

Nano-Hydroxyapatite Modified by Grafting Poly(lactide) and its Tissue Engineering Application

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Hydroxyapatite/poly(lactide) (HA/PLA) composite may have osteoinductivity and osteoconductivity due to the presence of HA. For this composite, the interfacial strength between HA fillers and PLA matrix is a critical factor to determine the mechanical properties of the composite. Several methods have been developed to improve adhesion between HA and PLA, such as utilization of surfactants. Here, we report a novel approach, i.e., direct graft-polymerization of lactide on HA surfaces.

The composite was prepared in three steps: (1) Nano-hydroxyapatite (*n*-HA) was reacted with lactic acid. The water formed was removed by azeotropic dehydration with toluene. After fully washed with THF and dried, surface modified nanosize needle-like HA crystals (*l*-HA) were obtained. (2) L-lactide was polymerized in the presence of *l*-HA with Sn(Oct)₂ as catalyst. The PLA homopolymer formed was removed by dissolving in chloroform and by centrifugation. The powders were washed 5 times with chloroform and vacuum-dried. They were designated as *p*-HA. (3) *l*-HA and *p*-HA were melt-mixed with neat PLLA (M_v=196,000) to get composites *l*-HA/PLA and *p*-HA/PLA, respectively.

These improvements could be ascribed firstly to the grafted-PLLA molecules which played a role of tie-molecules between the fillers and the PLLA matrix, and secondly to the grafted HAP particles which were uniformly distributed in the composites and played a role of the heterogeneous nucleating agents in the crystallization of the PLLA matrix. The *p*-HA/PLLA composites showed good mechanical properties and uniform microstructure. The tensile strength and modulus of the *p*-HA/PLLA composite containing 15wt% of *p*-HA were 68MPa and 2.1GPa, respectively, while those of the *n*-HA/PLLA composites were 43MPa and 1.6GPa, respectively. The *p*-HA/PLLA composites have better thermal stability than *n*-HA/PLLA composites and neat PLLA by isothermal TGA. The hydrolytic degradation behavior of the composites in PBS (pH7.4) was investigated. The *p*-HA/PLLA composites lost their mechanical properties less than *n*-HA/PLLA composites in PBS due to their reinforced adhesion between the HA filler and PLLA matrix.

Hydroxyapatite (HA)/poly(L-lactide) (PLLA) nanocomposites have received a great deal of interest in orthopedic and dental applications for their good osteoconductivity, osteoinductivity, biodegradability and high mechanical strength. HA/PLLA nanocomposites are usually used at fiber, sheet, rod, screw or micropore piece form. Recently, a great deal of attention focuses to the composite microspheres. Various approaches have been designed to prepare the composite microspheres to obtain the required properties and promising applications in many fields, including optics, electronics, mechanics, membranes, protective coatings, catalysis, sensors, biology, and others. Preparation of HA/PLLA nanocomposite microspheres has important significance in drug controlled system. In addition to being used as support carriers for cell cultures, such microspheres have potential applications in selective laser sintering (SLS) and porous scaffold materials.

The HA nanoparticle surface grafting with PLLA (*p*-HA) has relatively hydrophobic surface and the aggregation of HA nanoparticle in organic solution was overcome. The *p*-HA nanoparticles would rather be located in the organic solvent phase than in the water phase, hence *p*-HA nanoparticles could be finely incorporated in the *p*-HA/PLLA composite microspheres by using the oil-in-water emulsion solvent evaporation method. Furthermore, the *p*-HA powders could be easily dispersed into hydrophobic solvent to form a stable *p*-HA/chloroform suspension so that *p*-HA/PLLA composite microspheres with uniform micro size diameter will be obtained.

ESEM analysis revealed the morphology and size distribution of the composite microspheres (Fig. 1). The examination (Fig. 1A) showed HA/PLLA composite microspheres had wide size distribution ranging from 200nm to 7.7 μ m. During the emulsion process, HA nanoparticles would rather to precipitate from oil phase to water phase

and reduce the surface tension of oil emulsion drops thus part of the oil drops would combine with each other; therefore various size microspheres with ugly looks were obtained. ESEM image presented in Fig. 1B indicated that the effective encapsulation of *p*-HA nanoparticles took place and the composite microspheres with relative uniform diameter (over 80% of the microspheres were in the size range of 2-3 μ m) formed in the system.

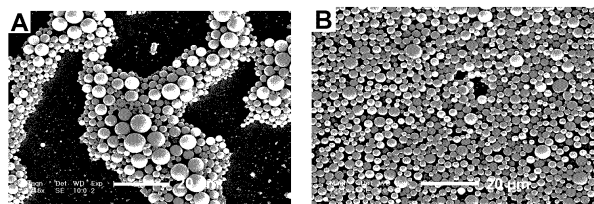


Figure 1. (A) and (B): ESEM photographs corresponding to HA/PLLA composite microspheres and *p*-HA/PLLA composite microspheres, respectively.

The *p*-HAP/PLLA composites also demonstrated improved cell compatibility due to the good biocompatibility of the HAP nanoparticles and the more uniform distribution of the PLLA-grafted HAP nanoparticles on the film surface. All of these results indicated that the *p*-HAP/PLLA nano-composites might have a promising medical application in bone repair and in bone tissue-engineering.

Blends of HAP particles and biodegradable polymers have been used traditionally in various forms in order to improve their biocompatibility and mechanical properties. However, this kind of physical adsorption between them only left lower stability and limited mechanical properties. A novel nano-composites of PLLA/gHAP were obtained through the hydroxyl groups on the surface of the HAP nanoparticles grafted with PLLA, and higher mechanical property and stability of its blends with PLLGA were achieved. With this blends, a porous scaffold for bone tissue engineering were prepared, and used to construct artificial bone seeded with autogenic MSCs of rabbits in vitro and grafted to repair the bone defects of rabbits. Morphological analysis and SEM proved MSCs grew well in scaffold in vitro; The animal test showed that new bones were formed and fused in the area of bone defects of rabbits with x-ray at 2 weeks after operation (Fig.2). This material showed broad prospects in clinical application with its good biocompatibility and rapid regeneration for bone damage.

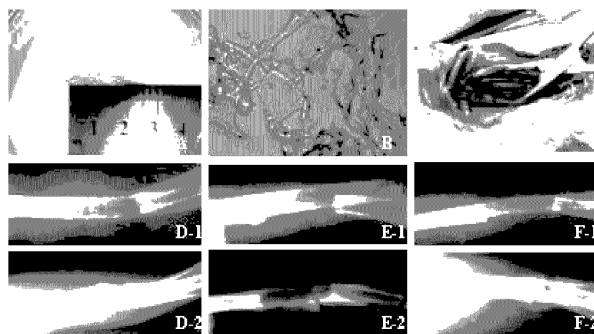


Fig. 2 A. Bone substitute with PLLA/gHAP seeded with MSCs; B. Histological analysis; C. Grafting operation in animal tests; D., E. and F. showed X-Ray examinations of bone defect repairing with the substitutes of PLLA/gHAP and PLGA, and the control group with no materials 0d after operation (in -1) and 2w after operation (in -2).

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