Scientific Report for Short Term Scientific Mission

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Development of biphasic and graded scaffolds for bone and osteochondral tissue regeneration

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Introduction

Development of biodegradable and bioactive 3D scaffolds as cell supports for *in vitro* or *in vivo* tissue engineering and regeneration requires a multidisciplinary approach utilizing the knowledge in life sciences as well as engineering principles. Particularly the challenging task is to produce scaffolds for implantation in osteochondral defects since cartilage and bone are adjacent tissues with distinctly different compositions, structures, and mechanical properties.

Scaffolds based on gellan gum (GG) reinforced with nanoparticulated bioactive glass (BAG) aimed for bone tissue engineering have been previously successfully developed [1]. Gellan gum is a biocompatible, natural polysaccharide soluble in hot water, which forms hydrogels by temperature decrease, at pH ~4 in the presence of divalent ions, such as Ca^{2+} [2]. It could be produced as a continuous or in a highly porous hydrogel with macro-pores. The continuous hydrogel is attractive for cartilage tissue engineering providing hydrophilic environment resembling the cartilaginous extracellular matrix. On the other hand, porous structure is attractive for cell seeding and regeneration of bone tissue. However, the porous hydrogel structure exhibits low mechanical properties, which could be improved by addition of BAG. Furthermore, BAG converts to hydroxyapatite (HAp) in the presence of physiological fluids, providing osteoconductive characteristics and supporting vascularization of the newly formed bone tissue.

Therefore, the aim of this STSM was to obtain scaffolds with graded concentration of BAG, which will be suitable for bone/osteochondral tissue engineering. Two strategies for scaffold preparation were investigated: use of the electrophoretic deposition (EPD) technique to obtain BAG concentration gradients and GG hydrogel casting over a porous GG-BAG scaffold so to obtain a bilayered (biphasic) scaffold. The EPD technique, considered as a "*green synthesis*" method due to the use of just several chemicals, has been used for years in the field of biomaterials as an efficient method for HAp coatings on metallic or other surfaces, but recently it has been extended to fabrication of 3D scaffolds aimed for tissue engineering.

During this STMS, 45 different scaffolds were prepared, while the most promising were lyophilized and characterized by SEM and EDS analyses.

Materials and methods

Gellan gum powder (Sigma, USA) was dissolved in distilled water under constant stirring at 90 °C to promote linearization of GG single chains. BAG powders were synthesized using the particulate sol–

gel technique and thermally treated at 600 °C to remove any organic residues. BAGs with70% SiO_2 and 30% CaO (% n/n) were used.

The EPD process was optimized in terms of applied voltage (in the range of 2-10 V), temperature (50-85 °C), time of deposition, stirring rate (50-200 rpm), concentration of GG (1-2 % w/w), and concentration of BAG (0.5-2 % w/w).

After deposition, the obtained deposits were stabilized in phosphate-buffered saline (PBS, Sigma, USA) during 48 h. To produce dried scaffolds, deposits were frozen overnight at −80 °C and freeze-dried for 2 days in a Lio-5 freeze-dryer (Kambič, Slovenia). Microstructure and composition of the obtained scaffolds were analyzed by field-emission-gun scanning electron microscope (FEG-SEM) (JEOL JSM 7600F, USA) equipped with an energy-dispersive X-ray spectrometer (EDXS) system from INCA Oxford Instruments. For that purpose, a thin carbon layer was sprayed over cross-sections of the scaffolds.

The bilayered scaffolds were produced by casting warm 2 % w/w GG solution over porous GG-BAG scaffolds made in Petri dishes. . After gelation by cooling to room temperature, the bilayered scaffolds in the form of discs (12 mm in diameter, 5mm thick) were cored out.

Results and discussion

The voltage value suitable for uniform deposition was at first optimized for production of polymer scaffolds (GG), only. Typical images using the stereo zoom microscope of GG deposits prepared under different voltage values are presented in Figure 1.

Figure 1. Stereo zoom microscope images of cross-sections of freeze-dried GG deposits obtained under low (~ 2 V) and medium voltage (~ 5 V)

When medium voltage was applied, deposition reproducibility was achieved under all other parameters unchanged (temperature, time of deposition and stirring rate). The deposit weight was 9.6 ± 0.9 g and generally, more uniform and thicker deposits were obtained as compared to those obtained at lower applied voltages.

However, the SEM characterization at low magnification (25 x) has shown non-uniform macroporosity within one sample (Fig. 2A) as well as among different samples (Fig.2B). Such non-uniform porosity could be attributed to the manipulation procedure after deposition (freezing and freeze-drying process) and/or high sensitivity of the deposition process by small changes in processing parameters.

