The cultured cells were fixed in 4% paraformaldehyde for 1 h, then washed in PBS and stained with acridine orange solution (1 mg/mL). Examination of the viability and proliferative activity of stem cells was carried out using the test Alamar Blue (Sigma Aldrich, Germany). To the culture medium DMEM / F12 Ham's with 10% bovine serum and 1% antibiotics was applied.

In vivo studies of peripheral nerve regeneration were carried out on groups of male rats (Wistar, each approx. 300 g), and the regeneration of the spinal cord was observed on a group of leopard gecko after implantation in the animal's tail.

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THE INFLUENCE OF PRE-COARSENING ON ARCHITECTURAL AND MECHANICAL PROPERTIES OF HIGHLY POROUS TITANIUM DIOXIDE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Introduction

Highly porous titanium dioxide (TiO_2) scaffolds are very interesting among other bone substitutes. TiO_2 is proved to be fully biocompatible with bone tissue and it promotes proliferation of bone cells [1]. TiO_2 scaffolds can be manufactured by polymer sponge replication method, which enables obtaining materials with preferable pore architecture. However, high porosity significantly reduces compressive strength of the scaffolds [2]. In ceramics, a lot of defects are introduced to the material during sintering, in which powder particles are irreversibly fused into one piece. Any inhomogeneity in green body can lead to formation of microstructural flaws and reduction of mechanical strength of the material [3]. Pre-coarsening of the material can eliminate the smallest pores and particles and allow obtaining more homogenous and fine grained ceramics [4-6].

In the present study, pre-coarsening was used in order to improve mechanical properties of the TiO_2 scaffolds manufactured by polymer sponge replication method. To this end four different dwelling times (2, 5, 10 or 30 h) at 1100°C were tested prior final sintering at 1500°C for 20 h. The main goal of the study was to find out what is the most preferable time of pre-coarsening treatment from the point of view of increase in compression strength.

Materials and methods

Manufacturing of titanium dioxide scaffolds

TiO_2 powder (Kronos 1171, Kronos Titan GmbH, Leverkusen, Germany) was cleaned prior to use in order to remove phosphate ions from the surface of the particles. Raw powder was soaked in 1M NaOH solution and then rinsed several times with deionized water. After drying, powder was sieved and particles between 0-100 μm were collected for further processing. For preparation of the slurry, 65 g of cleaned TiO_2 powder was gradually mixed with 25 ml of deionized water at low stirring rate (1000 pm). After 10 min of initial homogenization, pH of the slurry was measured and adjusted with 1M HCl solution to 1.5-1.7. Stirring was continued for 2.5 h at 15°C and stirring rate of 5001 rpm. Polyurethane sponge (60 ppi, Bulbren S, Eurofoam GmbH, Wiesbaden, Germany) was cut into cylinders (10 mm in height and 12mm in diameter), washed and dried. Templates were immersed in a slurry and compressed few times. Excess slurry was removed by squeezing templates between two polyurethane foam sheets using a self-made device. Dried scaffolds were hated up at 0.5°C/min to 450°C and kept for 1h in order to remove polymeric material. Then scaffolds were heated up at 1°C/min to 1100°C, dwelled for 2, 5, 10 or 30 h (20 samples for each group) and cooled down at -5°C/min. 10 samples from each group were withdrawn, the remaining ones were sintered at 1500°C for 20 h.

Material characterization

In order to investigate the influence of pre-coarsening on architectural parameters of the scaffolds, 5 sintered samples from each group were examined using micro-computed tomography (micro-CT). Samples were scanned using desktop 1172 micro-CT imaging system (Skyscan, Kontich, Belgium). Data were reconstructed using standard SkyScan software (NRecon) and analyzed with standard SkyScan software (CTan). The microstructure of the non-sintered and sintered scaffolds was analyzed using scanning electron microscopy (Hitachi TM3030, Hitachi High-Technologies Corporation, Tokyo, Japan). The main interest was put in compressive strength of the scaffolds. All of the samples were examined using Zwicki (Zwick/Roell, Ulm, Germany) according to DIN EN ISO 3386. Statistical analyses were performed using SigmaPlot 12.0 software (Systat Software Inc., San Jose, United States of America).

