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Properties of calcium carbonate-containing composite scaffolds

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Bone grafting in skeletal reconstruction has become a common task of orthopedic surgeon. Three-dimensional, porous, degradable scaffolds are often used to provide support while the new tissue can be formed in situ. There are numerous materials and techniques involved; however, each has certain drawbacks. One of the patented ceramic bone grafts is made of coral that has many benefits, e.g., its chemical and surface structure similar to that of the cancellous bone, extremely good biocompatibility and optimal pore-size. The drawback, being difficult to overcome, is the manufacturing to the desired shape. In order to maintain the advantageous chemical composition, but to overcome these difficulties, we have manufactured polymer-ceramic scaffolds both by solvent casting and by melt mixing and particulate leaching. The scaffold morphology was examined using scanning electron microscope (SEM), while the compressive properties were chosen to validate these substrates mechanically.

Key words: biocomposite, bone grafting, calcium carbonate, scaffold, tissue engineering

1. Introduction

With over 800,000 grafting procedures carried out each year in US alone, the need for surgical reconstruction or replacement is often the result of trauma, pathological degeneration, or congenital deformity of the tissue [1]. Current treatments, although fairly successful, do not provide optimal therapy. These treatments typically rely on donor tissues obtained either from the patient or from another source. The former raises the issue of supply, whereas the latter poses the risk of rejection and disease transfer. This has prompted orthopedic surgeons and scientists to look for viable alternatives [2].

Tissue engineering is one of the most rapidly developing fields of the health care. There are several definitions of tissue engineering; one of the earliest by WILLIAMS is "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function" [3], or a more certain definition by LANGER is "the application of biological, chemical and engineering principles to repair, restore or regenerate living tissues, used biomaterials, cells and factors alone or in combination" [4]. Although each definition is slightly different, each shares common elements: the repair or replacement of living tissue, the application of interdisciplinary principles, and the use of natural and/or synthetic polymers. The aim of tissue engineering is to create tissues, organs and synthetic grafts under laboratory conditions to overcome the difficulties resulted from the lack of donors and current options.

The biomaterial which can be used as a bone substitute is a fast developing discipline of tissue engineering. The guided bony tissue regeneration has major importance, as this tissue is repaired oftentimes. It has relatively simple structure; this simplicity could be beneficial, as there is no need to force the different sort of cells to penetrate in other directions; "only" the

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bone forming cell (osteoblast) needs to penetrate and create the bony tissue.

The guided bone tissue regeneration has different tasks. The basic idea is to create a 3D porous network with desired pore-size, pore-interconnectivity, porosity, mechanical, physical and chemical properties, biocompatibility and degradation characteristic. Although there are several choices of the substances, none of them is appropriate, that is why a progress is needed. Many approaches have been adopted, e.g., surface modification, reinforcements, fillers, blends, etc.

In general, about the scaffold we can state that it must be highly porous with interconnectivity, should have adequate mechanical properties, and appropriate degradation characteristic. Porous scaffolds can be obtained by several methods, e.g., particulate leaching techniques [5], electrospinning [6], freeze-drying [7], and solvent casting/particulate leaching [8] – throughout the manuscript the solvent casting and phase separation process are used as synonyms. Each has certain benefits and drawbacks; for example, the particulate leaching technique do not require highly sophisticated equipments to obtain highly porous scaffolds; furthermore the pore-size is dependent on the porogen fraction and can be combined with several methods such as solvent casting, phase separation or melt processing. However, significant amount of slat crystals can stayed back in the scaffold resulting in inflammatory reactions.

The targets of our study are to incorporate an inorganic phase into polycaprolactone scaffolds in order to enhance their biodegradative and mechanical characteristics. For scaffold preparation, solvent casting methods were chosen based on acetone as the solvent of polycaprolactone (PCL). The pure PCL specimens obtained were the reference, and the composite scaffolds made of calcium carbonate and PCL mixture were mechanically – based on compressive properties – compared to them. Composite scaffold obtained by melt mixing will be shortly introduced here as comparison; however, its detailed discussion will be the topic of another paper.

2. Materials and methods

2.1. Materials

PCL of a nominal number-average molecular weight M_n = 80 kDa and weight-average molecular weight M_w = 130 kDa was obtained from Sigma-Aldrich. It was dried under reduced pressure at 40 °C before use.

Calcium carbonate ($CaCO_3$), in the form of calcite powder, was purchased from Fulka, and was dry grinded before use. Sodium chloride (NaCl) was used as pore-forming agent, with an average grain-size of 125–250 μm. Acetone and distilled water were of analytical grade.

