Investigation of Calcium Carbonates Enhanced Poly(ε-caprolactone) Materials for Biomedical Applications

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Summary: In this study poly(ε-caprolactone) – calcium-carbonate composites were obtained by melt-mixing. Two crystal-modifications of calcium-carbonate were used, namely calcite and aragonite. Compressive and tensile tests were executed on samples with various compositions to analyze the effect of filler content and particle geometry. Both minerals improved the compressive modulus and strength significantly, however the influence of calcite was superior. The tensile modulus was also highly increased. The elongation at break remained high even at 50 wt% aragonite filling, but decreased with two orders in the case of calcite. Biocompatibility tests were also carried out with human osteoblast cells and the results were promising. The relative cell number increased due to calcium-carbonate. Both filler material is able to enhance the mechanical and biological properties of poly(ε-caprolactone) significantly. Aragonite samples remained more ductile compared to calcite ones, but the calcite filled scaffolds are stiffer, stronger and slightly more biocompatible than aragonite filled materials.

Keywords: aragonite; biodegradable polymers; calcite; mechanical properties; poly(ε-caprolactone)

Introduction

Degradable polymeric biomaterials are thought optimal candidates for medical devices such as sutures, coronary stents, bone plates and screws, controlled/sustained release drug delivery vehicles and three-dimensional porous scaffolds for tissue engineering. However each is important, bone tissue engineering, which is one of the most rapidly developing fields of medicine, has a major relevance of, because the lack of donors.[1] Bone is a unique 'dynamic' tissue due to its continuous remodeling. Under mechanical load it builds up, but without stress it disintegrates. Therefore, in bone tissue engineering the mechanical properties (especially the compressive ones) of grafts are utmost important to create a bone-mimic material.

The compressive modulus of cancellous bone varies from 50 to 600 MPa, and its compressive strength ranges between 2 and 12 MPa. The properties of bone however particularly depend on age, sex, loading, porosity etc.[2] To match these basic characteristics various metals, ceramic and polymers were used and synthesized. The disadvantage of ceramics is their brittleness and too high stiffness, while polymers mostly have good toughness and processability, but low strength, as well. The properties of implant materials might be tailored through the prosperous use of these two material classes.

Poly(ε-caprolactone) (PCL) is a semicrystalline polymer, and is of great interest because it is easily processible, soluble in a wide range of organic solvents, has a low melting point (55–65 °C) and glass transition temperature (−60 °C), in addition to its ability to form blends with a wide range of

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polymers. The tensile modulus of PCL is around 300 MPa, but it is a very ductile material; its elongation at break is over 700%. PCL has good biocompatibility comparable to those of tissue culture polystyrene. The polymer undergoes hydrolytic degradation because of the presence of hydrolytically labile aliphatic ester linkages; however, the rate of degradation is quite slow, 24–36 months. According to Hutmacher[3] the bone remodeling lasts at least for 6 months, thus the scaffold must retain its properties at any rate for this time. Since the total loss of mechanical properties occurs after 9–12 months in PCL, this material might be adequate for bone tissue engineering approaches.

The influence of mineral fillers has been studied in numerous papers. In our previous work[4] calcium-carbonate reinforced scaffolds were manufactured by phase-exchange technique. In that work the aragonite reinforced samples provided both better compressive modulus and strength values than the neat or the calcite filled PCL templates. In contrast, Calandrelli et al.[5] found that PCL reinforced with the powder form of hydroxyapatite (HA) proved better tensile properties than those with HA whiskers. The results were interpreted by the poor adhesion between the matrix polymer and whiskers.

The main target of the current study is to investigate the mechanical properties of calcite and aragonite filled PCL samples, to clarify differences for further studies, and to test their influences on the biocompatibility of these substrates.

Experimental Part

Materials

PCL of a nominal number average molecular weight $M_n = 80\,kDa$ (density: $1.15\,g/cm^3$) was supplied by Perstorp Caprolactones, Perstorp UK Ltd. Before use the polymer was dried at 40 °C over a day.

