Editorial on the original article entitled "3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration" published in the *Biomaterials* on February 14, 2014

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Abstract: The paper entitled "3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration" published in the *Biomaterials* recently illuminated the way to make particular scaffolds with calcium phosphate (CaP) powder, phosphoric acid, type I collagen and Tween 80 in low temperature. After the optimal concentration of each component was determined, the scaffolds were evaluated in a critically sized murine femoral defect model and exhibited good material properties. We made some related introduction of materials applied in 3D printing for bone tissue engineering based on this article to demonstrate the current progress in this field of study.

Keywords: Three-dimensional (3D) print; bone tissue engineering; material; scaffolds

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It is known that the body itself cannot heal the large-scale bone defects although the osseous tissue has well self-healing abilities (1). To overcome this clinical obstacle, autografts and allografts are the two common treatment options. However, both the two operations have limitations including the amount of graft material, donor site morbidity, high risk of infection, chronic pain and lengthy rehabilitation (2).

Due to these complicated reasons, methods of synthesizing and/or regenerating bone to restore, maintain or improve its function *in vivo* have become hot research topics in bone tissue engineering (3). Materials and structures are the two crucial factors that could have significant influences on biocompatibility, mechanical strength and cell viability of scaffolds. Scaffolds made by appropriate materials in three-dimensional (3D) biocompatible structures can mimic the properties of extracellular matrix and provide a template for bone tissue formation *in vivo* through biochemical and mechanical interactions (1,3).

A paper entitled "3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration" published in the *Biomaterials* expounded a kind of new method of making scaffolds with 3D structure in low temperature by composite materials including calcium phosphates (CaPs), type I collagen, Tween 80 (a non-cytotoxic surfactant) and phosphoric acid (4). This study gave an interpretation of the production and identification of materials as well as the *in vivo* testing through a series of rigorous experiments.

To made the composite powder consisting of hydroxyapatite (HA) and α-tricalcium phosphate (α-TCP), the solution contained $Ca(NO_3)_3{\cdot}4H_2O$, $(NH_4)_2HPO_4$ and carbohydrazide inside was combusted at 500 ℃ and subsequently calcined at 1,300 ℃. The binder solution for 3D printing was composed of different concentration of type I collagen, phosphoric acid and Tween 80, which enhanced the mechanical strength of the materials without compromising the biocompatibility. The authors demonstrated some results of cell viability, maximum flexural stress and micro-CT to explain how they determine the optimal binder solution acidity and powder particle size.

The scaffold was made of CaP powder with a size ranging from 30 to 150 μm through a ZPrinter 450 under low temperature and selectively bound by the 8.75 wt% phosphoric acid solution containing 0.25 wt% Tween 80 and 1.5 wt% collagen which was delivered by a HP thermal inkjets. According to the results of the scanning electron micrographs, the 3D printed scaffolds confirmed pore sizes in the range of 20-50 μm with layer thickness of 89 μm. For the purpose of determining the functional performance of type I collagen, which is one of the key structure proteins of the bone extracellular matrix attributing to its assembling into fibers, some scaffolds were bound by the solution without collagen but were coated with a 0.5 wt% neutralized collagen gel that dried into a film on the surface.

The *in vivo* massive bone defects' healing was evaluated by a murine femoral defect model. A 2 mm osteotomy was created at the femoral mid-shaft in 13-15 weeks female mice and an allograft or a 3D printed scaffold [calcium phosphate scaffolds (CPS), CPS with collagen binder, CPS with collagen coated] was placed into the defect to heal for 9 weeks. X-rays was taken weekly to monitor the progress of bone healing and micro-CT was used to measure the mineralized volume, mineral density and mineral content. The biomechanical properties, especially torsion, were tested by using an EnduraTec TestBench instrument.

Although the maximum flexural strength, toughness and cell viability improved in both CPS with collagen binder and CPS with collagen coated in *in-vitro* studies, the result differed in *in-vivo* experiment. The scaffolds coated with collagen tended to facilitate less new bone formation and ingrowth as measured by the mineral content and scaffold engraftment despite the levels of new bone formation was similar between allografts and 3D printed scaffolds. Compared with 3D printed scaffolds, the allografts had the greatest net mineralized volume and higher maximum torque values otherwise the slower period of dissolving or resorbing. However, host-host unification was observed in none of the 3D printed scaffolds or allografts. Cause for this phenomenon could be the sufficient osteoconductive and insufficient osteoinductive of scaffolds, which resulted in bone formation into the engraftment with incomplete healing.

