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COMPOSITES THEORY AND PRACTICE

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CHANGE OF MECHANICAL PROPERTIES OF PCL-BASED TERNARY COMPOSITE SCAFFOLDS FABRICATED BY SOLID FREEFORM FABRICATION TECHNIQUE DURING *IN VITRO* DEGRADATION

The number of critical bone defects caused by injury, cancer or aging of the world population is increasing. Techniques currently used to repair these defects suffer from several disadvantages, such as a lack of mechanical and biological matching of bone characteristics, the requirement of second surgery and the risk of pathogen transmission. Scaffolds made of bioresorbable polymers are a promising alternative as they temporarily support regeneration of the damaged site and undergo complete degradation after new tissue is formed. The goal of the present study was to determine the changes of the mechanical properties of fibrous PCL-based nanocomposite scaffolds during *in vitro* degradation. The composite scaffolds containing PCL, 5 wt.% of hydroxyapatite nanoparticles, HA, and different concentrations of PLGA were prepared by combined solvent casting and solid freeform fabrication techniques. The composite scaffolds were subsequently put to a dynamic degradation test. After fixed periods of time, the mechanical properties, mass loss of the scaffolds and change of the surface morphology (SEM) were determined. It was observed that the addition of PLGA accelerated the degradation of the scaffolds. However, the mechanical properties increased during the first weeks of incubation.

Keywords: poly(ε-caprolactone), PCL, nanocomposites, bioresorption, mechanicaltest

ZMIANY WŁAŚCIWOŚCI MECHANICZNYCH RUSZTOWAŃ WYKONANYCH METODĄ SZYBKIEGO PROTOTYPOWANIA Z KOMPOZYTÓW POTRÓJNYCH O OSNOWIE PCL PODCZAS DEGRADACJI *IN VITRO*

Liczba krytycznych ubytków kostnych powstałych w następstwie wypadków, nowotworów czy też na skutek chorób wynikających z wydłużania się przeciętnej długości życia ciągle wzrasta. Techniki ortopedyczne używane obecnie do ich leczenia to alloplastyka oraz auto-, allo- i ksenoprzeszczepy. Techniki te posiadają jednak wady, takie jak możliwość ulegania korozji w przypadku alloplastów (implanty metalowe), ograniczone rozmiary autoprzeszczepów, czy ryzyko przeniesienia czynników chorobotwórczych przy transplantacji allo- i ksenoprzeszczepów. Rusztowania wykonane z polimerów bioresorbowalnych nie posiadają wyżej wymienionych wad, ponieważ ich zastosowanie jest przewidziane jako tymczasowe, a uleganie w środowisku biologicznym resorpcji eliminuje konieczności przeprowadzania dodatkowej operacji. Rusztowania bioresorbowalne mogą być dodatkowo zasiedlone materiałem autogennym pobranym od chorego podczas biopsji. Polikaprolakton (PCL) jest biodegradowalnym poliestrem o bardzo dobrej biozgodności. Jego dodatkowymi zaletami są bardzo dobre właściwości reologiczne oraz wysoka stabilność termiczna. Niestety, polimer ten wykazuje znaczną hydrofobowość oraz bardzo długi okres resorpcji (do 4 lat). Jednym ze sposobów na przyspieszenie degradacji bioresorbowalnych polimerów jest wytworzenie kompozytów z bioaktywnym napełniaczem nieorganicznym lub mieszanki z szybciej degradującym polimerem, np. kopolimerem kwasu glikolowego i mlekowego (PLGA). Przy projektowaniu rusztowań do zastosowań w inżynierii tkankowej niezmiernie ważne jest dopasowanie szybkości resorpcji rusztowania do tempa regeneracji tkanki, tak aby rusztowanie mogło zapewnić stabilność mechaniczną do momentu odbudowy ubytku. Celem niniejszych badań była charakteryzacja procesu degradacji porowatych rusztowań wykonanych z kompozytów potrójnych o osnowie z PCL ze szczególnym uwzględnieniem zmiany ich właściwości mechanicznych. Badania rozpoczęto od wytworzenia materiałów kompozytowych, zawierających nanocząstki hydroksyapatytu oraz różne stężenia PLGA, przez wylanie z roztworu. Następnie z tak przygotowanych kompozytów wytworzono porowate rusztowania za pomocą techniki szybkiego prototypowania. Rusztowania te poddano procesowi degradacji w płynie symulującym środowisko fizjologiczne (ang. Simulated Body Fluid, SBF). W określonych czasowych punktach pomiarowych próbki wyjmowano z roztworu, myto w wodzie destylowanej, suszono w suszarce próżniowej i ważono w celu wyznaczenia ubytku masy. Następnie w statycznej próbie ściskania badano właściwości mechaniczne rusztowań w celu określenia ich zmiany. W początkowym okresie inkubacji obserwowano wzrost sztywności oraz granicy plastyczności dla wszystkich badanych rusztowań kompozytowych. Po 9 tygodniach inkubacji w SBF nastąpił spadek właściwości mechanicznych rusztowań zawierających najwyższe stężenia PLGA. Dodatek PLGA miał zatem istotny wpływ na szybkość degradacji rusztowań oraz w konsekwencji zmianę właściwości mechanicznych.