2.2. Scaffold processing

In order to prepare pure PCL and composite scaffolds, two types of manufacturing methods, i.e., solvent casting and particulate leaching techniques, were applied. In the case of solvent-based processes, the manufacturing began with dissolving PCL in acetone at the acetone to polycaprolactone weight ratio of four to one. In our earlier study [9], this concentration was found to have superior properties compared with the scaffolds made of either less or more concentrated solutions. Later, in the case of PCL scaffolds, the solution was poured into a stainless steel tool, which was immersed into water untill the polymer had underwent solidification. In the case of composite, first the solution was mixed with calcium carbonate to obtain a homogeneous slurry, and then this slurry was put into the tool, which was immersed into water until total solidification had occurred. Prior the experimental tests the scaffolds were totally dried.

The particulate leaching technique was combined with melt mixing. In order to obtain PCL-salt or PCL-salt-$CaCO_3$ mixtures, Brabender type internal mixer was utilized at 120 °C until torque equilibrium had been reached. The prepared mixture was hot pressed by the Collin type compression moulding equipment (at 100 bar pressure for 10 min, then cooling) to obtain specimens. The specimens were immersed to water for salt extraction, and after the salt-extraction the sample was dried under reduced pressure at 40 °C until the mass-change had not been measurable.

2.3. Analytical techniques

Compressive tests were performed according to ISO standard. The test conditions were set as suggested. The test was carried out at room temperature at 1 mm/min crosshead speed by a Zwick Z020 universal testing machine. The Young's modulus was calculated:

$$E_c = \frac{H}{A} \cdot \frac{F_h - F_l}{L_h - L_l}, \quad (1)$$

$$\sigma_c = \frac{F}{Z}, \quad (2)$$

where:

E_c – compressive Young's modulus,
H – initial height,
F_h – higher force,
F_l – lower force,
L_h – higher strain,
L_l – lower strain,
σ_c – compressive yield strength,
Z – cross-section.

Scanning electron microscopic images were taken about the surface and cross-cut morphology of the specimens by JEOL 6380LA instrument. Prior the test JOEL JFC-1200 fine coater was used to form a metal layer on the specimen. The magnification ranging from 18 to 20000× was applied. The pores were investigated in the range of 100 nm up to 1000 μm. In order to investigate the correlation between the pore and processing parameters, the built-in software was used.

3. Results

The structure of PCL scaffolds prepared by solvent casting is dominated by micropores, which appear as a result of solvent evaporation (figure 1). The micropores, in the range of 10–50 microns, are randomly distributed in the structure with high interconnectivity. The drawback of the pure sample is the unpredictable structure of the product; the shrinkage of polymer is greatly affected by the homogeneity of the polymer solution, but it also depends on the speed of processing (during processing the solvent can evaporate resulting in a concentrated solution with lower shrinkage).

The composite scaffold was obtained by the same method, and a continuous polymer-mineral matrix formed is shown in figure 2a. No formation of separate large particles was observed, the calcite grains were more uniformly dispersed in the polymer matrix, and the interfacial connection in these scaffolds proved to be better than previously. On the micron scale (figure 2b) the porous structure of the sample can clearly be seen. The synthesized samples are microporous. The porosity of calcite-filled composite scaffold was found to be only slightly dependent on the filler content. The values were in the range of 50–60%, i.e., even somewhat lower than in filler-free specimens obtained by the same method. It seems that the filler influences, to some extent, the solvent casting process. Despite the fact that most of the pores appear as a result of phase exchange, some increase in the porosity of composite with the filler seems to indicate that the filler itself at higher concentration does contribute to pore formation, probably due to the formation of free spaces between its particles which are not penetrated by the PCL solution and there remain the voids of polymer after the process is completed. Nevertheless, the overall porosity of these samples is lower than that observed for solvent cast and particulate leached scaffolds, where both micropores and macropores are formed.

The second manufacturing method, applied both in the case of polymer and composite, was the particulate leaching combined with melt mixing. The structure of the polymer and the composite scaffold is nearly the same (figure 3A and 3B). These scaffolds have both micro- and macropores, being reported by several authors [2], [5], [10] as important factor of cell attachment. The macropores, formed due to the pore-forming agent, mainly dominate the structure and correlate well with its shape. The micropores can appear due to the porogen cleavage during the manufacturing or due to the grains, which are not located in the same plain as the majority of porogen crystals.