Calcium carbonate was used in two crystal forms. Calcite filler was purchased from Sigma-Aldrich Inc. The median diameter of spherical particles was measured $2.3\,\mu m$ (on the left of Figure 1). Aragonite filler (on the right of Figure 1) in the form of needle-like crystals, called whiskers, was kindly supplied by Bioceramic Department of the Institute of Glass and Ceramics (Warsaw, Poland). The median diameter of crystals was $7.9\,\mu m$, the length varied between 30 and $50\,\mu m$. The major differences of properties are originated of different lattice structures. Calcite has rhombohedral lattice and a density of $2.71\,g/cm^3$. Aragonite has orthorhombic lattice, and its density is $2.95\,g/cm^3$. It is noteworthy that the naturally occurring form of calcium carbonate is calcite; therefore precipitation method was applied to obtain aragonite whiskers.

Specimen Processing

In order to have exact compositions, analytical balance (OHAUS Explorer,

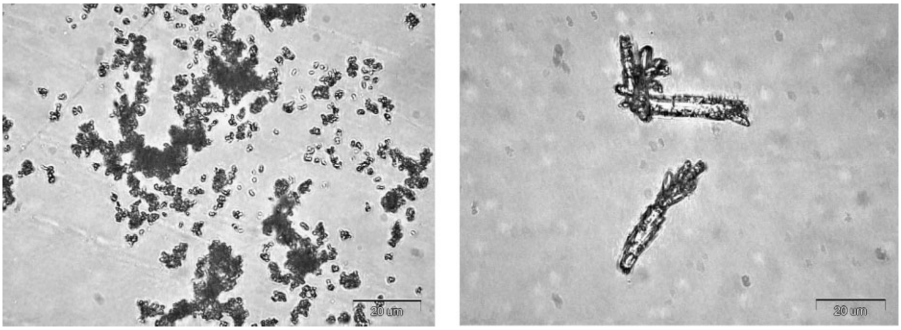

Figure 1.
Calcite (left) and aragonite (right) particles by optical microscope.

accuracy of ±0.1 mg) was applied to measure the materials prior processing. The polymer pellets were used neat or dry-mixed with appropriate amounts of calcium carbonate. The neat-PCL, or the mixture was kept in an internal-mixer (Brabender PL2000) at 100 °C and 25 RPM for 15 minutes. Following the homogenization, the mixtures were hot pressed at 100 °C temperature and at 50 bar pressing-pressure using a COLLIN P-200E-type compression molding machine. Hot-pressing of samples was followed by 10 minute water-cooling. Three types of specimens were obtained.

First, cylindrical samples, which have diameter of 6 mm and height of 7 mm, were pressed. These specimens were used to determine the compressive properties of the compositions.

The second type of specimens was designed type 1BA tensile specimens according to ISO 527–2:1993. In this case, 2 mm of plates were pressed, and from the pressed plates the specimens were worked out. Samples had dumb-bell shape, having a length of 100 mm and a width of 5 mm.

For biocompatibility tests 1.5 mm thick disks having 6 mm diameter were prepared in medical grade stainless steel tool. The specimens were sterilized by 25 kGy dose radiation in cobalt chamber.

Analytical Techniques

The compressive properties of specimens were measured at ambient conditions (23 °C, RH = 45%) at crosshead speed of 2 mm/min by a Zwick Z020 universal testing machine. The test conditions were set and the results were also determined according to ISO 604:2002 standard.

Tensile tests were performed according to ISO 527 standard. Both the sample geometry and the test conditions were set as suggested. The test was done at room temperature at 100 mm/min cross-head speed by a Zwick Z020 universal testing machine. The initial modulus was calculated between $\varepsilon = 0.0005$ and $\varepsilon = 0.0025$.

Scanning electron microscopic images were taken of the surface and the crosscut 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 20000x was applied.

Human ostecaromic cells were used to investigate the biocompatibility of PCL matrices. The experiments were performed in 24-well polystyrene culture plates (TCPS-tissue culture polystyrene, Sarsted). Prior the cell inoculation the sterile samples were pre-treated with RPMI1640 medium (Sigma) supplemented with 10% foetal calf serum, 1% antibiotic mixture (Sigma) in humidified carbon dioxide atmosphere. The cells were seeded at 10^5 cells/ml/well onto sterilized PCL. After two days the medium was changed. For the next seven days the cells were maintained in culture medium at 37 °C in a humidified carbon dioxide atmosphere. At the fixed time, the cells were re-suspended by Trypsin-ETA solution (Sigma) and the cell-growth was determined by cell numbering in Bürker chamber.