Słowa kluczowe: polikaprolakton, PCL, nanokompozyty, bioresorpcja, testy mechaniczne

INTRODUCTION

Tissue engineering is a very dynamically developing field of science and is emerging as an alternative to traditional treatment therapies. It combines materials engineering and life sciences in order to fabricate substitutes of damaged parts of a human body. Artificial matrixes, so called scaffolds, are used to ensure proper structural regeneration of the injured site. A scaffold suitable for bone tissue engineering should be made of a biocompatible, bioactive and bioresorbable material. Furthermore, it should exhibit sufficient mechanical properties, high open porosity and a tailored degradation rate [1, 2].

Poly(ε-caprolactone), PCL is a polyester which meets most of the aforementioned requirements. Additionally it is highly processible and therefore of great interest for scaffolds fabrication. The disadvantage of the polymer is its lack of bioactivity and very low degradation rate [3, 4]. In order to overcome those drawbacks, bioactive, inorganic fillers (like hydroxyapatite) are added to the polymer matrix [5]. The concentration of the filler is, however, limited due to its agglomeration and the reduction of tensile strength of the composite [6, 7]. Therefore other approaches, for example, the addition of a faster degrading polymer, have to be applied in order to increase the resorption rate of PCL. The degradation rate has to be, however, tailored carefully to ensure mechanical stability of the scaffold during the formation of the neotissue.

In this paper, the changes of the mechanical properties of novel nanocomposite materials during degradation in Simulated Body Fluid (SBF) are presented.

MATERIALS AND METHODS

Materials

Poly(ε-caprolactone) (PCL, M_n = 80 kDa, Sigma-Aldrich, USA) and poly(D,L-lactide-co-glycolide) (PLGA, lactide:glicolide molar ratio of 50:50, Resomer RG 504 H; i.v. 0.45 dl/g, Boehringer-Ingelheim, Germany) were used. Hydroxyapatite, HA (Specific Surface Area = 64.45 $m^2 \cdot g^{-1}$, density = 2.93 $g \cdot cm^{-3}$) was purchased from Merck, Germany. Methylene chloride was purchased from Chempur, Poland.

TABLE 1. **Composition of prepared materials**
TABELA 1. **Skład wytworzonych materiałów kompozytowych**

Name	PCL [wt.%]	PLGA [wt.%]	HA [wt.%]
PCL	100	0	0
PCL95	95	0	5
PCL90	90	5	5
PCL80	80	15	5
PCL70	70	25	5

Preparation of composite

Four composite materials (see Tab. 1) were prepared by the solvent casting technique. PLGA was dissolved in methylene chloride, then HA was added and ultrasonicated in order to disperse the nanoparticles. Subsequently, PCL was dissolved and the resulting suspension was cast into Petri dishes. The solution was first dried in a fume hood for 2 h and then in a vacuum dryer (100 mbar, 40°C, 48 h). Afterwards, the membranes were cut into pieces and used to fabricate scaffolds.

Fabrication of scaffolds

A solid freeform fabrication device (Bioscaffolder, SYSENG, Germany) was used to form 3D scaffolds at 95°C with a volume dispensing speed of 200 rpm and at a dispense pressure of 5 bars. The inner diameter of the needle was 250 μm. The scaffolds were cuboids with dimensions of 5 x 5x 6.5 mm.

Degradation experiments

The degradation experiment was carried out in Simulated Body Fluid (SBF) at 37°C, pH = 7.4 and low tangential agitation over a period of 20 weeks. SBF was changed at 2 week intervals. At fixed time points, the samples were removed from SBF, washed with distilled water and dried in the vacuum dryer (100 mbar, 23°C) for 48 hours and the mass of the dry samples was determined. The mass loss was calculated according to equation

$$\Delta m = \left(m_0 - m_{dry}\right) / m_0 \cdot 100\% \quad (1)$$

Mechanical testing of scaffolds

A compression test of the scaffolds was carried out using a Zwick/Roell Z005 mechanical tester at a crosshead speed of 1 mm/min. The samples were pre-loaded with an initial force of 1 N. The test was run until the strain reached 50%. The yield strength was calculated as stress at 1% strain. The Young's modulus was calculated from the linear part of the stress-strain curve.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) images were taken with a TM-1000 microscope (Hitachi, Japan) in order to determine the changes of the surface morphology. The micrographs were recorded at an acceleration voltage of 15 keV and in the BSE mode.