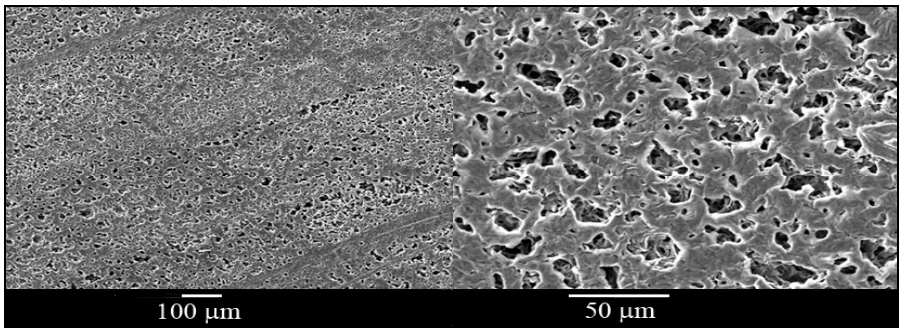

Fig. 1. SEM image of surface morphology of pure PCL scaffold (made of 20 w% PCL- acetone solution), A: 80× magnification; B: 50× magnification

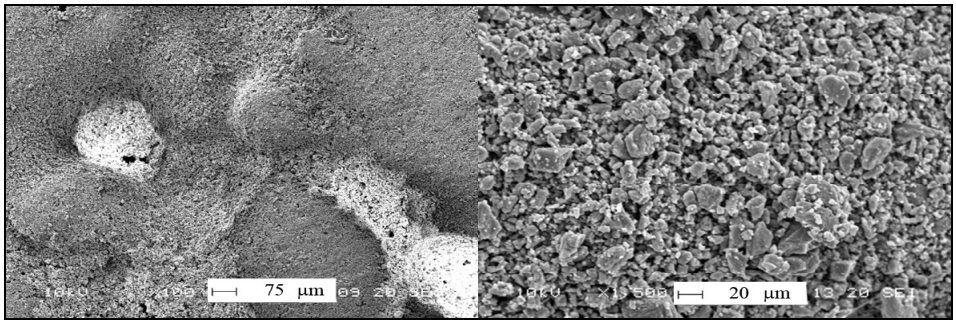

Fig. 2. SEM images of the cross-section morphology of calcite-filled composite scaffold prepared by solvent casting, at calcite powder content of 33 w% and solution concentration of 20 w%, A: 1500× magnification; B: 100× magnification

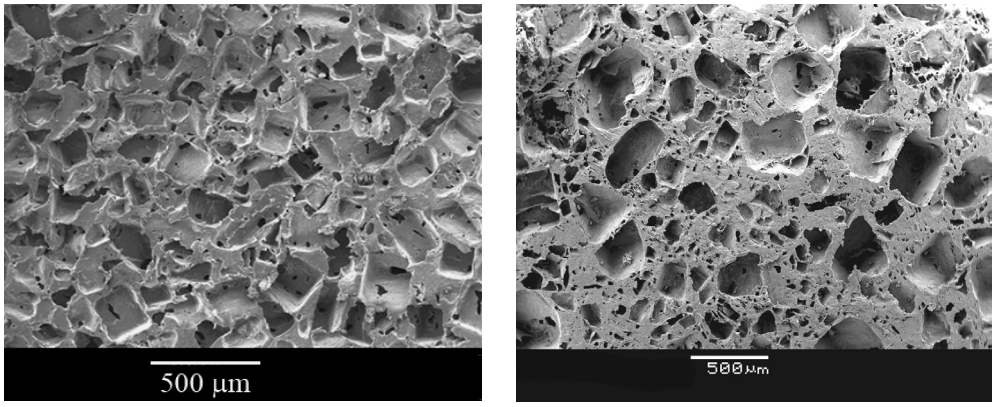

Fig. 3. SEM images (100× magnification) of the cross-section morphology of scaffolds prepared by particulate leaching, A: pure PCL, B: calcite-filled composite

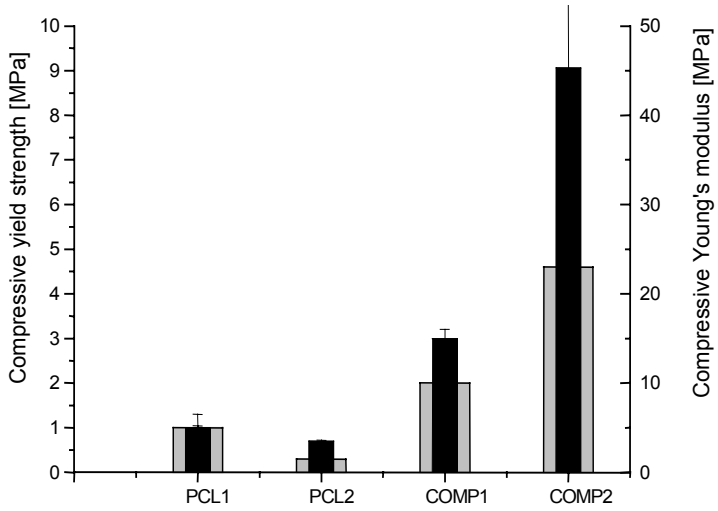

Fig. 4. Compressive characteristics of various scaffolds:
PCL1-pure PCL scaffold by solvent casting, PCL2-pure PCL scaffold by particulate leaching,
COMP1-composite scaffold by solvent casting, COMP2-composite scaffold by particulate leaching