CaPs are common substitutes in bone tissue engineering due to they are osteoconductive and good mechanical strength. The most commonly processing method with this material is high temperature sintering (5,6) to achieve higher mechanical strength but less bioactivity as nearly all the bioactive substances cannot suffer the temperature as high as 1,200 ℃ or higher. An *in vitro* study showed that scaffolds made by biphasic calcium phosphate (BCP) containing HA as well as TCP in varying ratios were cytocompatible and enhanced the cell viability and the cell proliferation, as compared with pure TCP (6). To maintain the biological activity, dicalcium hydrogen phosphate and a bioactive glass were mixed with CaPs during the heat treatment, the reactions between these three components can generate the phases $CaNaPO_4$ and $CaSiO_3$ with bioactive potential of biodegradation (5). Human mesenchymal stem cells (hMSC) associated with sintered BCP particles induced osteoclastogenesis and osteogenesis after implanted in the paratibial muscles of nude mice after 4 weeks (7). On the other hand, low or normal temperature 3D printing provides the potential to create composite scaffolds with proteins, growth factors and collagen to attain the combinational therapies of inducing new bone formation as well as enhancing osteoconductive and osteoinductive characteristics (8).

As with CaPs, HA is another inorganic material widely used in almost all kinds of 3D printing like direct ink writing, laser-assisted bioprinting, selective laser sintering (SLS), selective laser melting (SLM) and robotic assisted deposition (8). A kind of water based binder solution with layer thickness ranging from 100 to 300 μm is considered as the optimal condition for making scaffolds and the bending strength ranging from 0.69 to 76.82 MPa based on diverse rapid prototyping (RP) techniques (9-12). Ceramic scaffolds made up of HA powder in 3D structure exhibited good cell viability as well as good proliferation behavior (13). In a previous study published in 2012, capillaries and vessel formation that accompany the homogeneous osteoconduction from central channels have been observed in 3D-printed HA blocks with the application of bone morphogenetic protein 2 (BMP-2) (14), which can be regarded as another successful example for combinational therapies. The attachment, proliferation and osteogenic differentiation as well as the expression of angiogenic factor of adipose derived stem cells were be systematically investigated while cultured with HA bioceramic scaffolds with nanosheet, nanorod and micro-nano-hybrid surface topographies (15).

Apart from inorganic materials, synthetic polymers such as polycaprolactone (PCL), poly lactic-coglycolic acid (PLGA), polylactic acid (PLA), polyethylene glycol (PEG), poly L-lactic acid (PLLA) and polypropylene (PP) are widely used in scaffold development (8) within orthopedics due to the highly biocompatible and degrades into harmless by-products metabolized in the tricarboxylic acid cycle of

these polymers (16). The preferred option of processing method is fused deposition model (FDM), another kind of RP technology, which allow complex shapes for scaffolds' fabrication directly from a computer aided design (CAD) file to accurately mimic the different void dimensions of cortical bone or cancellous bone (16,17). Direct ink writing, SLS, stereolithography (SLA) and robotic assisted deposition are also suitable for polymers (8). Since the diversity of characteristics and manufacturing methods between inorganic materials and polymers, scaffolds made by polymers offer low mechanical strength while good biocompatibility (4). A cranial bone defect model in female Danish Landrace pigs was utilized to verify the application of PCL, the result demonstrated that the purely PCL scaffold without any cells, growth factors or BMP significantly induce bone formation and osteoconductive effect as well as slight degradation of scaffold volume *in vivo*, although the osseointegration and biocompatibility were not as pronounced as the autografts *in vitro* (16). Compared with other polymer scaffolds, permeability in PCL scaffolds increased with higher pore volume and resulted in better bone regeneration, blood vessel infiltration and compressive strength *in vivo*. Combined application of rhBMP-2 and collagen with PCL/PLGA scaffolds showed the best healing quality without inflammatory response at 8 weeks as well as controlled release of rhBMP-2 up to 28 days after implantation in a rabbit radius defect model (18). To heal the rat femur massive full-thickness defect with critical-size, a uniquely PLGA scaffold seeded with MSCs pre-differentiated *in vitro* into cartilage-forming chondrocytes was fabricated and exhibited excellent bone union with biomechanical strength ranging from 75% to 100% compared with normal rat femur (2).

Some commonly used materials were not mentioned in this paper like alginate, chitosan (19,20) and so on. Scaffolds can be made in more precise layer thickness, pore size, porosity and Young's modulus with combined application of various materials due to the rapid development of the 3D printing technology in biomedicine. Fabrication of scaffolds with not only biological activity but also mechanical strength in low or normal temperature has become the hot topic in current research of bone tissue engineering.

Generally speaking, much more kinds of biological or synthetic materials can be applied to make grafts with controllable structure, size as well as shape through very diverse 3D printing technologies for the application of bone tissue engineering with the development of materials science and the numerical control technology.

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