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Bone Tissue Scaffold Technologies Based on RP Adopted Droplet Assembly[1]

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ABSTRACT

Tissue engineering tries to grow replacement tissues to repair damaged bones. In this paper, the fabrication technology of Multi-nozzle Deposition Manufacturing (MDM) was adopted to fabricate scaffolds of a tissue engineered bone at low temperature. The composite of poly(L-lactic acid) and tri-calcium phosphate (TCP) was chosen to form bone tissue engineering scaffolds. The new computer aided manufacturing process can make porous PLLA/TCP scaffolds. A new surface processing technology of apatite coating on bone tissue engineered scaffolds was also adopted. This digital forming technology was based on rapid prototyping (RP), in which a digital droplets assembly technology was introduced. The MDM technology of 4 nozzles was developed based on the layer-by-layer manufacturing principle of Solid Freeform Fabrication (SFF) in our laboratory. The bone scaffolds made by the multi-nozzle deposition process in the MDM system have good biocompatibility and bone conductive properties as a molecular scaffold for bone morphogenic protein (BMP) in the implantation experiment of repairing segment defects in rabbits' and dogs' radiuses.

INTRODUCTION

Tissue Engineering has been developed and applied widely recently for the ability to provide medical implantations which repair, maintain and promote the function and morphology of injured tissues or organs. As one of the keystrokes of tissue engineering, scaffolds are important for the following functions: directing the growth of cells migrating from surrounding tissue or of cells seeded within the porous scaffold and providing a substrate for cell attachment, proliferation, differentiated function and, in certain cases, cell migration; for an article cartilage/bone transplant, the scaffold must have the proper mechanical properties to support the normal physiological functions. Research on scaffolds has been a hot arena[1-5]. The structure of human bone is built up with multi-phase composites of cells. There are three kinds of main structural elements: cells which constitute functional units, the extra-cellular matrix (ECM) and bone scaffolds. Traditional materials such as metals, ceramics, polymers and composites are always exotic materials in the human body. Implants with these materials could only replace functions partially.

Traditional scaffold manufacturing technologies include fiber bonding, solvent casting, particulate leasing, membrane lamination, melt molding and emulsion freeze drying. These can hardly make customized scaffolds for different patients with specially designed functional

gradient structure including gradient material structure and gradient morphology structure. Those features of bone scaffolds are important for the regeneration of the structural tissue. After careful consideration, this paper presents PLLA/apatite/Collagen as a materials system for Rapid Prototyping and manufacturing of bone tissue engineering scaffolds[6-8]. This system has the ability to manufacture scaffolds with good bio-compatibility, bioactivity, higher strength and workability. Chemical reaction methods are put forward to deposit apatite coating on scaffolds. The hybrid structure of bone tissue engineering scaffolds was used in the PLLA/apatite/Collagen system, and this structure was made by rapid prototyping and manufacturing for bone tissue engineering scaffolds. Two kinds of collagen-apatite composites have been prepared with alkali reaction and mechanical mixing methods. Collagen has been coated on the surface of PLLA/TCP tissue engineering scaffolds through the vac-sorb method, and then apatite has been deposited on scaffolds with an unbalanced reaction method, thus a hybrid structure of PLLA/apatite/Collagen has been prepared. SEM observation shows that collagen sponges are adhered on the pores of the scaffolds and apatite is deposited on the collagen sponge. Tests show that the hybrid structure has good bio-compatibility.

In this paper, the rapid prototyping manufacturing (RPM) technology based on droplet assembly has been adopted. There are two important characteristics for this RPM technology: one is that bio-materials were deposited at low-temperature, and the other is that bio-materials were deposited droplet by droplet precisely[9-12]. This kind of bio-manufacturing method could be used in some other fields, and there are some other researches and applications[13-16]. We introduce different RP technologies in different applied fields of bio-manufacturing.