Results and discussion

TABLE 1 presents selected architectural parameters of the sintered scaffolds. No statistically significant differences were found between different groups, which proves that the pre-coarsening treatment does not affect architecture of the scaffolds.

FIG. 1 presents microstructure of the selected samples. In the case of non-sintered samples (upper panel), it can be found that after 2 h of dwelling powder particles are barely connected with each other, while after 5 and 10 h, necks between particles are much wider. After 30 h initial sintering of particles occurred. The microstructure of sintered samples is very similar, with the presence of large and irregular grains (lower panel). However, scaffolds heat-treated for 5 h at 1100°C seem to be more uniform than the others.

TABLE 1. Selected architectural parameters of the sintered scaffolds dwelled at 1100°C for different period of time and sintered for 20 h at 1500°C (n=5).

Sample	Strut thickness [μm]	Pore size [μm]	Closed porosity [%]	Open porosity [%]
2 h at 1100°C 20 h at 1500°C	63.8 ± 6.7	437 ± 7	0.91 ± 0.28	90.1 ± 1.0
5 h at 1100°C 20 h at 1500°C	61.6 ± 3.1	427 ± 22	0.78 ± 0.29	90.3 ± 1.1
10 h at 1100°C 20 h at 1500°C	61.4 ± 8.1	434 ± 11	1.23 ± 0.39	90.1 ± 1.0
30 h at 1100°C 20 h at 1500°C	62.0 ± 5.1	431 ± 24	0.97 ± 0.29	89.7 ± 1.0

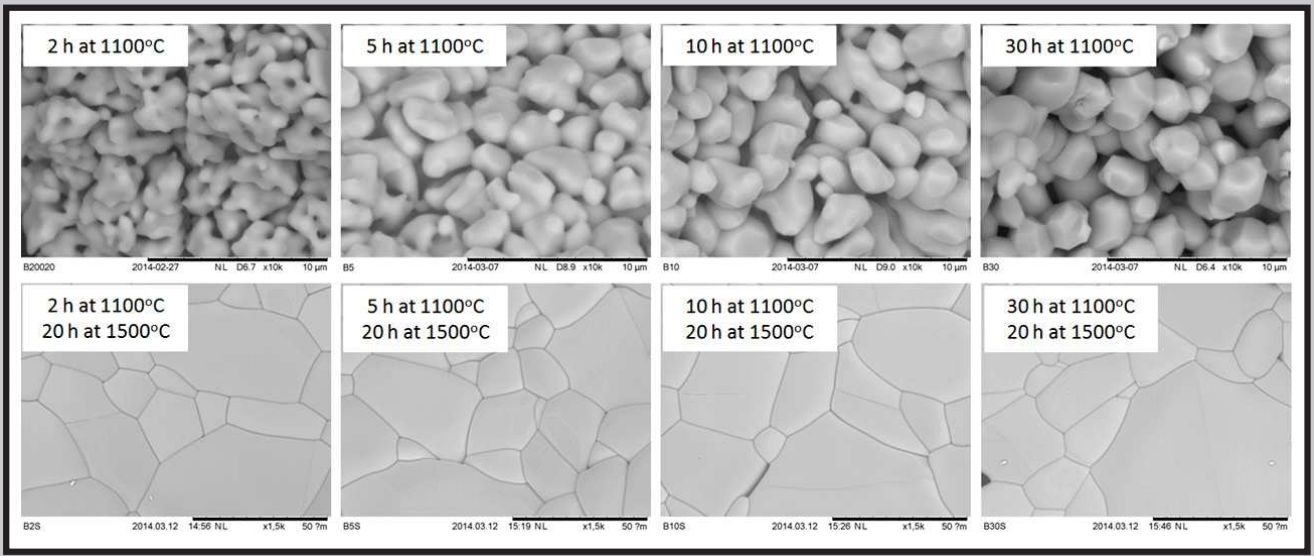

FIG. 1. SEM images of samples after pre-coarsening phase (upper panel) and corresponding samples after sintering (lower panel).

