Poly(\(\varepsilon\)-caprolactone) urethane/calcium carbonate composite porous scaffolds for bone tissue engineering

Porous biomaterials have proved to be important for bone replacement and regeneration. Many porous polymers, ceramics and polymer – bioceramic composites have been prepared for orthopedic applications. Poly(\(\varepsilon\)-caprolactone) is commonly used as a soft segment in polyurethanes, known to be biocompatible, slowly hydrolytically and enzymatically degradable. An aliphatic isocyanate and a poly(\(\varepsilon\)-caprolactone) diol were used for fabrication of polyurethanes to prepare porous scaffolds. Scaffolds made from these polyurethanes were highly elastic, with good biocompatibility, however the process of degradation was too slow and bioactivity was too low. The way of minimizing the problems of porous polyurethane scaffolds could be the usage of a biodegradable polymer/bioactive ceramic composite. In the present work, two types of foam scaffolds were fabricated by the salt leaching/polymer coagulation method. The first type was made from PUR/calcium carbonate composite obtained in a polymerization process, the second type from PUR and calcium carbonate mixed during the process of creating pores. Poly(\(\varepsilon\)-caprolactone) urethane and the PUR/calcium carbonate composites were synthesized without the use of solvents and catalysts. Introduction of 5% aragonite and calcite into the PUR matrix during polymerization causes a significant increase of the foams stiffness.

**Key words:** biomedical composite, polymer-bioceramics composites, poly(\(\varepsilon\)-caprolactone) urethanes, orthopedic applications, porous scaffolds, bone tissue engineering

Porowate podłoża z kompozytu poli(\(\varepsilon\)-kaprolaktono)uretanu i węglanu wapnia przydatne w inżynierii tkanki kostnej

Porowate biomateriały pełnią ważną rolę w zastępowaniu i regeneracji kości. Wiele rodzajów porowatych polimerów, ceramik i kompozytów ceramiką–polimer jest wykorzystywanych w ortopedii. Poli(\(\varepsilon\)-kaprolaktono)diol jest często używany jako segment giętki w syntezie poliuretanów (PUR), jest on biokompatybilny, powoli rozkłada się w wyniku procesów degradacji hydrolicznej i enzymatycznej. Ten poliol i alifatyczny izocyjanian zostały użyte do wytworzenia porowatych rusztowań do zastosowań ortopedycznych. Rusztowania takie cechuje wysoka elastyczność i dobra biokompatybilność, ale często proces ich degradacji okazuje się zbyt powolny i bioaktywność za niska. Sposobem na wyeliminowanie tego problemu jest zastosowanie kompozytów z biodegradowalnych polimerów i bioaktywnej ceramiki. W przedstawionej pracy metodą koagulacji polymeru z roztworu w poliuretanu, drugi w trakcie procesu kształtowania porów. Poli(\(\varepsilon\)-kaprolaktono)uretan i kompozyty PUR/węglan wapnia były syntezowane bez użycia rozpuszczalników i katalizatorów. Dodatek do poliuretanu w trakcie polimeryzacji 5% mas. aragonitu lub kalcytu prowadził do wzrostu sztywności otrzymywanych pianek.

**Słowa kluczowe:** kompozyty biomedyczne, kompozyty polimerowo-bioceramiczne, poli(\(\varepsilon\)-kaprolaktono)uretany, ortopedia, rusztowania porowate, inżynieria tkanki kostnej
1. Introduction

Since 1963, when the first works were published concerning the usage of porous inert biomaterials, there has been a growing demand for new porous materials for medical applications [1]. The therapy of damaged or lost tissues and organs include tissue or organ transplantation, surgical reconstruction, drug therapy, synthetic prostheses, and medical devices. Porous biomaterials have proved to be important for bone replacement and regeneration [2]. Many porous polymers [3-5], porous ceramics [6-9] and porous polymer – bioceramic composites [10-20] have been prepared for orthopedic applications. These materials with clinical applications constitute an interesting field of research and development in the production of useful materials for implant fabrication. Different types of polymers: degradable (PCL, PLA) and nondegradable (PE, PMMA) are used as a matrix for composites [2, 10, 21].