MATERIALS SYSTEM FOR BONE TISSUE ENGINEERING SCAFFOLDS AND ITS PREPARATION

Materials system

Fig. 1 is a list of some Materials of Bone Tissue Engineering Scaffolds. A scaffold should exhibit the following characteristics [1]: 1) three-dimensional and highly porous structure with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; 2) biocompatibility and bioresorbability with a controllable degradation and resorption rate to match cell/tissue growth in vitro and /or in vivo; 3) suitable surface chemistry for cell attachment, proliferation, and differentiation; 4) mechanical properties to match those of the tissues at the site of implantation; and 5) ease of processing to form a variety of shapes and sizes.

Biodegradable biomaterials, poly(L-lactic acid) (PLLA), tricalcium phosphate (TCP) and their composites, have already been used to fabricate bone tissue engineering scaffolds by many different fabrication technologies including fiber bonding, solvent casting, particulate leaching, membrane lamination, melt molding, etc. PLLA grains with an average molecular weight of approximately 53,000 were kindly offered by Institute of Chemistry, Chinese Academy of Science. TCP powder was purchased from Forth Reagent Factory of Shanghai, China. Fig. 2 shows a Scaffold of large bone tissue engineering using rapid prototyping technology. Left is the

Carrier scaffold of PLLA/TCP with multi-pores on 3-D orthogonal directions (×30), and right is the Carrier scaffold of PLLA/TCP for dog radio-bone (×5).

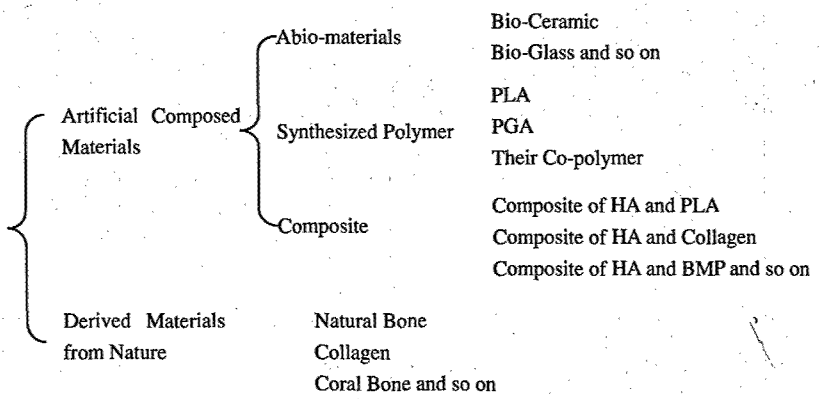
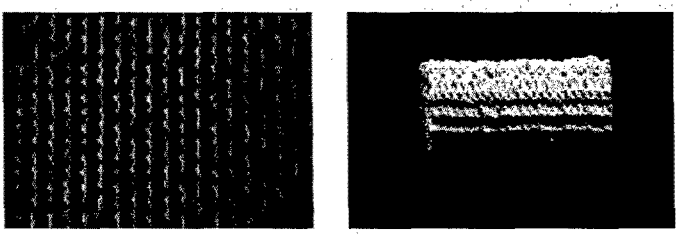


Figure 1. Materials of bone tissue engineering scaffolds.

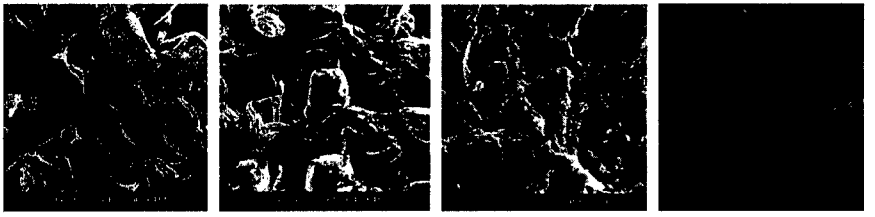


(a) (b)

Figure 2. Scaffold of large bone tissue engineering using rapid prototyping technology. (a) Carrier scaffold of PLLA/TCP with multi-pores on 3-D orthogonal directions (×30). (b) Carrier scaffold of PLLA/TCP for dog radial-bone (×5).