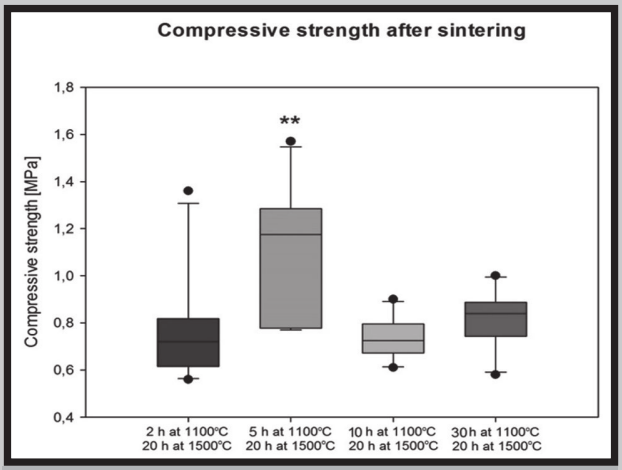

FIG. 2. Comparison of compressive strength of scaffolds after sintering (P < 0.01 against all other groups). The bottom and the top of the box represent the first and third quartiles, the band inside the box - the second quartile (median). The whiskers stand for the minimal and maximal values, excluding outliers (values lower than 3/2 of the first quartile or greater than 3/2 of the third quartile, marked with dots).**

Compressive strength of non-sintered scaffolds was very similar for all groups, but in the case of sintered samples significant differences were found (FIG. 2). The highest compressive strength was measured for the samples dwelled at 1100°C for 5 h. One-way ANOVA confirmed that difference between group pre-coarsened for 5 h and the other groups was significantly higher with the probability level $P<0.01$. No significant differences had been found between groups dwelled for 2, 10 and 30 h.

Improved compressive strength of the scaffolds pre-coarsened for 5 h at 1100°C resulted probably from more uniform microstructure. Higher concentration of grain boundaries inhibited propagation of the cracks and strengthened the material. When large grains were present, cracks were more likely to propagate through the grains and they spread more easily in the whole volume of the material.

What is noteworthy, closed porosity of the scaffolds pre-coarsened for 5 h was slightly lower than in the other samples, which indicates that densification of the material and porosity reduction was the most successful after 5 h of pre-coarsening. Increase in dwelling time could cause initial sintering of particles, isolation of pores and in the end, might preclude effective densification.

Conclusions

Pre-coarsening did not affect architectural parameters of the titanium dioxide scaffolds such as pore size or porosity. However, it significantly improved the compressive strength of the scaffolds. 5 h of dwelling at 1100°C followed by sintering at 1500°C for 20 h was found as the most favorable in terms of microstructural and mechanical properties of the scaffolds.

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CARBON FIBROUS MATERIAL FOR CARTILAGE TISSUE TREATMENT

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Abstract

The work presents in vitro and in vivo experiments related to the evaluation of the biological properties of the two groups of carbon fibrous (micro, nano) materials. We investigated the carbon materials in the form of a biomimetic scaffolds made from carbon nanotubes and a composite membrane made from carbon micro-fiber and biocompatible polymer to induce regeneration of missing cartilage tissues. Evaluation of biological properties of both materials clearly showed that carbon fibrous material is biocompatible with cartilage cells and stimulates regeneration of cartilage tissue.

Keywords: *cartilage, chondrogenic materials, tissue engineering, carbon fibrous composite*
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Introduction

Reconstruction of upper respiratory tract in case of neoplasms, traumas (of mechanical, thermal or chemical origin), as well as post-intubation and post-trauma stenoses is a medical problem, which still remains without a solution. The materials used so far (autogenic as well as plastic ones) fail to give results that would be satisfying for patients as well as doctors, in early as well as late follow-up. Developing a biologically active material for making up the defects in upper respiratory tract shall allow to reconstruct the defective structures, which occurred in patients due to neoplasms, as well as in treatment of stenoses or traumas of mechanical, thermal and post-intubation origin. As is it evident from our previous studies and papers of other authors, the carbonaceous material in fibrous forms (micro and nano) were successfully applied in the treatment of defects of cartilage. Numerous findings support the hypothesis that fibrous carbon components due to their unique chemical and physical properties (biomimetic form, functional groups on the surface, electrical conductivity, thermal conductivity, mechanical properties) can act as chondrogenic materials [1-7].