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Application of β-1,3-glucan in production of ceramics-based elastic composite for bone repair

Research Article

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Abstract: Background: Unsatisfactory surgical handiness is a commonly known disadvantage of implantable granular bioceramics. To overcome this problem, β-1,3-glucan, biotechnologically derived polysaccharide, has been proposed as a joining agent to combine granular ceramics into novel compact and elastic composite. Hydroxyapatite/glucan elastic material was processed and evaluated as a potential bone void filler Methodology: The procedure of composite formation was based on gelling properties of glucan. Its properties were studied using X-ray microtomography, SEM-EDS, FTIR spectroscopy, compression test and ultrasonic method. Sorption index was determined in phosphate buffered saline; bioactivity in simulated body fluid; sterility in growth broth and human blood plasma; implantation procedure in dog model. Results: HAp/glucan composite is sterilizable, flexible and self-adapting to defect shape. It exhibits bioactivity, good surgical handiness, high sorption index and profitable mechanical properties, resembling those of spongy bone. Results of pilot clinical experiment on animal (dog) patients of a local clinic of animal surgery suggested good healing properties of the composite and its transformation into new bone tissue within critical-size defect. Conclusions: The results obtained in this study confirm that flexible HAp/glucan composite has potential as a bone-substituting material. Promising results of pilot clinical experiment suggest that further *in vivo* experiments should be performed.

Keywords: *Hydroxyapatite ceramics • Bone filler • Sorption index • Sterility • Bioactivity*

1. Introduction

Hydroxyapatite (HAp), especially in a porous form, is appreciated as a bone filler due to its biocompatibility, bioactivity, osteoconductivity, minimal risk of appearance of allergic reactions, lack of carcinogenic properties and lack of sensitivity to sterilization processes [1-3]. There are numerous commercially available bone graft materials based on biologic and synthetic HAp, including ProOsteon® (Interpore Cross International, USA), Endobon (BIOMET Orthopaedics, Switzerland), Cerapatite (Ceraver Osteal, France), Synatite (SBM, France) and others. HAp may serve not only as a bone filler but also as a carrier of active substances: antibiotics, chemiotherapeutics, growth factors, *etc.* [4-9]; it can also be used in composites, as a factor increasing their cytocompatibility, bioactivity, osteoconductivity, adhesion of coatings and compression modulus [10-14]. On the other hand, HAp application is often limited due to its relatively poor resorption and slow replacement by the host bone after implantation, substantially high Young modulus, and low fracture toughness [15,16], although these properties may be improved by addition of elasticity-increasing polymers. It is considered that polymer-ceramic composites reveal superior properties (at least in some aspects) over polymer and ceramic

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alone [17]. However, most polymers used for bone filler fabrication meet only some of the criteria for implantable materials.

Bone replacing material such as ceramics or crushed bone offers an insufficient surgical handiness, especially when the orthopedic surgeon has to handle granules (in case of defects in maxillary bone) or scaffolds (rigid and non-adaptable to the implantation site). Some commercially available biomaterials contain the artificial or natural polymers serving as plasticizing agents and thus significantly reduce this inconvenience. Among them, EasyGraft™ (Degradable Solutions SA, Switzerland), Plexur M and P (Osteotech, USA) and Cerapatite-Collagen (Ceraver Osteal, France) can be listed. EasyGraft™ contains PLGA-coated β-TCP granules which hardens into a compact mass *via* partial and temporary PLGA dissolution by organic solvent; Plexur M and P are composed of cortical bone and resorbable artificial polymer; Cerapatite-Collagen, made from HAp granules and naturally-derived collagen fibers, becomes elastic after hydration with blood or saline. Bone or ceramic granules and powders may also be turned into a paste using collagen gels (e.g., Tecnoss® Gel O; Tecnoss, Italy) or fibrin glue [18,19]. However, the natural polymeric compounds used in commercially available composites (collagen, fibrin glue) originate from animal sources; this can be considered as a limiting factor because of the increased risk of transmissible contamination (e.g. viruses) and undesirable immune responses. Thus, the necessity of careful purification of these animal polymers exacts the high price of final product.

To solve the problems originating from low elasticity of HAp ceramics and the risk of negative effects of biologic factors mentioned above, β-glucan may be applied as polymeric phase in such materials, due to its specific gelling properties. β-glucan is a natural, relatively cheap, non-animal and nontoxic polymer biotechnologically produced by *Alcaligenes faecalis*, a bacterium commonly found in soil, water and human-associated environments. It was used in production of dietetic and diabetic food as thickener and stabilizer. In recent years, this polysaccharide attracted growing attention in biomedical and pharmaceutical applications [20]. Encapsulation of theophylline, salbutamol sulfate, prednisolone, indomethacin, doxorubicin and epirubicin with β-glucan has been shown to improve the pharmacokinetics of drugs [21-24]. Moreover, numerous studies reveal positive effect of β-glucans and its derivatives on health, particularly in the field of immunology: they improve wound healing and show antioxidant, antiviral, antibacterial, anti-inflammatory and DNA-protecting activity [25-28]. It also possesses anti-coagulant activity [29]. Morikawa *et al*. [30] found that β-glucan-injected mice produced a high level of macrophages and polimorphonuclear leukocytes, which were spontaneously cytotoxic to mammary carcinoma cells *in vitro*; macrophage stimulation has been also observed *in vitro* in contact with β-glucan-treated plates [31]. However, the use of glucan as a component of implantable bone filler has not yet been reported, although – on a base of available knowledge - it could show profitable healing properties.

The aim of this study was to prepare a biphasic HAp/glucan composite of elastic properties, which would allow for easy manipulation and good adaptation to the shape and dimensions of even large bone defects. Some of its physical and biological properties were examined and presented. Pilot clinical experiment concerning the repair of critical bone defects (oronasal fistula) in animal patients with HAp/glucan material was performed to initially evaluate its healing properties.

2. Experimental Procedures

2.1 Composite preparation and structure studies

HAp-glucan composite samples were prepared according to the procedure described in Patents [32,33]. Briefly, the granules and β-glucan in appropriate proportions were mixed carefully and baked at 100°C for 10 minutes. The samples tested in described experiments contained porous HAp granules and glucan at ratios within the ranges: 43.3-90.9 wt % for HAp and 9.1-56.7 wt % for β-glucan. Microporous HAp granules (0.2-0.6 mm; open porosity 68%; unimodal pore size distribution; surface area 24.94 m/g; average pore size of 0.1 μm) with high ability for water absorption were obtained according to the method described in Patent [34]. β-1,3-Glucan from *Alcaligenes faecalis* (DP 450) was supplied by Wako Chemicals, Japan. Composition of tested samples is presented in Table 1. Space distribution of HAp granules within the structure of composite (sample 3A) and volume of granules (in vol %) were evaluated by 2D X-ray microtomography using Skyscan 1174 apparatus with UDS 1.3Mp FW camera (50 kV; 800 μA; pixel size of 12 μm). Structure and chemical composition of cross-section of 3A sample was studied using SEM-EDS technique. FT-IR spectra of pure gelled β-glucan, HAp ceramics and composite (sample 3A) were obtained using IR spectrometer (Vertex 70, Bruker, USA) in ATR mode, 32 scans with 4 cm^{-1} resolution, at wavenumber range of 370-4000 nm.