Surface coating processing

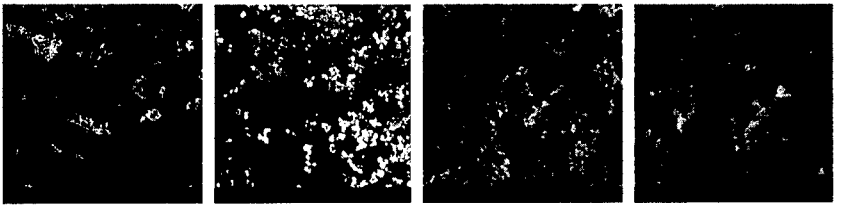
There are two methods of surface coating with tricalcium phosphate. In the first, PLLA is put into simulated body fluid (SBF), and then TCP is deposited onto its surface. This is a near-equilibrium chemical deposition process. In the second method, a film of scaffold material PLLA is made and put into solutions of TCP, where deposition occurs by means of non-equilibrium reaction (Fig. 3). The porous morphology of the scaffolds was studied by scanning electron microscopy at 20 kV. The dimension of macro pores is about 300~500 μm. There is micro porous structure around the macro pores. The average dimension of the micro pores is about 5 μm. It is shown that the minute TCP particles are embedded in the PLLA walls and can be distinguished.



(a) (b) (c) (d)

Figure 3. Multi-pore structure of PLLA tissue engineering scaffold and the surfaces of the scaffold after dipping in solutions of TCP. (a) Horizontal section of the scaffold (×50). (b) Perpendicular section of the scaffold (×50). (c) Scaffold surface after 3 Days (×2500). (d) Scaffold surface after 10 days (×2500).

The PLLA film was cut into strips of 3 mm. Then, three kinds of solutions were obtained with $CaCl_2$ and $K_2HPO_4 \cdot 3H_2O$. Solution A was of 1.12M $CaCl_2$, solution B was of 0.56M K_2HPO_4, and solution C was of 0.28M K_2HPO_4. KOH was put into solutions B and C, so that the pH was about 14. A strip of PLLA was put into solution B for about 20 min, with the ratio of solid against liquid being 1:30 (w/v). Then, it was put into solution A with a ratio of solid against liquid of 1:50 (w/v) at 37 °C for a reaction time of 3 min, 5 min, 10 min, 15 min, 30 min, 1 h or 2 h respectively. The sample was then washed with de-ionised water. This is the so called Process B+A. Processes C+A and A+C were similar to this process. Three samples were obtained for each time. Fig. 4 shows SEM photos of processes B+A. The morphologies of this composite in different times for process B+A were as follows: there is no deposition in 3 min; there are some island-like depositions on the PLLA surface in 10 min; the surface is almost covered by deposition material in 2 h and the size of the deposited particles is about 1 μm. Fig. 5 is the evolution of the mole ratio of Ca/P in the mineralization processes for processes C+A and B+A. The difference is mainly in the first 1 h. Fig. 6 shows the morphology of the surfaces for process of B+A for 10 min. It is known that a pre-process of isopropanol is suitable for deposition of TCP.



(a) (b) (c) (d)

Figure 4. Processes of B+A for: (a) 3 min (×3000); (b) 10 min (×3000); (c)2 h (×3000); (d) 2 h (×3000).

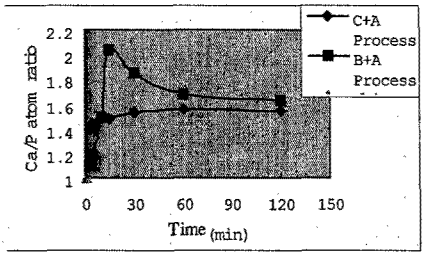
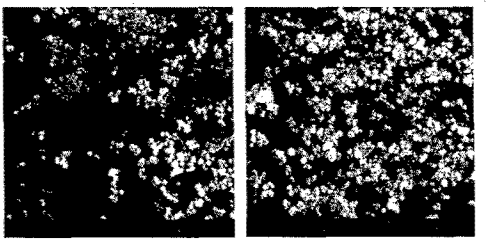


Figure 5. Evolution of mole ratio of Ca/P in the mineralization process.



