Results and discussion

Adding the nano-filler to the chitosan matrix changes the electrical potential of the material in a significant way. Using 1%wt of unmodified nanotubes set the material resistivity at the level of 350-78 kΩ, while the CNTs modified with carboxyl groups increased the value to 8-9 MΩ. It was established that additional surface modifications with different forms of carbon increase the resistivity of the system only if the modified material is carbon nanotubes. However, this change is rather slight, as compared to the initial material's value in the applied conditions (increase by 15-20% only). Meanwhile, the presence of fillers has a strong influence on the shape and size of pores in the liofilized systems. Introducing the carbon nanotubes results in elliptical and irregular pores. Introducing graphene and graphite oxide makes the pores more circular and homogenous (FIG. 1).

Conclusions

The proposed nanocomposite systems: active electrical foil and porous fulfilling nanocomposite material seem to meet the requirements for material used in guided nerve regeneration concerning damages in the peripheral nervous system.

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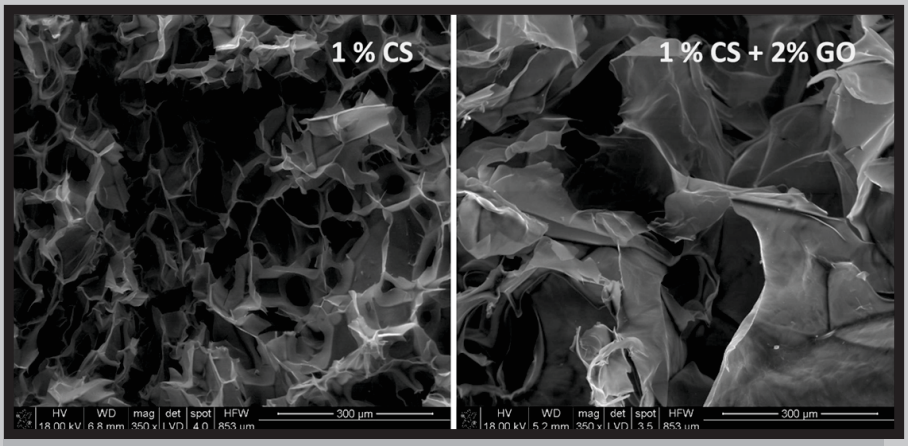

FIG. 1. Mictrostructure of liophilised chitosane and chitosane witf graphite.

COMPARISON OF DURABILITY OF RESORBABLE POLYMER PINS IN IN VITRO AND IN VIVO CONDITIONS. PRELIMINARY STUDY

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Abstract

The work focuses on polymeric cartilage/bone pins (pegs) that were manufactured and tested to assess their application in meniscus injuries. The bone pins measuring 1,2 and 4 mm in diameter were produced from certified resorbable PLDLA by means of hot pressing (at 126°C). In order to establish the material characteristics, tests of mechanical properties, structural testing and stability tests were performed in vitro (an immersion medium: water/ PBS buffer). It was established that after three months of incubation the initial implant's bending strength (120 MPa) decreased by 35%, whereas its tensile strength (52 Pa) weakened by 60%. The degree of degradation did not affect the pH of the immersion fluid. The observed physical changes of the implant, such as: the mass decrease, the change of shape, the increase of crystallinity (DSC/TG), the number of polymer terminal groups (-OH, -COOH), proved the advanced degradation process of PLDLA pins. Implants of particular behaviour were inoculated into the tibia of a New Zealand rabbit. In vivo tests were conducted to confirm the changes observed in vitro. Monitoring of the degradation process was performed after three months following the implantation by means of control X-ray and computed microtomography (μCT).

Keywords: *meniscus, bone pins, polylactide, regeneration*

[Engineering of Biomaterials, 128-129, (2014), 87-89]