Bioceramic materials used for bone regeneration as a filler in composites, can be classified in two groups: bioinert and bioactive. Bioinert ceramics have almost no influence in the surrounding living tissue. Bioactive ceramics, by contrast, are capable of bonding with living osseous tissues and their finest example would be Bioglass®, sintered hydroxyapatite and glass ceramic [10].

To serve as a scaffold for bone tissue engineering, the material must be biocompatible, mechanically integrable, osteoconductive, and have a macroporous structure [10].

Polyurethanes (PURs) remain one of the most popular groups of biomaterials applied for medical devices [22, 23]. Their popularity has been sustained as a direct result of their segmented block copolymeric character, which endows them with a wide range of versatility in terms of tailoring their physical properties. These polymers can be fabricated from various groups of substrates, due to this it is possible to obtain degradable and nondegradable polymers. Potential applications of such biodegradable elastomers may be in cardiovascular implants, repair of articular cartilage, adhesion barriers and artificial skin. Biodegradable polyurethane elastomers are expected to be suited for any application requiring the use of flexible elastic material, such as soft [24-28] and hard [28, 29] tissue engineering.

Generally, polyurethanes are made by a reaction a polyol with a diisocyanates followed by a chain extension with a diol. Commonly used polyurethanes are based on aromatic isocyanates. These, however, lack biocompatibility due to toxic degradation products originating from the aromatic hard segment [30-33]. Therefore, aliphatic diisocyanates are preferred over conventional aromatic diisocyanates, essential for cell growth and differentiation [34].

Poly(ε-caprolactone) is commonly used as a soft segment in polyurethanes [35], known to be biocompatible, slowly hydrolytically and enzymatically degradable [36]. An aliphatic isocyanate and a poly(ε-caprolactone) diol was used for fabrication of polyurethanes to prepare porous scaffolds [37, 38]. Scaffolds made from these polyurethanes were highly elastic, with good biocompatibility, however the process of degradation was too slow and bioactivity was too low. Bioactivity of polyurethane-based scaffolds was low, which is why they were coated with Bioglass® particles [39]. Another way of minimizing the problems of porous polyurethanes scaffolds could be the usage of a biodegradable polymer/bioactive ceramic composite.

Poly(ε-caprolactone) urethane elastomers (PUR) are formed from liquid components during two step prepolymer process [37, 38]. Often in the synthesis of poly(ester)urethanes based on aliphatic diisocyanates, performed via bulk polymerization, the use of catalyst is essential, if full physical properties are to be developed. Without using catalysts polyurethanes could be prepared via solution polymerization, but the residue of catalyst and solvent could have an influence on the biocompatibility of polyurethanes [28, 29, 35].

Calcium carbonate has been recognized as a bone filling material and its good osteoconductivity has been approved in recent studies [40].

In the present work, two types of foam scaffolds were fabricated by the salt leaching/polymer coagulation method. The first type was made from PUR/calcium carbonate composite obtained in a polymerization process, the second type from PUR and calcium carbonate mixed during the process of creating pores. Poly(ε-caprolactone) urethane and the PUR/calcium carbonate composites were synthesized without the use of solvents and catalysts.

2. Materials and methods

2.1. Materials

The following reactants were used in the syntheses of polyurethanes: 4,4’-dicyclohexylmethane diisocyanate (HMDI), poly caprolactone diol (PCL diol) with molecular weight 530, purchased from Aldrich Chemical Co. (Germany). Polyol was dehydrated during mixing under vacuum for two hours at a 120°C. Ethylene glycol (EG) (POCH, Gliwice) was dried under a molecular sieve. The other chemicals were used as received. 1-methyl-2-pyrrolidone was supplied by Fluka, Germany and NaCl salt (crystal size ≤ 420 µm) was used as a pore former. As a filler two types of calcium carbonate: aragonite and calcite were used. Calcite (POCH, Gliwice) was obtained through grinding synthetic calcite, specific surface area of calcite was equal 2.12 m²/g and the diameter particle size of calcite powder was < 100 µm. Aragonite was obtained through precipitation of water solution of Na₂CO₃ with a presence of nucleus of crystallization with a dripping CaCl₂ solution method in hot temperature, specific surface area of aragonite was equal 1.21 m²/g and the diameter particle size of calcite powder was < 100 µm. Both fillers were prepared in the Institute of Glass and Ceramics, Warsaw.
2.2. Synthesis of polyurethanes, composites and foams