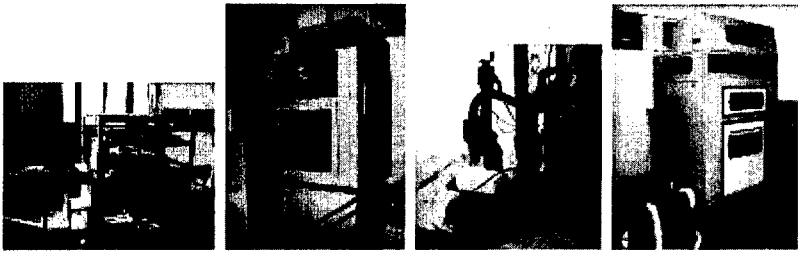
(a) (b)

Figure 6. Process of B+A for 10 min, (a) without pre-process of isopropanol (×3000), and (b) with pre-process of isopropanol (×3000).

FABRICATION BASED ON RP ADOPTED DROPLET ASSEMBLY

Introduction to manufacturing systems for bio-manufacturing

Four kinds of bio-manufacturing systems are built up in the Center of Laser Rapid Forming, Tsinghua university (Fig. 7). The fabrication methods for these four systems are based on RP adopted droplet assembly. There are a lot of different bio-materials for each bio-manufacturing system. Fig 7(a) is a platform for bio-manufacturing, and a lot of experimental results were obtained from this platform. For example, the particles of bio-materials could be deposited with their droplets transformed from solid state to liquid state based on a spraying technology. Fig 7(b) is the MedForm equipment. It is for repair a skull defects with polymers by means of melted extrusion modeling technology using bio-compatible materials. Fig. 7(c) is a desktop RP machine. It is for bio-manufacturing of ear bone for microtia patients. Microtia should be corrected or reconstructed with biomaterials, such as ultra-high molecular weight polyethylene, which is similar to the cartilage of ear. There are four nozzles on the TissForm system in Fig. 7(d). Droplets solidify and assemble at room temperature or low-temperature one by one. Three kinds of materials for bone tissue engineering scaffolds mentioned in this paper are provided and came from three nozzles.



(a) (b) (c) (d)

Figure 7. Four kinds of bio-manufacturing systems in CLRF, Tsinghua: (a) Bio-manufacturing platform; (b) MedForm system for materials with biocompatibility; (c) Open bio-manufacturing system; (d) TissForm system for tissue engineering materials.

Bone tissue engineering process

The material slurry was formed into frozen scaffolds in a low-temperature deposition manufacturing (LDM) system. Fig. 8 shows the structure of the LDM system. First, the material slurry was fed into the material supply that has a soft pipe connected to a screw pump nozzle. The diameter of the outlet of the nozzle is 0.3 mm. The LDM system built scaffolds layer by layer, directly computer-driven by 3-dimensional digital models. This was accomplished in low temperature environment under 0 °C in the refrigerator. The computer controlled the nozzle to move in the X-Y plane, extrude the material slurry out and deposit it onto the platform in the area defined by the digital models. The layer of deposited materials was frozen on the platform. Also under the control of the computer, the platform moved down 0.15 mm in the Z direction after the forming process of each layer. In this manner, the frozen scaffold was stacked up layer by layer. To ensure the forming of the pores of the vertical cross section, the extruded material was deposited into a series of parallel lines along the Y direction from the 1st to the 3rd layer while moving parallel to the X direction from the 4th to 6th layer; the scanning direction of the nozzle alternated every 3 layers as shown in Fig. 9. By changing the number of layers between changes of scanning direction, and the distance between the parallel lines in each layer, the pore dimensions can be adjusted. After the forming process, the frozen scaffolds made by the LDM system were freeze-dried in an ALPHA1-2 Freeze dryer for 38 hours to remove the solvent. After treatment by freeze drying, the scaffolds are in the solid state in normal atmospheric temperature.

