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Materials Science and Engineering C 25 (2005) 181–186

MATERIALS SCIENCE & ENGINEERING C

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Towards bone replacement materials from calcium phosphates via rapid prototyping and ceramic gelcasting

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Available online 4 March 2005

Abstract

Biomimetic porous scaffolds made of calcium phosphate mineral are promising structures to develop bone replacement materials. In order to fabricate scaffolds with a strut size of 450 μm, we used a stereolithographic technique which selectively polymerises photosensitive liquid resin by visible light to produce casting moulds for ceramic gelcasting. These moulds were filled with a water based thermosetting ceramic slurry which solidifies inside the mould. After burning the resin mould and sintering, hydroxylapatite structures with designed, fully interconnected macroporosity were obtained.

The preosteoblastic cell line MC3T3-E1, derived from mouse calvariae, was used to test for biocompatibility in cell culture experiments. The cells were seeded on the scaffolds immersed in the culture medium and cultured for 2 weeks. Thereafter the cells on the scaffold were fixed and investigated by histological methods. The osteoblast-like cells were found to cover the whole external and internal surface of the scaffold, they were embedded in collagenous extracellular matrix. The cells had in particular the tendency to fill any crack or opening and to generally smooth the exposed surfaces.
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Keywords: Bone replacement materials; Implants; Rapid prototyping; Ceramic gelcasting; Hydroxylapatite; Hydroxyapatite; Scaffolds; Macroporosity; Cell culture; Osteoblasts; MC3T3-E1

1. Introduction

The development of biomimetic bone replacement materials is a growing field of research for application in medicine [1,2]. The potentially best bone replacement materials are grafts derived from the patient himself. They are biocompatible, osteoconductive and osteoinductive, and there is no danger of immuno-rejection. There is, however, only a limited amount of autograft available for each patient and the extraction induces additional trauma [3–5]. With allografts, derived from donators, or xenografts from animal tissue, there is an additional risk of immuno-rejection and disease transmission [6–9]. In addition to these biogenic materials, metallic implants [10] as well as ceramic [11–15], polymer and composite [16–18] biomaterials have been developed for bone replacement applications. These implants should be well integrated into the remaining bone, which implies not only full biocompatibility (to avoid immunoreactions) but also osteoconductive properties in order to ensure a tight connection with bone [19–21]. Polymeric and ceramic materials can also be resorbable, ceramic materials having the advantage of higher strength and stiffness compared to the polymeric materials [22], although the intrinsic brittleness of ceramics limits their applicability. Among the ceramic materials, the calcium phosphates are known to have promising biological properties [23], in that they can be biocompatible, resorbable, osteoconductive and even osteoinductive under appropriate

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doi:10.1016/j.msec.2005.01.014

conditions [24–29]. Moreover, they can be used for bone cements and fillers [11,12]. Ideally bone replacement materials should, in addition, be remodeled into native bone, which we expect to be possible if the material is bioresorbable and provides sufficient access to bone forming cells [19].

Cell ingrowth behaviour strongly depends on the pore size [24,29,30] and interconnectivity [11,31] within the implant. A pore diameter of 150 to 500 μm is referred to be best for cell access [25,30,32–34]. On the other hand, nutrition of the cells within the structure has to be ensured for viability, which is possible via the flow of serum through micro- and/or macro-pores. Considerable success has already been obtained with porous ceramics serving essentially as a scaffold for bone regeneration and produced via different routes, e.g. from corals [3]. One difficulty with producing porous calcium-phosphate scaffolds is to control the porosity in order to match the biological requirements but also to provide sufficient mechanical stability.

The mechanical properties of cellular solids such as porous ceramics depend mainly on three parameters: the apparent density, the properties of the base material and the architecture of the structure [35]. The possibilities of optimizing the properties of the base material are limited by the requirements of biocompatibility and bioresorbability. Moreover, due to the biological need for interconnected porosity with pore sizes in the range of a few hundred microns, the apparent density cannot be increased beyond a limiting value. Significant improvement of the mechanical performance at given apparent density is possible, however, by adapting the architecture. In previous experiments we built several periodic three-dimensional cellular solids with constant apparent density and showed by compression testing that a simple change in the architecture of the unit cell can account for variations by almost a factor of three in strength and, independently, in defect tolerance [36].

Based on these results, we pursued the route of designing porous calcium-phosphate ceramics with predefined architecture and sufficient accessibility for bone forming cells. In a first step, we investigated the potential of rapid prototyping (RP) and ceramic gelcasting to produce three-dimensional scaffolds suitable for growing bone-forming cells. We chose a methodology starting with a computer-aided design of the structure, which gives the full freedom to obtain various types of structures with different mechanical properties [36]. Then resin moulds were constructed using a stereolithographic technique [37], and filled with a thermosetting ceramic slurry. After temperature treatment, we obtained cellular ceramic scaffolds with designed macroporosity. We report first results for hydroxylapatite scaffolds with 450 μm pore diameter and fully interconnected pore morphology, which were manufactured and tested in cultures of a murine preosteoblastic cell line.

2. Materials and methods

The first step in the production of ceramic structures was to virtually design the desired structure with a computer aided design (CAD) software. We used Pro/Engineer (PTC, Needham, Massachusetts, USA). An example is shown in Fig. 1.

We constructed structures consisting of layers of parallel struts with quadratic cross-section and a side length with the same physical dimension as the distance between two of them. Each layer was turned 90° with respect to the previous one. In this manner, 20 layers were superimposed. The diameter of the whole structure was 10 mm, the side length of one of the struts and the height of one layer was 500 μm, hence the height of the whole structure was 10 mm, too. The porosity of the structure was therefore 50 vol.%. The limitation of the strut diameter within the mould was given by the RP machine and not by the mould filling capacity of the ceramic slurry. For the rapid prototyping machine used, the obtainable minimum strut size would be about 300 μm, that is in the range of the size of the trabeculae of natural bone material [38,39].

This virtual structure was imported by the software that controls the RP-machine and decomposed into thin layers to be built sequentially in the rapid prototyping process. The RP-machine used was a perfactory mini (Envisiontec, Marl, Germany).

This system uses liquid photosensitive resin (envisiontec perfactory® resin) that is selectively hardened by visible light. A micro-mirror array lets the light pass where the photosensitive resin should solidify and stops it where the resin should remain liquid. After the first layer has been exposed, and by this, the first layer of the future part (or structure) has been built, the stage moves for one layer thickness, new resin is applied and the procedure starts again (Fig. 2). When the whole part has been built layer by layer, the remaining liquid resin is removed with alcohol and the part is post cured with UV light [37]. This device provides a resolution of 32 μm for objects with outer dimensions of a few centimetres in each direction. This offers the possibility to

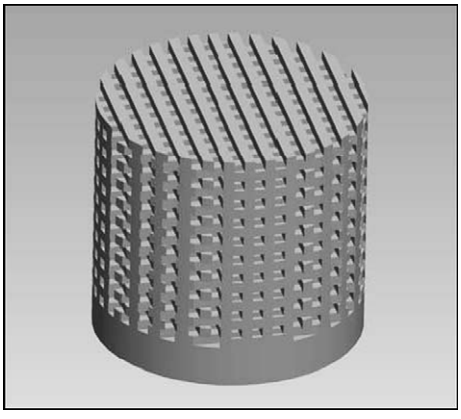

Fig. 1. Virtual structure designed with CAD (Pro/Engineer), for the description see text. This structure was reproduced in hydroxylapatite.

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