The mechanical performance of the scaffolds obtained was characterized by compressive yield strength and Young's modulus, because the most probable load of the human bony tissue is compression. Minimum of 20 independent samples were measured to determine the average values and the standard deviation. The porosity of specimens approximated to 65%. Figure 4 comprises the results.

The mechanical stability of pure PCL scaffolds, obtained either by solvent casting or by particulate leaching technique, is rather low. The higher value was measured in the case of scaffold obtained by sol-

vent casting method. This can be a result of its structure, as in the case of solvent casting no damages of the structure were found, because as the acetone is removed from the scaffold, the polymer solidifies. In the case of melt mixing and particulate leaching, failure cell-walls were found, which indicated that the deformation had begun, lowering the mechanical properties of scaffold.

In the case of composite scaffolds, the results were opposite. Young's modulus of the solvent casting-based scaffold approached 16 MPa, while that of the composite scaffold, prepared by melt mixing and particulate leaching technique, exceeded 50 MPa. In this case, the reverse relationship can be explained by the surface energy and aggregation of calcium carbonate. During melt mixing the calcium carbonate particles were homogeneously mixed in PCL due to a high shear stress, which was not possible during the hand mixing of the slurry preparation. Moreover, is should be stressed that the adhesion of PCL to filler particles decreased because of the solvent-based scaffold preparation.

In general, based on the results obtained, it can be inferred that pure PCL scaffolds cannot be used in load-bearing application. The composite scaffolds, however, have significantly better mechanical properties, and their strength and Young's modulus are over the lower limit of the human cancellous bone [2], [10], so they may be applied to replace a broad range of bones.

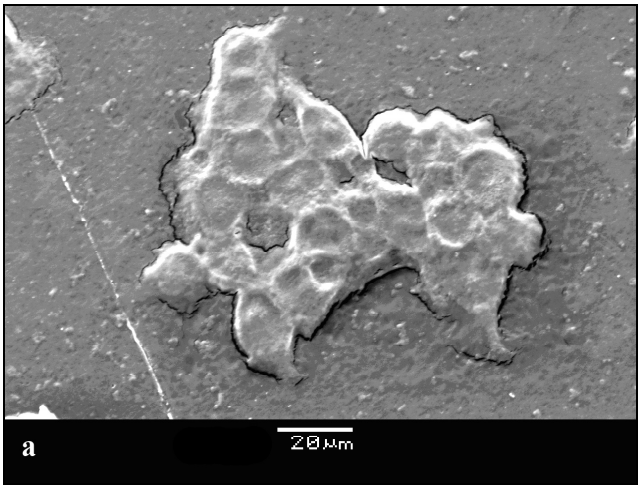

Fig. 5. Osteoblast cells attached to the surface

Preliminary biocompatibility tests were also carried out, and the osteoblast cell adhesion to scaffold surface was validated (figure 5). As it turned out, the human cells attached to these scaffolds due to their high biocompatibility. The calcium carbonate content made the rate of cell adhesion somehow higher compared to that of pure PCL, whose properties are comparable to those of tissue culture polystyrene.

4. Conclusions

Both polymer and composite scaffolds were produced either by solvent casting or by particulate leaching technique. These scaffolds were structurally and mechanically compared. The structure of PCL scaffold obtained by solvent casting can be characterized only by micropores, in the range of few ten micrometers; while the composite scaffolds obtained by the same method have pores in the range of few micrometers. Micropores are present, but the macropores are dominant in the case of scaffolds made by particulate leaching technique; and both polymer and composite scaffolds have the same structural properties. The major mechanical load is compression, therefore compression tests were performed. In the case of pure PCL samples, the solvent casting process was found to be superior to particulate leaching technique, as the compressive strength and Young's modulus were slightly higher in the case of this manufacturing technique. A small amount of calcium carbonate could enhance the mechanical properties of scaffolds significantly; however, the processing method had also a significant influence on the end-product properties. With melt mixing and particulate leaching techniques, it is possible to prepare the scaffolds of nearly the same compressive characteristics as those of the natural human cancellous bone [10]. The attachment of osteoblasts to the scaffolds produced was also confirmed.

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