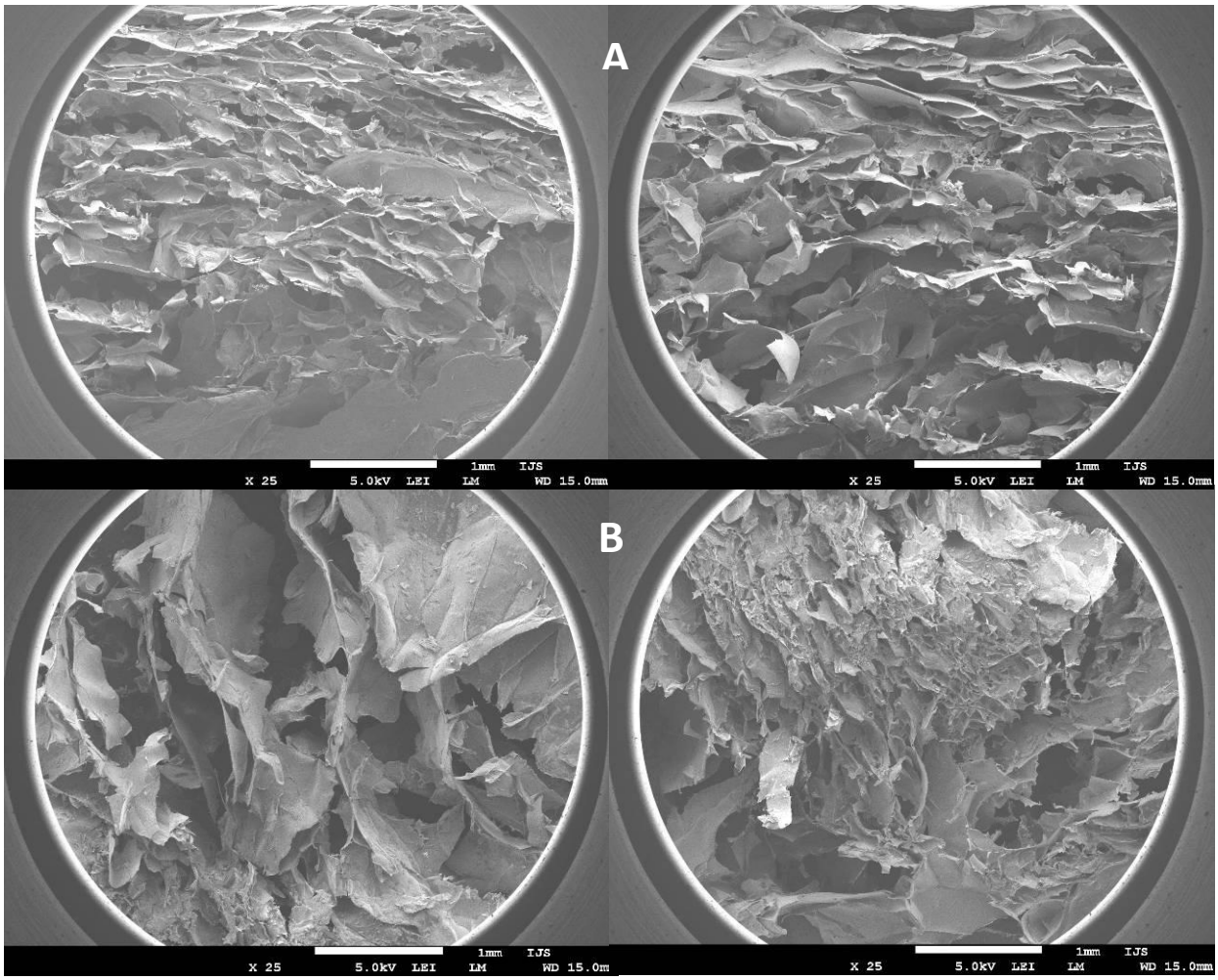

Figure 2. SEM images of different cross-sections of freeze-dried GG deposits obtained under medium voltage – sample A and sample B (scale bar: 1 mm)

The optimal deposition parameters defined for GG polymer only were used for deposition of GG-BAG. SEM characterization has confirmed the presence and uniform dispersion of BAG inside deposits (Fig.3B). However, porosity of the samples was still non-uniform (Fig. 3A).