Segmented polyurethanes with molar ratio of HMDI/PCL diol/EG 2:1:1 and a constant isocyanate index 1.00 were synthesized in moulds by the pre-polymer method. Soft segment based on a poly(ε-caprolactone) diol and hard segments of HMDI and EG were composed. These polymers contain about 52 wt % of the hard segment. Into the dewatered in a 120°C PCL diols the HMDI was added. The reaction was carried out at a 60°C for one hour, then EG was added and mixed for 15 minutes. The reaction was kept at a temperature 110°C for 8 h.

Polyurethane/CaCO₃ composites were prepared by in-situ polymerisation. Synthesis was performed with a prepolymer method. PCL diol and calcium carbonate was mixed under a vacuum for two hours at a temperature 100°C, afterwards the synthesis process proceeded in the same way as for PUR. The filler was added to the chosen polyurethane matrix in 5 wt % respectively to the whole weight of the polymer. Poly(ε-caprolactone) urethane and the composites were synthesized without the use of solvents and catalysts.

2.3. Foams preparation

Two types of porous scaffolds based on poly(ε-caprolactone) urethane were fabricated by the salt leaching/polymer coagulation method. Composites with aragonite or calcite for Type 1 scaffolds were prepared by in situ polymerization method, then were ground at liquid nitrogen temperature and dissolved in 1-methyl-2-pyrrolidone. The NaCl crystals were fractionated into two size ranges: ≤ 6 µm, and 300-420 µm, and incorporated into the polyurethane solution (15 wt %). The mass ratio of polymer solution to NaCl was 1:1. The polymer/salt/solvent mixture was poured into a mould (6 mm diameter) and immersed in distilled water for two days, where precipitation of the polymer and leaching of salt particles occurred simultaneously. Water was changed several times in order to increase salt leaching and solvent removing. The obtained porous composites was dried under vacuum.

The polyurethanes for Type 2 scaffolds were ground at liquid nitrogen temperature and dissolved in 1-methyl-2-pyrrolidone. Than fractioned NaCl crystals and aragonite or calcite were incorporated into the polyurethane solution (15 wt %). The mass ratio of polymer to aragonite or calcite was 100:5 and polymer solution to NaCl was 1:1. Afterwards the preparation process of this type of scaffold proceeded in the same way as for Type 1.

The foams preparation was described by Bil et al [39].

2.4. Characterization and in vitro studies

Fourier transform infrared (FTIR) spectroscopy was recorded with a Nicolet 6700 spectrometer (Thermo Electron Corporation). The microstructure of polyurethanes was investigated on microsections surfaces. The samples were microtomed using a glass knife with a microtome Leica RM 2165 with a system LN 21.

Thermal characteristics were performed using a differential scanning calorimeter (DSC Q1000, TA Instruments) an instrument equipped with a liquid nitrogen cooling unit. The samples scanned from –80°C to 200 °C, at a heating rate 1-10°C/min. Compression tests were carried out with a compressive mode DMA Q800 TA Instruments apparatus. The measurements were performed at a 1 Hz frequency, a 15 µm amplitude and a heating rate of 3°C/min from 20 to 120°C. Compression tests were performed on a disc shaped specimen with diameter of 3 mm and thickness 4 mm.

Dynamic mechanical analysis (DMA) were carried out with a dynamic mechanical analyzer Q800 TA Instruments apparatus. The structure of the foams before and after immersion in SBF were characterized by a scanning electron microscopy (SEM – Hitachi 2600). SEM observations were performed after coating the samples with a thin film of carbon.

