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Facile method of building hydroxyapatite 3D scaffolds assembled from porous hollow fibers enabling nutrient delivery

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Abstract

Nowadays, diffusion through scaffold and tissue usually limits transport, and forms potentially hypoxic regions. Several methods are used for preparation of 3D hydroxyapatite scaffolds, however, production of a scaffold including porous hollow fibers for nutrition delivery is difficult and expensive. In this study, we describe an easy and inexpensive method to create 3D hydroxyapatite structure containing porous hollow fibers via microtemplating. The fibers which are assembled into 3D scaffold and sintered, contain asymmetric membrane walls with flux suitable for nutrient delivery. These hollow fibers have good mesenchymal stem cell adhesion showing that the presented method has no negative influence on cell cytocompatibility. The proposed straightforward method for building 3D structures containing porous hollow fibers for nutritions can be suitable for in vitro bioreactors studies as well as for production tissue engineered or in vivo prepared bone grafts.

Keywords: A. Sintering; E. Membrane; Hydroxyapatite; Microtemplating; Cell adhesion

1. Introduction

The porosity and pore size are important morphological properties of biomaterial scaffolds, and play a critical role in vitro and in vivo tissue formation. For example, in bone tissue engineering – in vivo: lower porosity stimulates osteogenesis by suppressing cell proliferation and forcing cell aggregation. Contrary, in vivo, higher porosity and pore size result in greater bone ingrowth [1]. Then combination of macroscopic pores for cells growth and microscopic pores for cell adhesion and nutrition transport is necessary.

Synthetic foams are one solution for composition and structural control of bone filling materials [2,3]. The structure of foam is dependent on viscosity and surface tension, however, it is not easy to create macroscopic and microscopic porosity for the development of a network of blood vessels with the finer structure preferred for mechanical strength. Another possibility is to create scaffold using a rapid prototyping from fibers containing suitable material, therefore the 3D structure can be organized by orientation of fibers [4]. Both negative and positive scaffold biomaterials have been produced by rapid prototyping methods [5]. These established methods are dealing with polymer based materials allowing flexibility and solubility. Bioceramic materials have the rigid structure and direct formation of fibers is very limited. It is possible to design a hard tissue scaffold with hierarchies of pores, channels, and accompany smaller recesses which can host cells development as well as large voids structure for vascularization [6]. However, the vascular network is not separated from cells and only the size of voids differentiates vascular network from cells area. The separation of channels for nutrition delivery in hard tissue scaffold is desirable especially for construction of a bioreactor or in vitro preparation of a hard tissue. The absence of a nutrition delivery network capable of distributing gas, nutrients and removal

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of waste products is one major limiting factor in creating viable artificial tissues [7]. The concept of isolated microchannels (in hollow porous fibers) for nutrition delivery was already tested for a polymeric scaffold. The hollow fiber membranes integrated inside large three-dimensional (3D) scaffolds improved the cell density and cell distribution [8]. Nevertheless, the preparation of microchannels inside ceramic scaffolds is based on indirect building of channels via rapid prototyping techniques [9–11]. These techniques can build various shapes precisely, but the process is not facile and requires high investments. Furthermore, the obtained hollow structures are usually not rounded and a possibility of porosity tailoring is limited due to restrictions in selection of suitable powder materials.

Here we demonstrate an easy and economical method for production of porous and hollow fibers, which can be directly assembled for building the hydroxyapatite (HA) 3D scaffold. Polypropylene template fibers and other organic components were burn out during the thermal treatment and only inorganic HA structure remained. HA is bioactive ceramic material with relatively low hardness similar to human bone apatite and has been used as bone grafting material or active coating for dental implants [12,13].

The microchannels inside HA hollow fibers are tested for exchange of nutrients and the outer surface of the fibers can accommodate cells. Confirmation of scaffold cytocompatibility after processing was done by testing the metabolic activity of the cells on the hydroxyapatite fibers over the period of 7 days.

