In vitro mechanical and drug release properties of bioabsorbable ciprofloxacin containing and neat self-reinforced P(L/DL)LA 70/30 fixation screws

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Osteomyelitis is inflammation of the bone caused by a pathogenic organism. Both its acute and chronic forms are difficult to heal. Antibiotics are still the basic treatment for osteomyelitis. Bioabsorbable ciprofloxacin containing bone fixation screws based on self-reinforced (SR) copolylactide P(L/DL)LA 70/30 have been developed for local treatment of bone infections. These screws gradually released ciprofloxacin during the *in vitro* bulk degradation of the matrix polymer and at the same time have sufficient mechanical strength. All the loaded ciprofloxacin was released from the gamma sterilized screws during 44 *in vitro* weeks and the concentration of the released drug per day remained between 0.06 and 8.7 μg/ml after the start-up burst peak.

1. Introduction

Bone infections are serious clinical complications associated with surgical trauma or procedures [1]. Infection may be introduced to a compound fracture at the time of surgery, but more often, the infection is a result of contamination of the open fracture [2]. Antibiotics are the basic treatment for bone infections, but, for example, chronic osteomyelitis in most cases can be treated successfully only when all foreign bodies are removed and surgical debridement is used in combination with systemically administered antibiotic therapy [3, 4]. Especially for potentially infected cases prophylactic antimicrobial agent administration is very important [1].

Currently antibiotics are mainly administered intravenously. Systemic therapy is usually long lasting, e.g. for chronic osteomyelitis from 4 to 6 weeks, requiring multiple antibiotics [4]. Prolonged parenteral therapy is uncomfortable for the patient and expensive. Because bone infection results in bone necrosis and reduced blood supply to the bone [1], antimicrobial agents may not achieve therapeutic concentration at the site of infection. High doses of parenteral antibiotics have often been used to achieve adequate local concentrations, which may cause harmful systemic side effects such as an increase in toxicity [2, 5, 6]. Bone cements are also typical local controlled delivery systems for antibiotics. Polymethylmethacrylate (PMMA) beads impregnated with gentamicin have been successfully used for prophylaxis and treatment of bone infections since the 1970s. Because PMMA is a biostable polymer, second surgery is needed to remove the beads once the gentamicin is depleted [7]. Synthetic biodegradable polyesters are potential candidates for application in local drug carrier systems to avoid the removal operation. Polylactide (PLA), polyglycolide (PGA) and their copolymers, especially low molecular weight polylactide-co-glycolide (PLGA) are the most widely studied and used for controlled release systems [8, 9]. Biocompatibility of these polymers is well established [10]. Polylactides degrade through bulk hydrolysis *in vivo* and their degradation products are finally eliminated from the body through the normal metabolic pathways as carbon dioxide and water [11, 12, 13].

Self-reinforced (SR) screws based on high molecular weight bioabsorbable polylactides have been widely studied and used for bone fixation both in orthopaedic [14] and craniomaxillofacial surgery [15, 16]. Initial mechanical properties of these screws coincide with that of bone and during the healing they degrade gradually and slowly transfer load to the healing bone [17]. Our group has recently developed various antibiotics-containing self-reinforced bone fixation screws using different bulk degrading bioabsorbable high molecular weight polymer matrices. These screws gradually release the loaded antibiotic and retain sufficient mechanical properties *in vitro* hydrolysis. The mechanical and drug release properties of the neat and ciprofloxacin containing gamma sterilized SR-P(L/DL)LA 70/30 fixation

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screws are presented in this paper. Microstructure and degradation properties in vitro of these screws will be described elsewhere.

2. Materials and methods

2.1. Materials

The bioabsorbable matrix polymer in both neat and antibiotic containing fixation screws was the commercial Resomer® LR708 (Boehringer Ingelheim, Germany). It is an amorphous bioabsorbable synthetic copolymer of L-lactide and DL-lactide with monomer ratio 70L/30DL. Inherent viscosity of P(L/DL)LA 70/30 is 6.3 dl/g (0.1% chloroform, 25 °C) and weight average molecular weight (M_w) is 910 000 g/mol. The glass transition temperature of P(L/DL)LA 70/30 is 55–57 °C, the melting temperature range is 85–115 °C and the melting enthalpy is 9–12 J/g (DSC measurement, Perkin-Elmer Pyris 1).