Cells culture

The porous matrices were placed in 96-well tissue culture plates 5 mm in diameter and 2 mm high. After being sterilized in 70% alcohol overnight, the matrices were rinsed extensively with distilled water and washed by PBS and Dulbecco's Modified Eagle Medium (DMEM) medium twice separately. L929 cell suspension was supplied by Jishuitan Hospital of Beijing. 100 µl L929 cell suspension at a density of cells 5×10^5 /ml DMEM medium was dropped into each well.

Then it was incubated in a humidified atmosphere with 37 °C, 5% CO_2. The culture medium was changed after 1.5 days. After 2.5 days incubation, the matrices were fixed with 2.5% glutaraldehyde in phosphate-buffered saline (PBS) overnight at 4 °C.

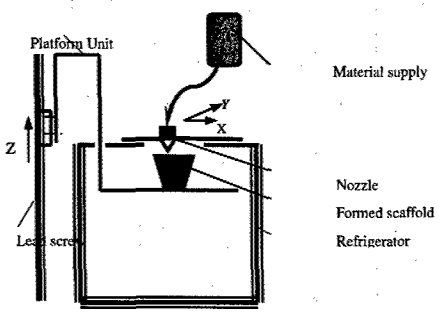


Figure 8. Schematic illustration of LDM.

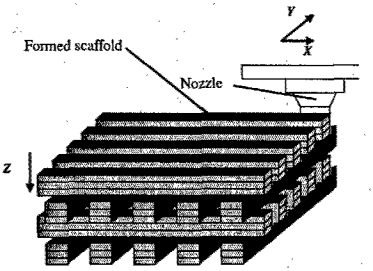


Figure 9. Illustration of the forming process of scaffolds in the LDM system.

After thoroughly washing with PBS, the cells were dehydrated by graded ethanol and critical point dried and examined by scanning electron microscopy (SEM). Table I shows the results of this experiment. The densities of different kinds of cells are almost the same. The densities of samples No. 3 and 4 are smaller a little, but the density for sample No. 2 is somewhat higher. The morphologies of cells growing on the surfaces of different materials were observed by microscopy. It is known that the growing conditions are good enough on surfaces of sample No. 1-5. It is concluded that these bio-materials have no toxicity. The adhesion and propagation of cells on the surfaces of materials are also good enough for samples of No. 1-5. But for the sample of pure PLLA (No. 6), the situation is different completely. There is no adhesion of cells on the surface of the material. This result shows that the bio-properties are improved for our material system.

Table I. Serial Numbers of Materials, Processing Methods and Cell Density

Number of Materials	1#	2#	3#	4#	5#	6#
Processing Method	No pre-processing, deposition in A for 15 min	No pre-processing, deposition in A for 80 min	No pre-processing, deposition in A for 12 h	Pre-processing in water, deposition in A for 10 min	Pre-processing with isopropanol, deposition in A for 50min	On pure PLA coating
Cell Density	5.6×10^5	6.0×10^5	3.9×10^5	3.2×10^5	5.0×10^5	4.9×10^5

CONCLUSIONS

Bone tissue scaffold technologies based on RP adopted droplet assembly were studied in this paper. In summary:

1. A new bio-material system was given for bone tissue scaffolds. This is the porous PLLA/TCP composite with BMP.
2. The fabrication technology of Multi-nozzle Deposition Manufacturing (MDM) was adopted to fabricate bone tissue engineered scaffolds in low temperature.
3. The cell culture experiments show that the bone scaffolds made by us have good bio-compatibility and bone conductive property as a molecular scaffold. It also gave good results in the implantation experiments for repairing segment defects in rabbits' and dogs' radiuses[11-12].

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