Gelatin/hydroxyapatite nanocomposite scaffolds for bone repair

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A specific combination of solvent-layer casting, freeze drying, and lamination techniques enables fabrication of biostructures with almost ideal properties for bone-tissue engineering.

The use of bioscaffolds for tissue repair is widely accepted. They can provide structural stability and a 3D system on which cells can grow new tissue. Natural bone is composed of organic (mainly collagen) and mineral biomaterials (predominantly carbonate hydroxyapatite, HA). In recent years, developments in 3D porous-scaffold manufacturing have increased hopes of successful fabrication of structures with ever closer similarities to bone matrix. Thus, most widely investigated composites for engineering tissue scaffolds are composed of natural polymers reinforced with HA ceramic particles. Gelatin (GEL), a derivative of collagen, is an attractive component for extracellular-matrix replacement, since it contains several biological functional groups that enhance osteoblast adhesion, migration, and mineralization.

A few years ago, synthesis of GEL/HA nanocomposites for bone-tissue engineering was the main focus of a number of research groups. It was shown that, in addition to the chemical structure of bioscaffolds, other properties—such as their porosity and texture (especially on micro- and nanoscales)—can dramatically influence the nature of the cellular reaction to the material.

Our aim was to design and fabricate a close-to-ideal scaffold for bone-tissue repair and study the effects of different glutaraldehyde (GA) concentrations as cross-linking agent.

We synthesized nanocrystalline HA powders through precipitation and, combining them with GEL, engineered a 3D nanocomposite scaffold. Our emphasis was on achieving uniform and proper pore size and increased scaffold strength, comparable with the mechanical characteristics of bone tissue. To engineer nanocomposite scaffolds, we prepared a homogeneous aqueous solution of microbiology-grade GEL (10% weight per volume, w/v) and added our synthesized HA nanopowder to obtain a GEL(70)/HA(30) weight composition. After homogenization through stirring, we cast a layer of this composite material into plastic petri dishes, which was subsequently frozen at $-20^\circ$C for three hours to solidify. To produce porous structures, we transferred the layers to a freeze drier. We cut composite layers at the desired sizes and laminated them by applying a GEL solution as binding agent. Next, we soaked samples in a cross-linking bath of 0.25–2.5%w/v GA for 24 hours to modify their mechanical properties and render them insoluble in water.

We investigated the physical, chemical, mechanical, and biological properties of the HA nanopowders and the nanocomposite scaffold using various analytical methods, including scanning- and transmission-electron microscopy, Fourier-transform IR spectroscopy, x-ray diffraction, and compressive mechanical testing. The results showed that nearly pure nanocrystalline HA can be successfully synthesized through precipitation with elliptically shaped crystallite with sizes of 8–12nm. Smaller sizes of HA powder used for scaffold preparation

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increased the surface area and, therefore, the level of osteo-induction, as well as the mechanical properties of the scaffolds, compared with conventional GEL/HA composites. The results obtained from the nanocomposite showed that it was characterized by a porous, 3D interconnected structure with pore sizes ranging from 300 to 500 μm and approximately 85% porosity (see Figure 1). The scaffolds exhibited typical honeycomb behavior. Increasing GA concentration to 1% w/v in the cross-linking solution caused a partial enhancement in the elasticity modulus to ~48 MPa, with a decrease for further increased GA concentrations to approximately 23 MPa. Fracture occurs less frequently in these nanocomposite samples because of the increasing GA concentrations.

Cytotoxicity evaluation showed cellular toxicity for the scaffold cross-linked with 2.5% w/v GA, but using GA as cross-linking agent at concentrations below 2.5% w/v will not threaten cell viability. Therefore, from a mechanical-data perspective, nanocomposites treated with 1% w/v GA solution are most appropriate for scaffold construction.

In summary, we used a specific combination of common techniques to engineer a scaffold with almost ideal properties for bone-tissue engineering. These scaffolds have potential for use in solid, free-form applications and can, therefore, be formed as required by the defect size and shape. We are currently working on further biological evaluation, including in vitro cell culture and in vivo implantation in animal models. Preliminary results indicate the feasibility of our fabricated scaffold for bone-tissue regeneration applications.

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References