The antibiotic was ciprofloxacin, which is a synthetic fluoroquinolone. Ciprofloxacin base is a light yellow crystalline powder with a molecular weight of 331.4 g/mol. Solubility of ciprofloxacin into water is low, i.e., < 50 μg/ml. It is active against a wide range of gram-negative and gram-positive bacteria including two common osteomyelitis causing bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* [18]. Therapeutic *in vitro* level of ciprofloxacin is 1.0–6.3 μg/ml depending on the organism [3]. The minimum inhibitory concentration (MIC) of ciprofloxacin against the most strains is < 2.0 μg/ml [19, 20, 21].

2.2. Fabrication of self-reinforced antibiotic screws

Matrix polymer and antibiotic were extruded with a small laboratory scale mixer into billets, which were die-drawn into self-reinforced rods. Screws with the same geometry as that of BioSorbFX® 2.0 Screws (Bionx Implants, Ltd.) and length of 12.0 mm were machined from the self-reinforced rods. The thread diameter of the screws was 2.0 mm and the core diameter was 1.45 mm. In addition, rods with threads and length of 40 mm were machined for three-point bending test. The finished screws (Fig. 1) and rods with threads were washed, dried under vacuum, packed into aluminum foil pouches in a dry N_2 atmosphere and then gamma sterilized.

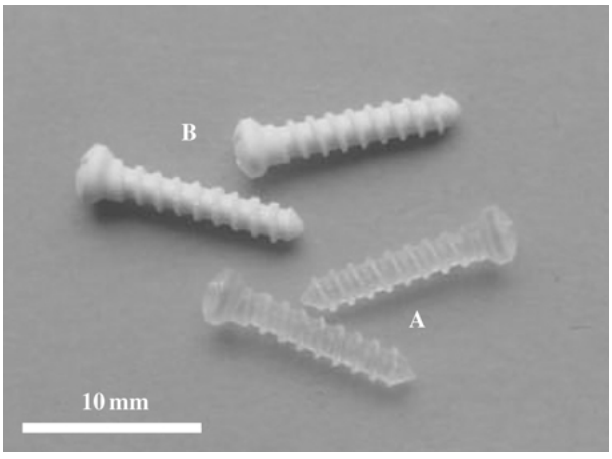

Figure 1 Bioabsorbable neat (A) and ciprofloxacin-containing (B) self-reinforced P(L/DL)LA 70/30 fixation screws.

2.3. Measurement of antibiotic release properties

Altogether 14 screws (500 mg) were placed into 50 ml of phosphate buffer (KH_2PO_4 and NaOH) at pH of 7.4 to determine released ciprofloxacin concentrations *in vitro*. Five parallel samples in brown drug bottles were kept in an incubator shaker at temperature of 37 °C. At the specific sampling times the buffer was replaced with fresh buffer and released ciprofloxacin concentrations were measured using a Unicam UV 500 spectrometer (Unicam Instruments, Cambridge, UK) at $\lambda = 270.5$ nm according to the Beer–Lambert Law.

2.4 Measurement of mechanical properties

To determine bending strength and modulus, and shear strength of the screws *in vitro*, the samples were placed in the phosphate buffer solution (KH_2PO_4 and NaOH) at a pH of 7.4 and kept stationary in the closed flasks at temperature of 37 °C up to 52 weeks. The buffer solution was changed each week or every 2 weeks and the pH was measured using a Mettler Toledo MP225 pH meter. The screw samples were kept in the brown drug bottles or containers. Specimens were removed from the solution, rinsed with distilled water and tested immediately.

All mechanical properties were measured with an Instron 4411 universal testing machine (Instron Ltd., High Wycombe, England) at room temperature. Five or six parallel samples were tested at each sampling time. Initial samples were tested dry and *in vitro* samples wet. Shear strength of the screws was measured with a tool, which was constructed by modifying the device of the standard method ASTM B 769-87 [22]. The crosshead speed was 10 mm min^{-1}. Bending strength and modulus of the samples were determined with the three-point bending test according to the modified procedure of the standard methods ASTM D 790-84 and DIN 53452 [22]. The crosshead speed was 2 mm min^{-1} and the gauge length was 22 mm. Radius of the loading nose and the supports was 5.0 and 1.5 mm, respectively.

