(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 $\times$ 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 $\times$ 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.

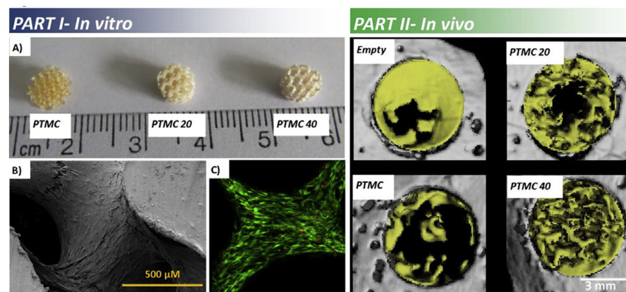

Figure 1 *In vitro and in vivo characterization of PTMC/nHA scaffolds* PART I-A) Macroscopic observation of the PTMC, PTMC 20 and PTMC 40% nHA scaffolds, and B) SEM and C) Live and Dead illustration of hBMSCs colonizing the scaffolds in vitro. PART II- MicroCT monitoring of neo-bone formation following 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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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