Results and Discussion

The most probable mechanical load in human bone is the compression. As mentioned above PCL has good biocompatibility but its mechanical performance is rather limited, therefore mineral filler materials were applied to overcome this issue. Generally, the filler material can increase the modulus of polymers because the mobility of molecular chains is significantly detained.

The compressive strengths and modulus' are plotted against the filler content in Figure 2. Both compressive modulus and strength was increased significantly due to the filler incorporation. Composites containing 50 wt% of calcium carbonate had modulus' from 170–220 MPa, which is high compared to those values of neat PCL, c.a 80 MPa. The compressive strength values were improved from 10 MPa to 17–22 MPa. Each compressive characteristic was

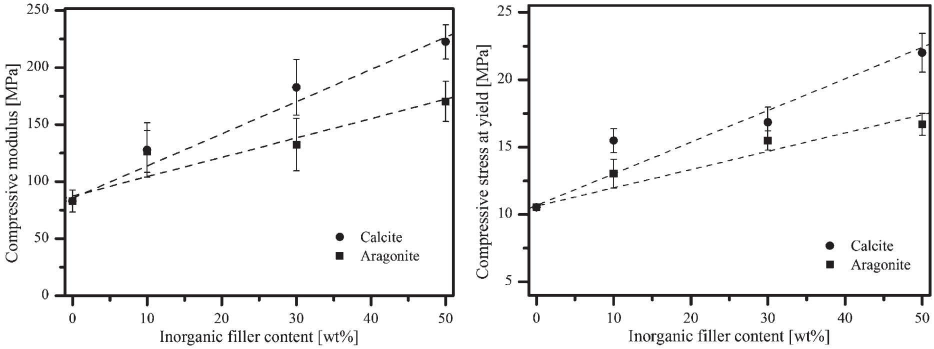

Figure 2.
Compressive modulus (left) and compressive yield strength (right) of samples as a function of filler content.

enhanced more by calcite than by aragonite. These findings are contradictory to our prior observations[4] that the aragonite had superior influence on mechanical properties compared to calcite. This phenomenon can be explained by different processing methods applied in the two studies. At phase separation of scaffolds[4] both fillers were immersed in the same solvents which can reduce the surface energy of the filler lowering the interfacial adhesion. As both fillers were treated the same that may have resulted in equivalent interfacial properties. In this study, the fillers were not opposed to any chemicals during scaffold processing. Aragonite was obtained from calcite by participation method, which resulted in worse surface properties of such fillers.

Although the main macroscopic load in bone tissue is the compression, the tensile properties can not be neglected, either. In cell walls of porous bone replacements complex microscopic stresses occur which are a superposition of compression, tension and bending; therefore each of these characteristics should be known to determine the expected performance of implant material.

The tensile modulus is plotted versus mineral content in the left of Figure 3. The tensile modulus of aragonite filled polymers increased nearly linear, but that of calcite filled samples fit to an exponential curve. If the adhesion is adequate the modulus increases as an exponential function of the filler material, and the modulus linearly in case of worse interfacial adhesion. Both fillers increased the compressive modulus of PCL, significantly. 50 wt% mineral content samples match the upper limit of characteristics of cancellous bone and thus they are promising candidates for further scaffold processing.

In the right of Figure 3 indicates the yield characteristics of samples. Pure PCL deviates from trend in both cases which suggests filler induced material structure or morphology change. It may be assumed that the particles induced interfacial crystallization of polymer which resulted in different morphological structure and disparate properties. The yield strength of aragonite filled materials decreased with increasing filler content. In the case of calcite filled polymers the experimental results were inverse. Pukánszky and Vörös[6] described similar observations. It was assumed that yielding occurs in the filled system when the maximum stress reaches the yield stress of matrix (σ_{y0}). The external load (σ) necessary to induce yielding is:

$$\sigma = \sigma_{y0}\frac{1-\varphi}{1-k\varphi}, \tag{1}$$

where φ is the volume fraction of filler and k is matrix/filler interaction related constant. In the case of composite containing rigid particles adhering to the matrix, a signifi-