3. Results

3.1 Antibiotic release properties

The cumulative percentage and concentration per day of released ciprofloxacin from the 14 SR-P(L/DL)LA 70/30 screws are shown in Figs. 2 and 3, respectively. All loaded ciprofloxacin was released from the screws in the 44 *in vitro* weeks. The release rate of ciprofloxacin changed significantly with time. It took approximately 9 weeks before the water had penetrated into the polymer matrix and the released ciprofloxacin concentrations per day increased significantly. The release level of ciprofloxacin was low in the first 9 *in vitro* weeks indicating poor ciprofloxacin diffusion due to slow water intrusion into the polymer microstructure. The maximum concentration value of released ciprofloxacin was measured in the 15th *in vitro* week. The concentration of released ciprofloxacin decreased almost linearly to the

TABLE I Initial mechanical properties of gamma sterilized SR P(L/DL)LA 70/30 screws

Sample	Shear strength (MPa)	Bending strength (MPa)	Bending modulus (GPa)
SR-P(L/DL)LA 70/30 screw	185 ± 10	171 ± 5	4.5 ± 0.5
SR-P(L/DL)LA 70/30 + ciprofloxacin screw	152 ± 15	151 ± 10	4.2 ± 0.2

value of 2 μg/ml/day after 16 weeks. At the end of the elution study, starting from the 26th week, the released ciprofloxacin concentration remained at a level of 2 μg/ml/day until almost all loaded ciprofloxacin was depleted.

3.2 Mechanical properties

The measured average initial shear strength of the neat and ciprofloxacin containing screws were 185 and 152 MPa, respectively. The initial bending strength of the both studied, neat and ciprofloxacin containing SR-P(L/DL)LA 70/30 screws are higher than, e.g. the one of the injection molded P(L/DL)LA 70/30 pin. The initial bending strength of the latter is reported to be 126 MPa [23]. The summary of the initial shear strength, the bending strength and the bending modulus of the studied screws are presented in Table I.

The *in vitro* shear and bending strength retention of the neat and ciprofloxacin containing screws are shown in Figs. 4 and 5, respectively. The neat SR-P(L/DL)LA 70/30 screws retained their shear strength at over 80 MPa at least for the first 36 weeks and bending strength at over 100 MPa for the first 44 weeks. During the first 9 weeks, the shear and flexural strength of the ciprofloxacin containing screws remained above 80 and 100 MPa, respectively.

4. Discussion

Biodegradable polymers are widely used as matrix materials in controlled drug release devices, which typically carriers no load [8, 9, 24–26]. Many factors effect bioabsorption, for instance chemical structure, molecular weight and molecular weight distribution, processing conditions, shape, surface area of implant, site of implantation, absorbed compounds, and mechanism of hydrolysis. Most biodegradable polymers undergo bulk hydrolysis in an aqueous environment through cleavage of the ester linkages. These polymers

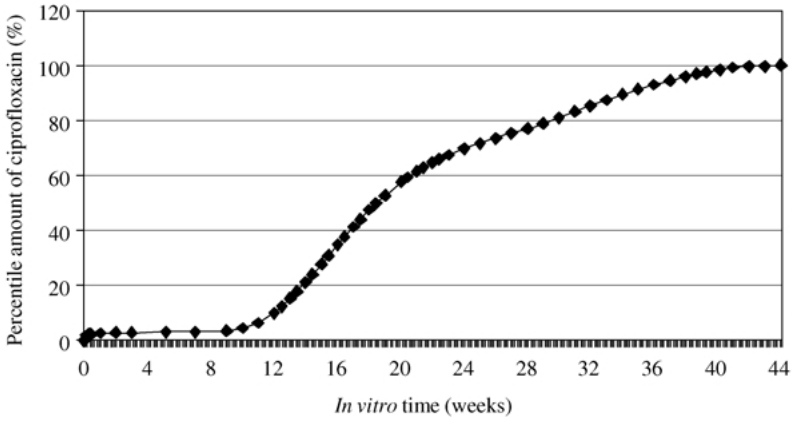

Figure 2 Cumulative percentage of released ciprofloxacin from studied bioabsorbable self-reinforced gamma sterilized P(L/DL)LA 70/30 fixation screws.