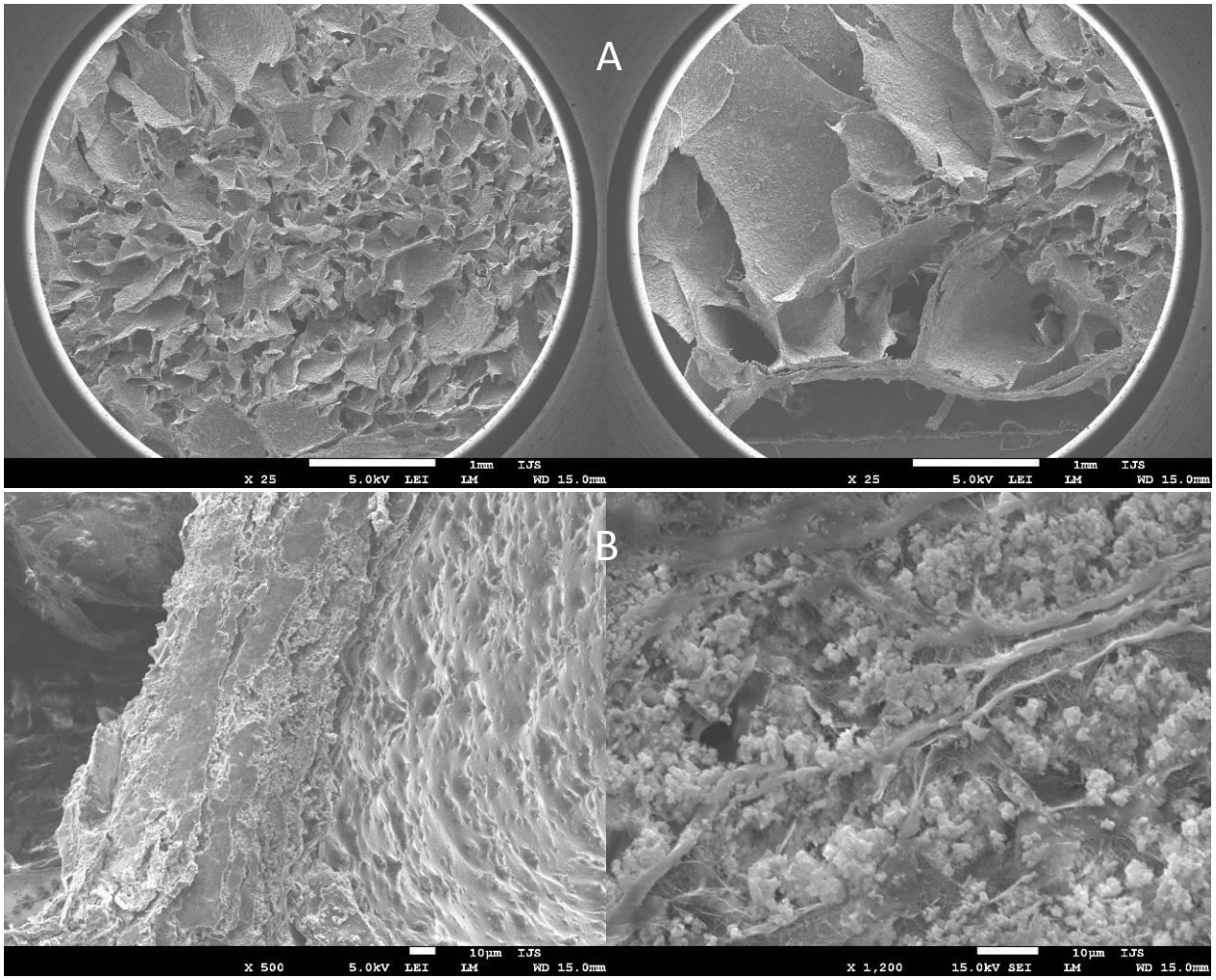

Figure 3. SEM images of cross-sections of freeze-dried GG-BAG deposits confirming the presence of BAG (B, scale bar: 10 μm) and non-uniform porosity of the deposit (A, scale bar: 1mm)

However, graded concentration of BAG within deposits was obtained and confirmed by EDS showing the increasing Si signal (Fig. 4).