EDS and SEM analysis were used to verify if hydroxyapatite (HA) had formed on the surface of the samples treated in SBF. In vitro studies were carried out in concentrated simulated body fluid (1.0 SBF), which is a modified simulated body fluid with ion concentrations 1.0 times those of standard SBF, which contains ion concentrations nearly equal to those of human blood plasma [41]. Incubation of samples in 1.0 SBF was carried out at controlled temperature of 37°C. 1.0 SBF was prepared by dissolving the reagents NaCl, NaHCO₃, KCl, KH₂PO₄·3H₂O, MgCl₂·6H₂O, CaCl₂·2H₂O, and Na₂SO₄ into distilled water. The solution was buffered to pH 7.25 at 37°C with tris(hydroxy-methyl)aminomethane and hydrochloric acid. Composite foams were dipped in 10 ml 1.0 SBF for time periods of 5 and 90 days. Prismatic specimens of nominally the same dimensions were used for these experiments. The solution was changed after 45 days. After the right time periods samples were extracted from the solution, rinsed gently with distilled water and left to dry at 37°C to stable mass.

The weight loss of composites during immersion in SBF was calculated as:

\[ \Delta m = (m_i - m_f)/m_0 \times 100\% \]

where \( m_i \) and \( m_f \) were the weights of the specimen before and after degradation, respectively, after \( t \) days of immersion in SBF.

3. Results and discussion

3.1. Properties and microstructure of foams

FTIR spectroscopy was used to investigate the degree of hard and soft segment interaction in polyure-
thanes and composites with aragonite and calcite obtained in two different procedures. FTIR spectra of investigated PUR and composites showed characteristic bands of urethane groups at $3320–3330 \text{ cm}^{-1}$ ($\text{N–H groups stretching}$), $1700–1725 \text{ cm}^{-1}$ ($\text{NHCOO stretching}$), $1530–1533 \text{ cm}^{-1}$ ($\text{C–N stretching, combined with N–H out of plan bending}$) (Fig. 1 and 2).

The absence of absorbance at $2267 \text{ cm}^{-1}$ indicated a lack of unreacted isocyanate groups [22, 23]. Two main spectral regions are the source of information about hard and soft segment of polyurethanes interaction: N–H and C=O absorption bands. A strong band, assigned to the free N–H stretching vibration, is present at $3325 \text{ cm}^{-1}$ in all investigated PURs and composites [22]. The band in region $1680–1740 \text{ cm}^{-1}$ is due to a free and hydrogen bonded urethane carbonyl (CO) but in researched materials it was no possible to estimate the level of absorbance of characteristic absorp-

Figure 1. FTIR – ATR spectra of the surface of polyurethane (1), composite PUR/aragonite Type 1 (2) and Type 2 (3) scaffolds.
Rys. 1. Widma FTIR – ATR powierzchni materiału rusztowań: poliuretanów (1), kompozytów PUR/aragonit typu 1 (2) i typu 2 (3)

Figure 2. FTIR – ATR spectra of the surface of polyurethane (1), composite PUR/calcite Type 1 (2) and Type 2 (3) scaffolds.
Rys. 2. Widma FTIR – ATR powierzchni materiału rusztowań: poliuretanów (1), kompozytów PUR/kalcyt typu 1 (2) i typu 2 (3)
tion bands connected with a free and hydrogen bonded urethane carbonyl, and as result is was not possible to estimate the degree of phase separation [42].

Characteristic absorption bands of the calcium carbonate were observed at 2512 cm\(^{-1}\), 1447 cm\(^{-1}\), 1160 cm\(^{-1}\), 874 cm\(^{-1}\), 851 cm\(^{-1}\) and 712 cm\(^{-1}\). These bands were not observed on the spectra of the surface composites with aragonite and calcite.

Fig. 3 shown the thermal properties of PUR and composite with aragonite, as obtained in a first DSC heating scan. Table 1 reports the glass transition temperature (T\(_g\)). DSC results show that the T\(_g\) of PURs is higher than the T\(_g\) of composites. We can assume that aragonite has an influence on the polymerization of composites Type 1. Particles of aragonite make phase separation in PUR more difficult. T\(_g\) of PUR/aragonite composites Type 1 is higher than Type 2. After fabrication of foams Type 2 from solvent of PUR and aragonite the interactions between macro particles are weaker and T\(_g\) of this PUR is lower.