Introduction

Meniscus injuries concern mainly young and physically active people. Doing competitive and amateur sports may result in meniscal tears. Injury to the meniscus occurs during the sudden bend-and-twist motion of the knee. Unfortunately, meniscal tears often happen at the same time other components of the knee are injured - a common injury among athletes involves simultaneously the anterior cruciate ligament (ACL) and the posterior cruciate ligament (PCL). Apart from the trauma-induced injuries, the congenital knee deformities (such as dysplasia or deviation of the knee joint) as well as degenerative process contribute to the incidence of meniscal injuries. The methods of treatment depend on the location of the injury, i.e. whether the injured area is well or poorly supplied with blood. In the first case the suture is usually recommended, using stitches, pins or staples made of plastic. The disadvantage of this method is the patient's longer immobilization and recovery time in comparison to meniscectomy (the surgical removal of all or part of a torn meniscus). In particular cases the faulty meniscus may be replaced by allogenic graft (from Tissue Banks) or the scaffolding implant. The porous material improves regeneration of the injured meniscus and may be applied in surgery. When the injured area is with the good blood supply, the preferable treatment is meniscectomy. The materials used in meniscus surgery are mainly polyhydroxy acids, among which the most popular ones are polylactide and polydioxanone (PDS). This polymer, depending on the isomer type (right or left, isomer mixture L/DL), is characterized by various time of durability and differentiation of mechanical properties tested in vitro/in vivo (strength, Young's modulus). Additionally, the thermoplastic material may be formed by means of heat treatment used in the polymer technology (injection moulding, drawing, blow moulding). Thus it is possible to form pins, pegs, staples, scaffolds or membranes used in surgical procedures of meniscus. The well recognized products on the market such as RigidFix pin, Steinman pin are manufactured mainly from polylactide due to its high endurance and crystallinity. In order to expand the implant/tissue interface, the pins are often roughened (Steinman pin). The main problem indicated by orthopaedists is migration of the pin during the recovery time and long degradation of polylactide (over two years). Because of the possibility of combining the meniscus treatment with the ligaments reconstruction, it seems advantageous to apply the material suitable for the soft tissue/bone interface. Thus the tested pin was made from poly-L/DL-lactide (80:20, L/DL), whose durability tested in vitro is 12-14 months, according to the manufacturers. The material is approved by FDA for medical applications and the proposed processing does not affect its biocompatibility. The aim of this work was to verify the material both in vivo and in vitro after three months. To assess its durability the following properties were tested in vitro: mechanical, thermal and structural (FTIR), and macroscopic (shape/mass). For in vivo testing an animal model was used – a PLDLA pin was implanted into the tibia of a New Zealand rabbit. Monitoring of the degradation process was performed by means of control X-ray and X-ray computed microtomography (μCT).

Materials and method

Polymer implants (PLDLA, by PURAC Biochem) were manufactured by means of hot pressing (at 160°C). Three types of the nozzle were used measuring respectively: 1, 2 or 4 mm in diameter. All the implants had 2cm in length. The prepared implants were measured, paying special attention to the mass and shape. The immersed medium were: PBS and water (1:100). Every month the pH and conductivity values were registered to establish the degradation rate. The mechanical bending and tension tests were performed first on the initial implants and after three months of in vitro incubation (37°C/water/PBS buffer) using Zwick 1435 machine. The initial values of tensile and bending strength were established. The thermal properties of the implant were measured in the atmosphere of nitrogen using the Netzsch thermal analyzer STA 449F3. The degradation rate of the material was established by means of analyzing the crystallinity alterations. The structural changes of polymer were monitored by means of FTIR-ATR (FTS 3000 Excalibur, Bio-Rad) equipped with a diamond ATR (PIKE Technology). This method made it possible to follow the changes taking place on the surface of the material (penetration depth of 2 μm).

In vivo testing was conducted on three groups of New Zealand rabbits. Under general anaesthesia (using xylazine 5 mg/kg and ketamine 25mg/kg) a lateral approach to the stifle joint was made in sterile conditions. Following lateral arthrotomy and medial patellar luxation, the femoral trochlea was visualized. A cylindrical hole (2 mm in diameter, 4 mm in depth) was drilled in the trochlear groove to imitate the osteochondral defect. Then the defect was filled with an implant using press-fitting method. The joint capsule, fascia and skin were closed in a routine manner. After the operation all rabbits were allowed to move freely in cages without any splints. The animals were sacrificed after three months of implant loading. The femoral trochleas were harvested, fixed in 4% paraformaldehyde solution and submitted to further analysis.

Skyscan 1174 system equipped with the dedicated control software (Bruker microCT, Belgium) was used for X-ray microtomography analysis. During scanning the sample was rotated within angular range of 0°÷180° with a step of 0.7°. After each step, three photographs were taken in order to average the exposure levels. In total, 257 photographs were taken. To eliminate possible artefacts, the sample was randomly moved for each projection. An aluminium 0.25 mm-thick filter was used at the source to reduce beam hardening effect. The images were then reconstructed into cross-sections using NRecon software (Bruker microCT, Belgium). After the reconstruction, the isotropic voxel size for the set of images was 18.3 mm in each axis. The set was then analyzed to establish geometrical measurements, using CTAn software (Bruker microCT, Belgium).