2. Materials and methods

2.1. Fabrication of the ceramic structure

Suspension for the dip coating was prepared by ball milling of following components: solvent (2-propanol), dispersant (solperse 20000), ceramic powder (hydroxyapatite), plasticizer (BBP), and binder (PVB) as listed in Table 1.

Polypropylene hollow and porous fibers were 4 times dip-coated in the suspension with withdrawing speed 0.29 m min^{-1}. The outside diameter of the micro template – polypropylene fiber was 300 μm, porosity and hollow space of the polypropylene fiber ensure collapse of the fiber shape before its thermal expansion may destroy green ceramic structure [14]. Each layer was fully dried between each dip-coating step and ambient conditions were always used for drying.

Table 1
Composition of suspension for the microtemplating [14], same suspension was used for connections of the green coated fibers.

Suspension components	Weight (%)	Volume (%)
2-propanol	52.8	77.3
Solperse 20000 (± 90% polymeric alkoxylate)	1.8	1.7
Hydroxyapatite (Sigma-Aldrich, BET 10–15 $m^2 g^{-1}$)	38.6	14.1
BBP (Benzyl butyl phthalate), S-160	0.9	1.0
PVB (Polyvinyl butyral) B-98	5.8	6.0

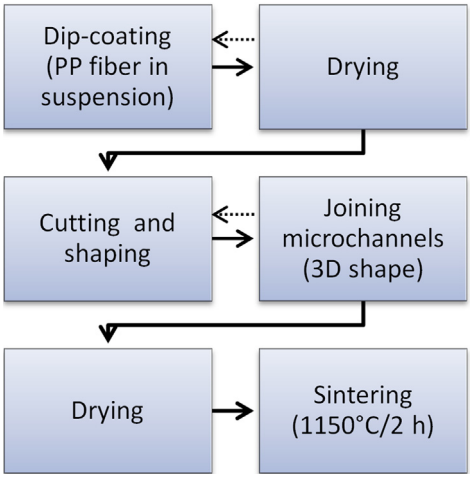

Fig. 1. Chart flow of describing the building process of ceramic 3D structure.

Furthermore, the prepared green fibers were cut, shaped and joined together. The cutting, shaping and joining of the coated fibers were done manually and distance between middles of the green fibers was 1 mm. Suspension with the identical composition (Table 1) was used to fix green coated fibers in intended positions (before sintering). Five vertical levels of the green coated fibers network were prepared, when the first layer contained eight parallel coated fibers and the following layers had seven parallel coated fibers. Sintering was done in air and subsequent heating or cooling steps were applied: 40–220 °C (1 °C min^{-1}), 220–600 °C (2 °C min^{-1}), 600–1150 °C (10 °C min^{-1}), 1150 °C/2 h, 1150–40 °C (5 °C min^{-1}). The chart flow in Fig. 1 describes the fabrication process of the 3D hydroxyapatite scaffold assembled from porous hollow fibers. The identical procedure, without 3D assembling step, was used for preparation straight self-supporting porous and hollow fibers for testing of membrane performance and biocompatibility.

2.2. Structural analysis and membrane characterization

Photography was used to demonstrate shape of the sintered hydroxyapatite structure. Hollow fibers after sintering were characterized with scanning electron microscopy (Jeol JSM 5600LV). SEM pictures of the membrane outer surface and cross sections were analyzed with image processing software to determine the porosity (ImageJ). Fluxes of gases (N_2, CO_2) and liquids (water, cell culturing medium) were measured in dead-end filtration mode using modules containing one single hollow fiber (microchannel inside). Gas permeability was measured using the manual low pressure gas permeation set up. The time needed to collect 25 cm^3 of gas was measured whereas the pressure was controlled at 1 bar, and temperature was 22 °C. The water flux of the HA fibers was determined by filtrating inside-out ultrapure water at the constant trans membrane pressure of 1 bar, volume increase of the filtrate was recorded as a function of time for at least 90 min.

The flux through the membrane (J, in 1 $m^{-2} h^{-1}$) was calculated from the slope of the filtrated volume (V) versus