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Development of glass–ceramic scaffolds for bone tissue engineering: Characterisation, proliferation of human osteoblasts and nodule formation

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Abstract

Glass–ceramic macroporous scaffolds for tissue engineering have been developed using a polyurethane sponge template and bioactive glass powders. The starting glass (CEL2) belongs to the system SiO_2–P_2O_5–CaO–MgO–Na_2O–K_2O and has been synthesised by a conventional melting–quenching route. A slurry of CEL2 powder, polyvinyl alcohol and water has been prepared in order to coat, by impregnation, the polymeric template. An optimised thermal treatment was then use to remove the sponge and to sinter the glass powders, leading to a glass–ceramic replica of the template. Morphological observations, image analyses, mechanical tests and in vitro tests showed that the obtained devices are good candidates as scaffolds for bone-tissue engineering, in terms of pore-size distribution, pore interconnection, surface roughness, and both bioactivity and biocompatibility. In particular, a human osteoblast cell line (MG-63) seeded onto the scaffold after a standardised preconditioning route in simulated body fluid showed a high degree of cell proliferation and a good ability to produce calcium nodules. The obtained results were enhanced by the addition of bone morphogenetic proteins after cell seeding.

Keywords: Scaffold; Glass–ceramic; Osteoblast; BMP; Bone graft

1. Introduction

Bone replacements are frequently needed due to trauma, bone neoplasia, stabilisation of spinal segments and more generally in many orthopaedic, maxillofacial and craniofacial surgeries [1–3]. At present, many injuries are not adequately treated because bone defects above a critical size cannot be repaired through the natural growth of bone tissue [4]. Furthermore, due to the increased mean population age and to ever more surgery being undertaken to remove tumors, bone regeneration is a clinical need of growing importance [5,6]. Autografts, allografts or xenografts can be used as bone substitutes. Autologous bone graft is still considered the gold standard due to its ability to support osteoinduction and osteogenesis. However, this procedure has currently insurmountable drawbacks associated with its availability, the need for further surgery and to donor site morbidity [7–9]. A promising alternative is represented by allografts and xenografts that can solve the above-mentioned problems, but, compared with autografts, are characterised by poorer bone induction properties, lower rates of integration and are also related to a non-negligible risk of contamination, immune rejection or viral transmission from donor [10,11]. For these reasons, artificial grafts (scaffolds) are interesting and challenging candidates for

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stimulating bone regeneration and for supporting the newly formed bone. Three-dimensional (3D) scaffolds should show a highly porous, open structure to allow a proper vascularisation of the implant, as well as the flow of nutrients and waste products through the scaffold. Specifically, the porous structure should be highly interconnected, with pore sizes in the range of hundreds of microns (100–500 μm), i.e. comparable to the size of blood vessels and osteoblasts, and with pores present in amounts higher than 50–60 vol.% [12–14]. Microporosity (pores below 10 μm) and pore wall roughness are also important for osteogenesis as these characteristics favour protein adhesion and thus cell attachment and proliferation. In addition, a porous surface favours mechanical interlocking between the scaffold and the surrounding bone tissue. Within ceramics, hydroxyapatite (HAp), β-tricalcium phosphate (β-TCP) and more recently bioactive glasses and glass–ceramic containing apatite and wollastonite are the most investigated materials as scaffolds for guided bone regeneration [15–19].

In this work, efforts were focused on new silica-based bioactive glasses of complex compositions. In fact, bioactive glasses possess unique properties if compared to ceramic materials such as HAp and β-TCP, as their composition can be tuned to obtain material with tailored reactivity in the human body, ranging from a slightly bioactive behaviour to a completely bioresorbable material. A complex composition also offers the unquestionable advantage of releasing ions known for their beneficial role on bone matrix mineralisation (i.e. calcium and magnesium) [20,21] and/or the achievement of a proper control of local pH during ion leaching (i.e. phosphate ions), thereby avoiding cell damage due to pH variation. Furthermore, it has recently been discovered that the dissolution products from bioactive glasses exert a genetic control over the osteoblast cycle, and more specifically silicon has been found to be the ion that contributes most to the mineralisation of bone and to gene activation [12,22–24]. In addition, glasses do not melt at a constant temperature but soften with increasing the temperature, and this is an advantage for the forming techniques.