The evaluation of storage modulus (E') has been studied by DMA, an example curves of E' are presented in Fig 4. Storage modulus was specified at 37°C and compared in Table 1. For PUR aragonite composite Type 1 modulus is the highest. E' for PUR and composites PUR/aragonite Type 2 is much lower. Introduction of 5% aragonite into PUR during polymerization causes a significant increase of the foams stiffness.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>T(_g) (°C)</th>
<th>E' at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUR</td>
<td>37.3</td>
<td>0.7 MPa</td>
</tr>
<tr>
<td>Composite PUR/aragonite Type 1</td>
<td>29.3</td>
<td>14.3 MPa</td>
</tr>
<tr>
<td>Composite PUR/aragonite Type 2</td>
<td>26.7</td>
<td>0.3 MPa</td>
</tr>
</tbody>
</table>

Figure 3. DSC trace for polyurethane (1), composite PUR/aragonite Type 1 (2) and Type 2 (3) scaffolds; the curves are separated.

Rys. 3. Termogramy DSC poliuretanów (1), kompozytów PUR/aragonit typu 1 (2) i typu 2 (3); krzywe zostały rozsuniête.
Figure 5. Structure of (1) polyurethane scaffold, (2) composite PUR/aragonite scaffold Type 1, (3) composite PUR/calcite Type 1 scaffold, (4) composite PUR/aragonite scaffold Type 2, (5) composite PUR/calcite Type 2 scaffold, before immersion in SBF at different magnifications

Rys. 5. Struktura podłoży z: poliuretanów (1), kompozytów PUR/aragonit typu 1 (2) i PUR/kalcyt typu 1 (3), kompozytów PUR/aragonit typu 2 (4) i PUR/kalcyt typu 2 (5), przed ekspozycją w SBF w różnych powiększeniach
The microstructure or polyurethane and composites scaffolds are illustrated in Figure 5. The salt leaching/polymer or composite coagulation method allows us to obtain scaffolds with an open and interconnected porous structure. The macropore size of polyurethane scaffolds is 400 µm (Fig. 1a). In the picture at a higher magnification the microporous structure of the pores walls was observed, the micropore size is about 8 µm (Fig. 5-1b). Foams from composites PUR/aragonite Type 1 has open pores, size 180 µm (Fig. 5-2a) and micropores size is about 10 µm, but there are much less interconnected macropores (Fig. 5-2b). Pore structure of PUR/calcite composites Type 1 scaffolds is similar to polyurethane foams, and open macropore have irregular structure and size is equal 250 µm (Fig. 5-3a); interconnected micropores size is about 6 µm (Fig. 5-3b). Scaffolds of Type 2 composites contain much less micropores connecting the macropores (Fig. 5-4b, 5-5b). The sizes of macropores in both types of composites are about 30 µm bigger than pores of Type 1 composites (Fig. 5-4a, 5-5a).

3.2. In vitro studies in SBF

*In vitro* studies in simulated body fluid were carried out in order to investigate how two types calcium carbonate filler influence the biodegradation process of polyurethane foams and improve the biocompatibility of the polymer and composites.

The biodegradation process was analyzed using thermal analysis DSC. Assessment of the changes occurring in the materials were monitored on the grounds of \( T_g \) changes of these materials. The results of the analyses are gathered on (Fig. 6). \( T_g \) of polyurethane does not change for 30 days in SBF, only after 90 days the drop of \( T_g \) was sharp. \( T_g \) of both composites rose systematically which indicates changes in the rigidity of these materials. The reason for these changes could be changes in chemical structure of these composites, changes on the surface can have a similar influence, for example emerging layers of sediment.

The weight loss studies showed that mass after 30 and 90 days in SBF of all investigated materials changes slightly within 1% of their mass (Fig. 7). These results indicate that together with a loss of composite mass the amount of sediment on the surface of these materials rose. The surface morphology of PUR and composites scaffolds was observed by SEM. The results of these observation are presented in Fig. 8. We observed that pores in scaffolds are bigger after 30 days in SBF than pores of the same foams after 90 days in SBF.
In Fig. 9 we compare the changes of macropores in the observed scaffold. The size of the foam macropores from Type 2 composites did not change. Also the changes of micropore sizes were analyzed (Fig. 10). Their size and amount drops together with the immersion time in SBF.