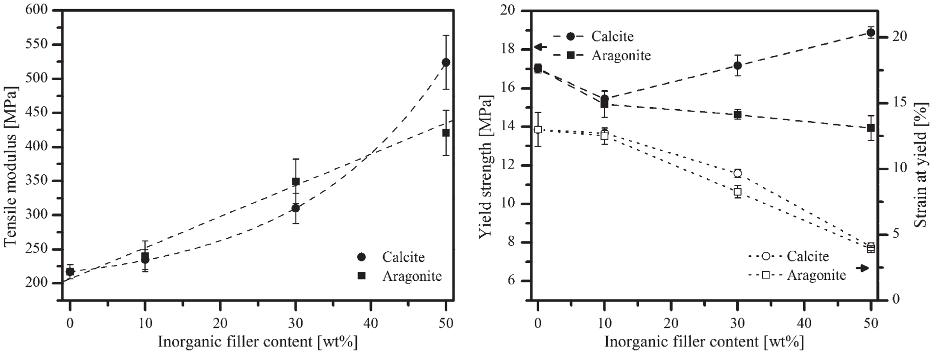

Figure 3.

Tensile modulus (left) and tensile yield strength and tensile strain (right) of samples as a function of filler content.

cant part of the load is carried by the particles, and yield stress increases with increasing filler content and $k > 1$. If, however, soft inclusions or voids are present, the entire load is carried by the polymer, and yield stress should decrease with composition; in such cases, $0 \leq k < 1$. The increasing yield strength of calcite filled materials suggests good adhesion between the particles and the matrix. In the case of aragonite slight decreasing is observable which suggests poor interfacial connection between the components. The aragonite filler act rather as void than reinforcing particle. This phenomenon can be explained either by surface properties (surface free energy) or by specific surface. Assuming calcite as sphere and aragonite as cylinder, the specific surface of calcite is approximately 20,000 cm^{-1} and that of aragonite is about 5,500 cm^{-1}. These values indicates that the applied calcite particles have an order of magnitude greater specific surface than the aragonite whiskers, and it is responsible for the different adhesion.

Figure 4 indicates the tensile properties at large deformations. The small increase in break-strain at 10 wt% filler content can be explained by either the previously mentioned structure change of matrix polymer or by toughening effect of rigid particles. The concept of toughening effect of rigid particles was described by Kim and Michler.[7] Rigid particles must debond and create free volume in the blend which is similar to the cavitation mechanism in rubber toughened systems. The formation and growth of voids through debonding results in energy dissipation that plays an important role for the activation of further plastic deformation.[8] The small filler content in this study act in the same way and improves the plastic deformation.

The calcite filling resulted in a relatively brittle material at 50 wt% mineral content. The reason is in connection with surface properties of filler. As SEM studies demonstrated big aggregates were present beside small particles (left of Figure 5). These aggregates reduced the local cross-section of material and decreased the overall failure strength and strain of specimens

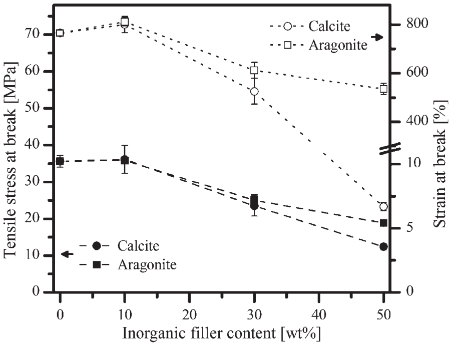

Figure 4.

Tensile break strength and strain of calcium-carbonate filled PCL samples.

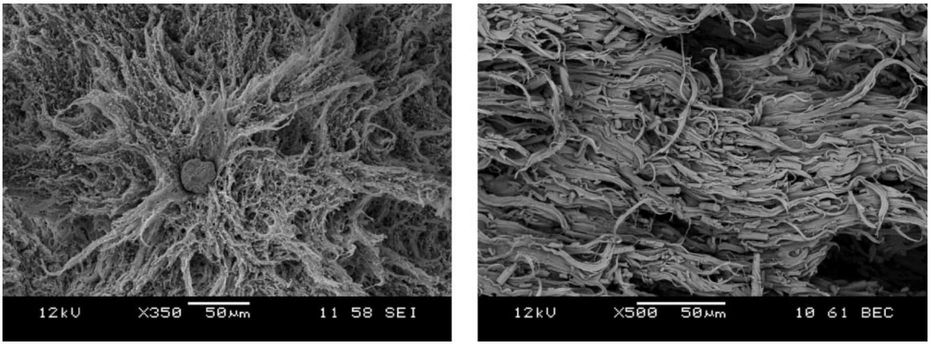

Figure 5.
SEM images of fracture surface of composite containing 50 weight% reinforcement, 50 weight% of calcite on the left, 50 weight% of aragonite on the right.

significantly. Additionally the adhesion of smaller calcite particles was better than that of bigger aragonite needles, as it was demonstrated previously, and at higher content these good adhering rigid particles inhibited the plastic flow.