Results and discussion

The pins had the same measurements in diameter and length (TABLE 1) and the mass of the implants was also comparable, which confirms the possibility to achieve the statistically homogeneous group for further research. The applied degradation time does not affect the pH values or the conductivity of the immersion medium (water/PBS buffer). A slight change is noted in the shape of implants, especially noticeable in pins initially measuring 1 and 2 mm in diameter. Yet such a change concerns only 10% of the tested pins. As for the 4- mm pins, no change of shape or mass was noted. The tests assessing mechanical properties of the thin pins (φ=1; φ=2 mm) revealed the tensile strength decrease by 60% and the bending strength fall by 35% in comparison to the initial implant (TABLE 2). The pins of φ=4 mm do not record strength decrease (following the applied degradation time). Additionally, the analysis of the curves in the force-deformation system shows that the implant material becomes brittle after three months of incubation.

TABLE 1.

	Pin φ=1	Pin φ=2	Pin φ=4
Size	d=0.85-1.05 mm	d=1.95-2.12 mm	d=3.75-4.15 mm
Size after durability test	d=0.98-1.26 mm	d=1.97-2.15 mm	d=3.83-4.05 mm

TABLE 2. Mechanical properties of pins after durability test.

	Rm [MPa]	E [GPa]
Pin φ=2	122±2.11	2,12±0.5
Pin φ=2 after durability test	41.2±2.62	0.95±0.7

The reason for such a significant decrease in durability is the growing crystallinity value – from the initial 18% up to 38% after three months of incubation. The advanced degradation of the implant is also visible in the analysis of FTIR spectroscopy data. In the range of wavelengths characteristic for the groups -OH (3200-3600 cm^{-1}) and -COOH (1240-1380 cm^{-1}) there is the increase in intensity. It confirms their increased concentration in the implant signifying the molecular weight decrease (groups -OH i -COOH are the terminals of the polymer chain PLDLA). The X-ray images of rabbits' bones taken three months after the surgery showed a visible outline of the implant. No negative changes were noticed in the implantation area. The three-month clinical observation of the rabbits did not reveal any pathology concerning the rabbits' knees, all the animals moved ably. The μCT confirmed the in vitro results – firstly the bone implant swells as the degradation proceeds (the pin's diameter changes from 1,78 to 1,94 mm). None of the implanted pins underwent the disintegration (FIG. 1). Probably the degradation process in vitro is faster than the one in vivo, which might be connected with the immediate surroundings of the implant. In the tissue the implant is press-fitted, while in the immersion fluid the penetration is unlimited and happens all over the surface.

Conclusions

To conclude the preliminary research on the durability of polymeric pins, it may be assumed that in vitro testing does not entirely reveal the changes taking place in the pin implanted in the tissue. Still such a testing "in glass" is a probable prediction concerning the degradation rate and the stages of the process. The presented results prove PLDLA to be a material of high mechanical potential and relatively short degradation time. The particular properties of the implant's material seem to recommend it for a bone pin.

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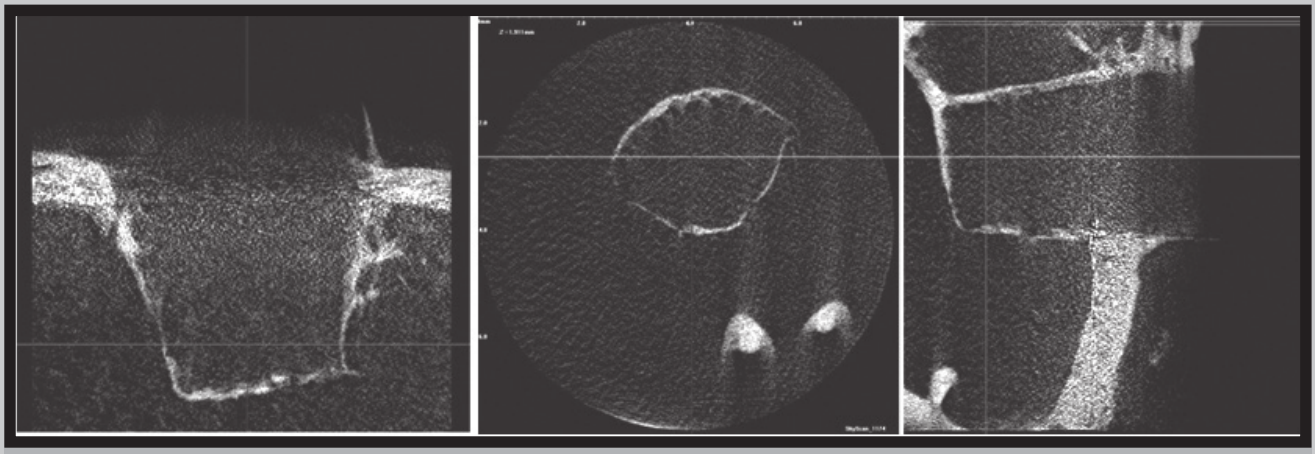

FIG. 1. Place of implanted pin in μCT photographs.