cells on rigid PAGs showed the greatest expression of collagen-I but the least of collagen-II and aggrecan. The gene expression of cells on middle PAGs was in between of those on soft and rigid PAGs. Expression of these genes in AFSCs cultured on DAFM-coated PAGs followed a similar substrate elasticity-dependent pattern. However, the responses of AFSCs to substrate elasticity appeared to be more prominent when they were cultured on DAFM-coated PAGs. The protein production of collagen-I, collagen-II and glycosaminoglycans (GAG) in each group was consistent with the specific gene expression of AFSCs.
Discussion and conclusion: In our pervious study, AFSCs would respond to the mechanical properties of substrate elasticity. Here, we combined the use of DAFM and scaffolds of gradient elasticity and found that the responses of AFSCs in gene expression and protein production appeared to be more prominent when they were cultured on DAFM-coated PAGs in every group with different substrate elasticity. These findings suggested that combined use of DAFM and scaffolds of gradient elasticity may represent a more efficient approach for AF tissue engineering.
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GENIPIN-CROSSLINKED DECELLULARIZED ANNULUS FIBROSUS MATRIX/CHITOSAN SCAFFOLDS FOR THE CULTURE OF ANNULUS FIBROUS-DERIVED STEM CELLS

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Background: While tissue engineering method has become an ideal approach for annulus fibrous (AF) regeneration recently, it remains challenging because of the heterogeneity of AF tissue. Decellularized extracelluar matrix (ECM) has been proposed as a novel tissue-specific biomaterial for tissue engineering, yet lacks sufficient mechanical strength. Chitosan, a linear polysaccharide which possesses characteristics of chondroitin sulfate and keratin sulfate, is similar to the glycosaminoglycans (GAGs) which are rich in native AF tissue. In this study, we used Genipin to crosslink decellularized AF matrix (DAFM) and chitosan (CS) to fabricate biomimetic scaffolds for AF tissue engineering. Importantly, the DAFM/CS samples have different elasticity to mimic the outer, middle and inner zones of AF.
Methods: Tensile tests, SEM and AFM were used to study the mechanical properties and surface characteristics of DAFM samples. After being cultured on the scaffolds, the proliferation of AF-derived stem cells (AFSCs) was examined by CCK8 tests. The morphology of AFSCs was checked by SEM and cytoskeleton staining. The genes expression (Col-I, Col-II, and Aggrecan) was measured using RT-qPCR. Cell traction forces (CTFs) of AFSCs were determined using CTF microscopy (CTFM).
Results: Biomimetic DAFM-CS composites were crosslinked using Genipin to fabricate scaffolds of various elasticity (47, 75, and 120 kPa, respectively). A highly porous and interconnected network structure was observed. After AFSCs were cultured on these scaffolds for 7 days, the gene expression and protein production of collagen-II and aggrecan decreased with the elasticity of scaffold, whereas the expression of collagen-I was exactly the opposite case. Similarly, the CTFs of AFSCs gradually decreased with the elasticity of scaffold. Cytoskeleton staining and SEM imaging showed that the morphology of cells was almost round on the scaffolds of low elasticity and spindle-like on the scaffolds of high elasticity.
Discussion and conclusion: DAFM/CS composite scaffolds with different elasticity were fabricated using Genipin-crosslinking. These scaffolds mimicked the outer, middle and inner zones of native AF tissue and markedly affected the differentiation of AFSCs. Importantly, the substrate elasticity-dependent changes (cell morphology, gene expression, and CTF) of AFSCs were similar to the cellular, mechanical and biochemical characteristics of cells from outer region to inner region of native AF tissue.
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PREPARATION AND PROPERTIES OF STRONTIUM DOPED NANO HYDROXYAPATITE

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Background: Osteoporosis (OP) is defined as a skeletal disorder that is characterized by a reduction of bone mass and deterioration of bone microarchitecture, with a consequent increase of bone fragility and decrease of bone strength that could induce increased risk of fracture. Hydroxyapatite (HA) is a widely used coating material due to its satisfactory biocompatibility and osteoconductivity. Strontium (Sr), which can increase the activity of osteoblasts while decrease it of osteoclasts, has been a research focus in osteoporosis.
Subjects and Methods: Pure nano hydroxyapatite (nHA) and a series of strontium-doped nano hydroxyapatites(Sr-nHAs)were prepared by our one-step method which mainly used the principle of homogeneous phase co-precipitation. The different Sr-nHAs were designed and prepared by doping Sr ions into nHA with an atomic ratio of Sr/(Ca+Sr)=1/20 (5%), 1/10 (10%), and 1/5 (20%), respectively. The properties of prepared nHA and Sr-nHAs were characterized by using FTIR, XRD, TEM and EDS.
Results: FTIR spectra showed that absorption bands of HA characteristic vibrations were observed in the four different HA material samples. Weak bands associated with carbonate (CO_3^{2-}) were also observed. Besides, with the increase of doped Sr, the intensity of absorption peak was decreased. As compared with the standard data, typical XRD diffraction peaks of HA were clearly identified in the prepared nHA and Sr-nHAs samples. The significant broadening of diffraction peaks indicated that the powders prepared in this study were nano-sized materials. Importantly, with the increase of doped Sr, special peak position shifting was observed. TEM images showed that the synthesized nanoparticles have a tiny rod-like feature, and the size of nanoparticles increased in accordance with the Sr-doping. Finally, EDS spectra clearly showed the presence of not only Ca, O and P but also Sr in all Sr-nHA samples.
Discussion and Conclusion: HA has been widely used in bone implants including the coating of prosthesis. However, for patients with osteoporosis, pure HA has little effects on promoting bone formation and suppressing bone resorption. As a result, the loose rate of pure HA coated implants is very high. On the other hand, strontium (Sr) can simultaneously promote bone formation and inhibit bone deterioration in osteoporosis. So we hope to improve implant coating by using Sr-doped HA, a novel biomaterial. In this study, both the pure nHA and Sr-nHAs were successfully synthesized by using our method and characterized. Importantly, on basis of this work, a series of Sr-nHA coated titanium (Ti) implants were prepared. Therefore, the Sr-nHAs synthesized in this study are expected to be further explored and optimized for biomedical applications in osteoporosis.
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