RESULTS AND DISCUSSION

PCL-based composites were successfully fabricated by the combination of solvent casting and fused deposition modelling techniques. The results of the mechanical tests are depicted in Figure 1. It can be seen that during the first 2 weeks of degradation, the Young's modulus and yield strength increased for all the tested materials. The increase was more pronounced for composite scaffolds containing 15 and 25 wt.% of PLGA.

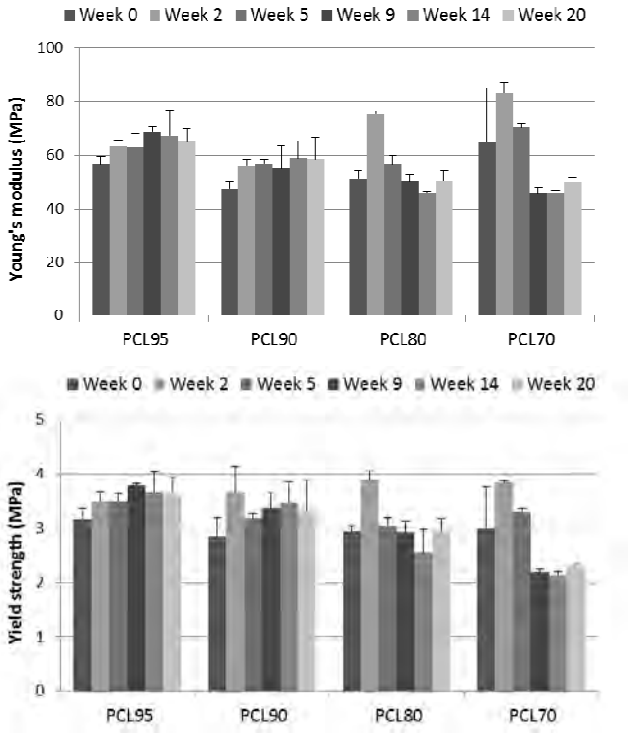

Fig. 1. Changes of Young's modulus and Yield strength of scaffolds during degradation in SBF

Rys 1. Zmiany modułu Younga oraz granicy plastyczności rusztowań podczas degradacji w SBF

The Young's modulus increased by 48% for PCL80 (76 MPa after 2 weeks of incubation) and by 28% in the case of PCL70 (83 MPa after 2 weeks of incubation). A similar trend was observed in the case of the yield strength of the scaffolds (increase of approximately 30%). This phenomenon could be caused by the increase in crystallinity of PCL. Yasin et al. observed similar behaviour in PCL-PHBV blends [8]. According to Yasin, hydrolytic scission of the amorphous regions of PCL led to higher chain mobility, which allowed for further crystallisation of the polyester. After the initial increase, the mechanical properties of ternary composite scaffolds PCL80 and PCL70 deteriorated up to week 9 of incubation in SBF. This drop was accompanied by an enhanced mass loss of these scaffolds (Figure 2). The mechanical properties of the binary composite scaffold PCL95 remained relatively constant during the next 18 weeks of degradation. All the ternary scaffolds retained their mechanical integrity during the entire degradation period.

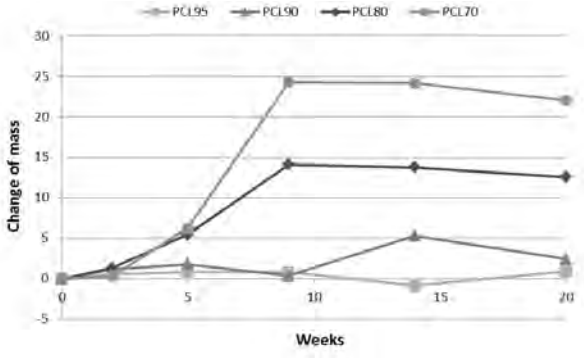

Fig. 2. Effect of PLGA concentration on mass loss of composite scaffolds. Each point represents average of 3 samples

Rys. 2. Wpływ stężenia PLGA na ubytek masy rusztowań kompozytowych. Każdy punkt jest uśrednionym ubytkiem masy 3 próbek