Different methods [3,12,17,25–32] can be used to prepare a scaffold, and of these, the sponge impregnation technique was chosen for the present work. Polymeric sponges possess an open, trabecular structure that can be used as a template for a ceramic replica through impregnation of the sponge with a slurry of ceramic powders and a subsequent thermal treatment. The ability to soften glasses can be successfully used to attain a good sintering of the ceramic particles while maintaining a sufficient viscosity and thus avoiding the risk of collapsing of the trabecular structure during the thermal treatment.

The scaffold's final properties will depend primarily on the nature of the biomaterial and on the processing parameters; other interesting properties can be attained through the preparation of hybrid materials obtained by loading the scaffolds with collagen, cells or more generally biomolecules. Tissue engineering is the branch of science that studies these hybrids. Osteogenic cells obtained from the host through a biopsy can be multiplied in vitro and seeded onto the scaffolds before implantation.

In addition to osteoproductivity and cell seeding, glass–ceramic scaffolds can be used as delivery vehicles of growth factors such as bone morphogenetic proteins (BMPs) that transform the host precursor cells into bone matrix producing cells [33–35]. In fact, BMPs cannot be successfully used by themselves since they quickly diffuse and disperse from the injection site due to their low molecular weight. Thus, BMPs need a suitable carrier to be maintained and properly released at the implantation site. In this work, new bioactive scaffolds were obtained; these were treated in SBF to deposit HAp onto their surfaces and were then colonised with human osteoblasts to induce bone synthesis. Thirty-eight scaffolds were used as a vehicle for BMP-2 and their effect on osteoblast attachment and on mineralisation was investigated in comparison with scaffolds colonised with osteoblasts alone.

2. Materials and methods

In this research work, glass–ceramic macroporous scaffolds were obtained using an organic template (polyurethane sponge) and bioactive glass powders.

The chosen glass belongs to the system SiO_2–P_2O_5–CaO–MgO–Na_2O–K_2O and has the following molar composition: 45% SiO_2, 3% P_2O_5, 26% CaO, 7% MgO, 15% Na_2O, 4% K_2O. The glass (hereafter referred to as CEL2) was completely characterised in a previous work [36]. Briefly, CEL2 was prepared by melting the raw products (SiO_2, $Ca_3(PO_4)_2$, $CaCO_3$, $4MgCO_3Mg(OH)_25H_2O$, Na_2CO_3, K_2CO_3) in a platinum crucible at 1400 °C for 1 h and by quenching the melt in cold water to obtain a frit that was subsequently ground by ball milling and sieved to a final grain size below 30 μm.

The differential thermal analysis (DTA7 Perkin–Elmer) carried out on CEL2 powders, showed a glass transition temperature ($T_g = 550$ °C), two crystallisation temperatures ($T_{x1} = 600$ °C, $T_{x2} = 800$ °C) and a softening range of 1000–1100 °C.

2.1. Scaffold preparation and characterisation

The chosen organic template is a commercial polyurethane sponge with an open, interconnected macroporosity.

The sponge was cut in blocks of $1.5 \times 1.5 \times 1.5$ cm^3, impregnated with a CEL2 slurry in water, compressed between cylinders to remove the exceeding slurry and then thermally treated to eliminate the organic phase and to sinter the inorganic one, obtaining macroporous glass–ceramic scaffolds.

Polyvinyl alcohol (PVA) was used as a binder to control the slurry viscosity and to optimise the ability of glass particles to coat the sponge.

The scaffold structure was investigated by X-ray diffraction (X'Pert Philips diffractometer) using Bragg Brentano