Oppositely, the failure of aragonite filled materials was remained ductile. The reason is that the aragonite has different lattice compared to calcite, and there is weak interfacial adhesion between aragonite and PCL. After processing the aragonite particles were not oriented in the material. During uniaxial tension the local stresses around the filler became triaxial and the stress concentration around particles results in local plastic flow and in debonding, forming of voids. The dissipated energy initiates further plastic flow. During the plastic flow the aragonite needles turn in the direction of strain (right of Figure 6) and the local stress decreases because of the smaller resisting surface. The oriented polymer is then strained further but the friction between the needle surface and the polymer matrix results in further energy dissipation, the local temperature raised and in this way the plastic flow was facilitated.

In order to confirm that the calcium carbonate filling can improve the biocompatibility of the pure polycaprolactone, $CaCO_3$ and PCL composite were prepared. The samples were studied by fluorescent microscope and cell numbering. By theory, the calcium carbonate can decompose at pH 7.4, and carbonate and calcium is released; these products can accelerate the

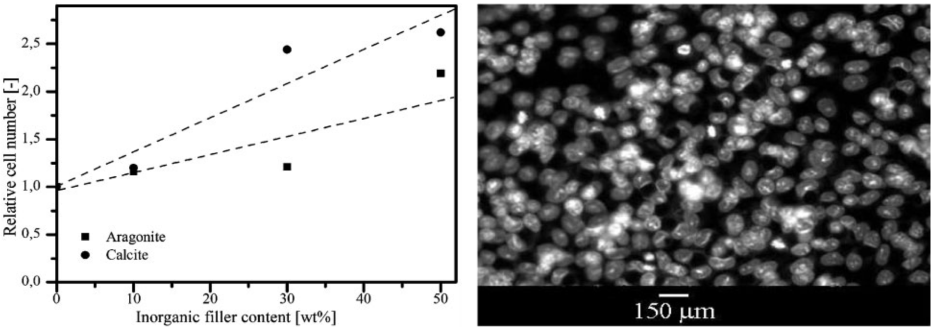

Figure 6.
Relative cell number as a function of filler content (left), and cell-adhesion to PCL surface by fluorescent microscope.

cell growth. However it was not aimed to confirm this theory, but the fluorescent pictures proved the cell adhesion to the surface (right of Figure 8), and the cell numbering showed that if the calcium content is increasing on the surface, the cell growth is increased significantly, and in case of 50 percent filling of calcium carbonate the cell number was nearly 3 times higher than in case of pure PCL (left of Figure 8). It means that not only the mechanical properties, but also the biocompatibility of composites are more advantageous than PCL samples.

Conclusion

This study was to analyzte the effect of two calcium-carbonate fillers – the spherical calcite and the needle-like aragonite – on the quasi static mechanical properties of PCL. These materials are highly biocompatible, biodegradable and are favorable candidates of guided bone regeneration.

The compressive modulus and strength of samples increased after both calcite and aragonite addition, but the calcite-containing materials proved better properties. However, each material is suitable as substratum for guided bone regeneration. The same can be established in the case of tensile properties. The better characteristics of calcite filled materials can be originated either from the different specific surface or from dissimilar surface properties (surface free energy). The tensile yield strength values confirmed the better adhesion of calcite versus aragonite in PCL matrix. The better adhesion resulted in decreased strain at break. At 50 wt% mineral content this property decreased with two orders in the case of calcite filling, but it remained in the same magnitude after aragonite reinforcing. The original strain at break of PCL was above700%, but the 50 wt% calcite content deteriorated it to about 7%. The mineral fillers also improved the biocompatibility, as it was showed by biocompatibility tests with osteoblast cells.

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