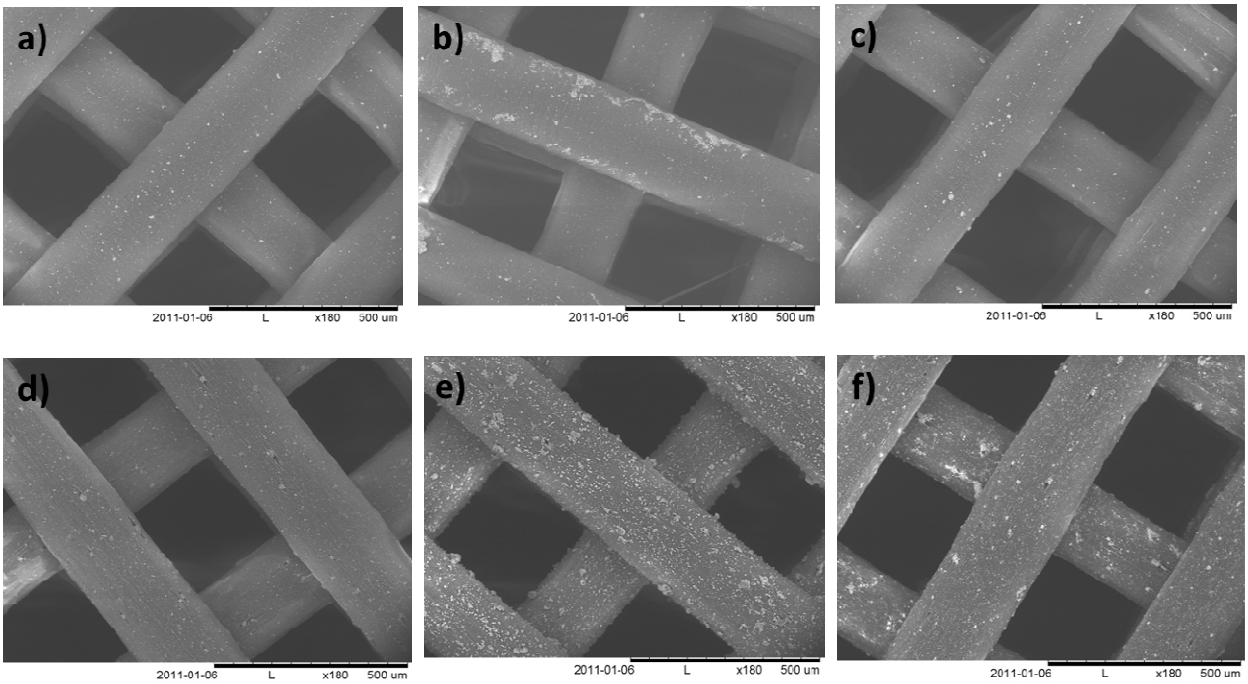

Fig. 3. SEM image of binary composite PCL95 and ternary composite PCL70 during degradation: a) PCL95 - week 0, b) PCL95 - week 5, c) PCL95 - week 14, d) PCL70 - week 0, e) PCL70 - week 5 and f) PCL70 - week 14. Scale bar 500 μm

Rys. 3. Zdjęcia SEM kompozytu podwójnego PCL95 oraz kompozytu potrójnego PCL70 podczas degradacji: a) PCL95 - tydzień 0, b) PCL95 - tydzień 5, c) PCL95 - tydzień 14, d) PCL70 - tydzień 0, e) PCL70 - tydzień 5 i f) PCL70 - tydzień 14. Marker 500 μm

Figure 2 shows the changes of the mass of all the prepared samples as a function of time and composition over the period of 20 weeks. It can be seen that the addition of PLGA accelerated the degradation of the scaffolds since the beginning of the experiment. A dramatic increase of the mass loss of PCL80 and PCL70 occurred between week 5 and 9. This time frame corresponds to the degradation of PLGA with a copolymer ratio 50:50 [9]. After 9 weeks of degradation, the mass loss stabilised at a level which corresponded to the initial weight fraction of PLGA in all the ternary composites.

The SEM micrographs (Fig. 3) revealed changes of the surface morphology of the scaffolds during incubation in SBF. The changes were more pronounced in the case of PCL70 due to swelling and subsequent degradation of the inclusions of PLGA.

The PCL-based scaffolds investigated in this study are intended for medical applications, and therefore should be sterile before use. Gamma-irradiation is one of the most commonly applied sterilization techniques, even though it can trigger changes of various properties of the irradiated material. However, recent studies of Walo et al. have shown that sterilization carried out at a low temperature (–78°C) allows one to reduce the extent of γ-induced degradation [10].

CONCLUSIONS

The addition of PLGA induced different surface morphologies of the composite scaffolds. Additionally, it accelerated the degradation rate of the ternary composites. Further research will focus on biological evaluation of the composites and the effect of sterilization on their degradation and mechanical properties.

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