On the surfaces of polyurethane scaffolds and composites Type 1 scaffolds we observed an occurrence of spherical particles. Their structure is shown in Fig. 11. The images of these particle show it is hydroxyapatite (HA).

The chemical composition of the layer was identified by EDS analysis. Calcium and phosphorus, as predominant elements in the EDS spectrum, confirm the presence of HA on the surface of polyurethane (Fig. 12), composite PUR/aragonit Type 1 (Fig. 13) and composite PUR calcite Type 1 scaffolds (Fig. 14). On the surfaces of composites Type 2 we did not observe a layer of spherical particles of HA (Fig. 15, 16).
4. Conclusion

Bioactive and bioresorbable composites were developed based on polyurethane and calcium carbonate. The results indicate the important role of the composites preparation process in the formation of micropores. The composites preparation process has an influence on the internal structure of the foams (SEM).

Figure 10. Micropore diameter of polyurethane (1a) after 30 days and (1b) 90 days of immersion in SBF; composite PUR/aragonite: (2a) after 30 days and (2b) 90 days of immersion in SBF; composite PUR/calcite: (3a) after 30 days and (3b) 90 days of immersion in SBF scaffolds.

Figure 11. Surface of (1) PUR, (2) composite PUR/aragonite and (3) composite PUR/calcite scaffold Type 1 after 90 days of immersion in SBF at different magnifications.

Rys. 10. Zmiana średnicy mikropor podłoży wykonanych z: poliuretanów – po 30 dniach (1a) i 90 dniach (1b); kompozytów PUR/aragonit typu 1 – po 30 dniach (2a) i po 90 dniach (2b); kompozytów PUR/kalcyt typu 1 – po 30 dniach (3a) i 90 dniach (3b) ekspozycji w SBF.

Rys. 11. Struktura – przedstawiona w różnych powiększeniach – podłoży z: poliuretanów (1), kompozytów PUR/aragonit typu 1 (2) i kompozytów PUR/kalcyt typu 1(3), po ekspozycji przez 90 dni w SBF.

Figure 10. Micropore diameter of polyurethane (1a) after 30 days and (1b) 90 days of immersion in SBF; composite PUR/aragonite: (2a) after 30 days and (2b) 90 days of immersion in SBF; composite PUR/calcite: (3a) after 30 days and (3b) 90 days of immersion in SBF scaffolds.

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Rys. 11. Struktura – przedstawiona w różnych powiększeniach – podłoży z: poliuretanów (1), kompozytów PUR/aragonit typu 1 (2) i kompozytów PUR/kalcyt typu 1(3), po ekspozycji przez 90 dni w SBF.
Figure 12. Surface and EDS analysis of PUR scaffold after 90 days of immersion in SBF
Rys. 12. Struktura powierzchni i wynik analizy EDS rusztowania z PUR po 90 dniach ekspozycji w SBF

Figure 13. Surface and EDS analysis of composite PUR/aragonite scaffold Type 1 after 90 days of immersion in SBF
Rys. 13. Struktura powierzchni i wynik analizy EDS rusztowania z PUR/aragonit typu 1 po 90 dniach ekspozycji w SBF

Figure 14. Surface and EDS analysis of composite PUR/calcite scaffold Type 1 after 90 days of immersion in SBF
Rys. 14. Struktura powierzchni i wynik analizy EDS rusztowania z PUR/kalcyt typu 1 po 90 dniach ekspozycji w SBF
were connected with matrix, is different than those with chemical bonds (Type 1) and different if there were only physical interactions (Type 2). Rigidity also differs between the foams from composite Type 1 and composite Type 2. Rigidity of the foams prepared by in situ was higher than in other materials. A layer of HA did not occur on the surface of Type 2 composites.

The in vitro studies in SBF indicated that calcium carbonate filler imparted high bioactivity to the polyurethane scaffolds by promoting the formation of a carbonate hydroxyapatite layer on their surface. Result of in vitro studies show that composites with calcium carbonate should simplify the process of bone forming in vivo conditions.

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