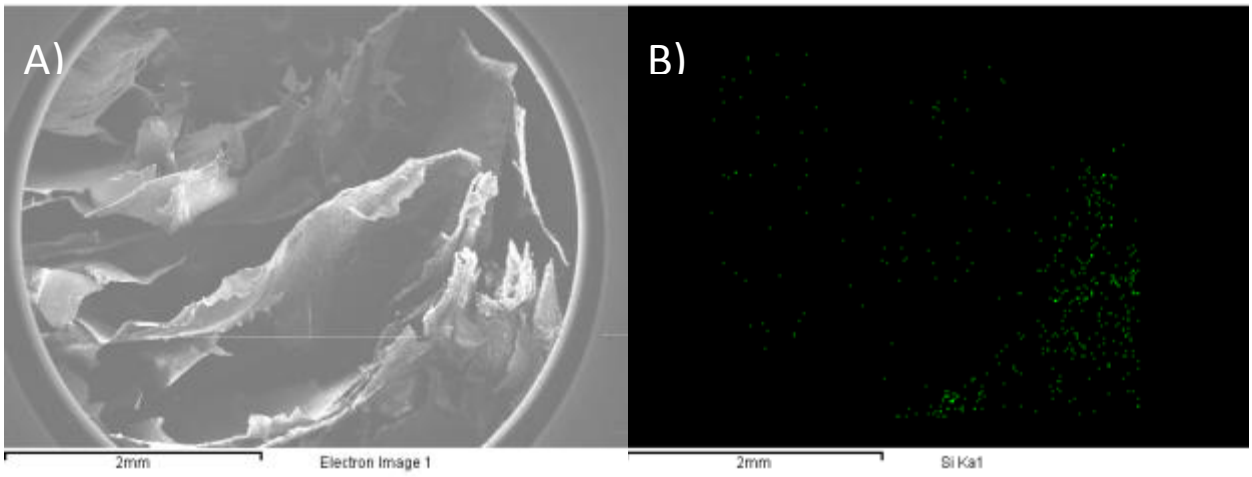

Figure 4. Gradient freeze-dried GG-BAG deposits: A) SEM image of the cross-section; B) EDS image of Si signal confirming the graded concentration of BAG (scale bar: 2 mm).

In the second experimental series, well integrated and compact bilayered scaffolds were produces by optimizing the casting procedure of GG solution over the porous GG-BAG scaffold (Fig. 5).

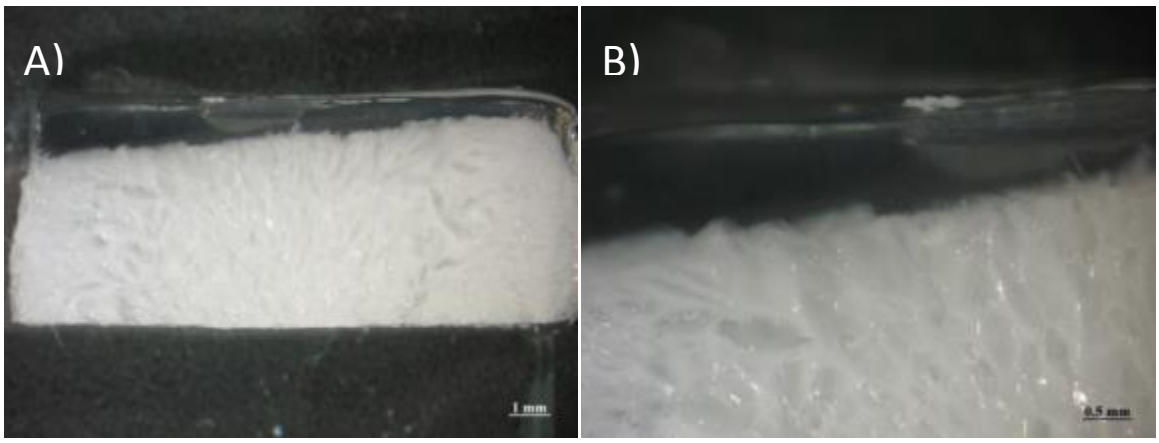

Figure 5. Images of cross-section of a bilayered GG/GG-BAG scaffold obtained by using a stereo zoom microscope: A) the scaffold at low magnification (scale bar: 1 mm); B) the interface between the GG hydrogel and the porous GG-BAG scaffold showing apparently very good integration (scale bar: 0.5 mm).

Good integration between layers and stability of the obtained scaffolds were preserved after 48 h in PBS solution.

Conclusion

During this STSM, new methods for production of graded and biphasic scaffolds based on GG and BAG were utilized and optimized. The obtained preliminary results confirmed the possibility to produce scaffolds by EPD technique based on GG with graded concentration of BAG aimed for osteochondral tissue engineering. We have obtained well integrated and stable deposits, but the non-uniform porosity in different scaffold parts is still an unresolved issue. In addition, stable bilayered scaffolds were produced by casting a layer of GG hydrogel over a porous GG-BAG base. A special advantage of this method is simplicity not requiring any sophisticated equipment. Further studies will be focused on characterization of the obtained scaffolds including studies in biomimetic bioreactors providing conditions imitating those *in vivo* in articular cartilage and/or bone.

[1] Gantar, A., da Silva, L., Oliveira, J., Marques, A., Correlo, V., Novak, S. and Reis, R. (2014). Nanoparticulate bioactive-glass-reinforced gellan-gum hydrogels for bone-tissue engineering. Materials Science and Engineering: C, 43, pp.27-36.

[2] Zhitomirsky, D., Roether, J., Boccaccini, A. and Zhitomirsky, I. (2009). Electrophoretic deposition of bioactive glass/polymer composite coatings with and without HA nanoparticle inclusions for biomedical applications.Journal of Materials Processing Technology, 209(4), pp.1853-1860.