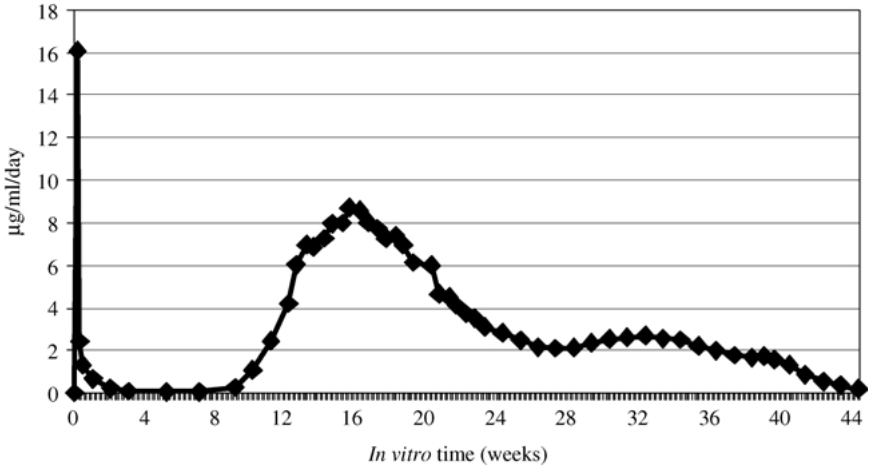

Figure 3 The concentration of released ciprofloxacin from studied self-reinforced gamma sterilized P(L/DL)LA 70/30 fixation screws per day.

degrade in a consistently throughout the matrix. Degradation time of polymer dictates the release rate of the drug together with drug diffusion [11, 12, 13].

It took approximately 9 weeks, before the water had penetrated into P(L/DL)LA 70/30 polymer microstructure, thereafter the dissolved ciprofloxacin was diffused through the matrix into the surrounding phosphate buffer. After the start-up burst peak and the first *in vitro* week, the measured average concentration per day of released ciprofloxacin from the 14SR-P(L/DL)LA 70/30 screws was in the range of 0.06–0.7 μg/ml during the following 8 *in vitro* weeks. Thereafter the concentration *per* day of released ciprofloxacin remained between 1.33 and 8.7 μg/ml. Depending on bacteria, the therapeutic *in vitro* level of ciprofloxacin is 1.0–6.3 μg/ml and the potentially detrimental *in vitro* level to osteoblast-like cells is more than 20 μg/ml [3]. The margin is quite narrow and it is difficult to keep the release rate between these two levels. The UV results show that the *in vitro* release level is not therapeutic between the first and ninth weeks of the drug elution test after the burst peak on the first day. However, the concentration of released ciprofloxacin did not exceed the detrimental level during the entire elution study. Bioactivity of ciprofloxacin after manufacturing, gamma sterilization, and long-term *in vitro* hydrolysis has been indicated with bacteriological tests for both antibiotic fillers and screws [27].

The requirements for polymer implants used to hold fracture or osteotomy fragments together are that they retain their strengths until bone union is achieved, normally about 4 to 8 weeks [28]. Ciprofloxacin containing SR-P(L/DL)LA screws retained their mechanical properties at least 12 weeks at the level, which ensures their fixation properties (Figs. 4 and 5). Because polylactides degrade faster *in vivo* than *in vitro* [22], the loss of mechanical properties will be faster *in vivo*.

5. Conclusions

Ciprofloxacin containing fixation screws based on self-reinforced P(L/DL)LA 70/30 have sufficient strength retention and drug release properties *in vitro*. They may

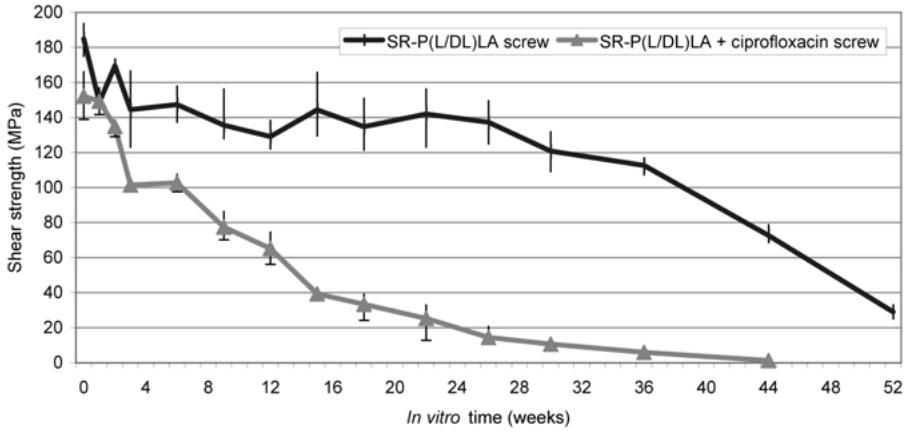

Figure 4 In vitro shear strength retention of studied neat and ciprofloxacin-containing bioabsorbable self-reinforced gamma sterilized P(L/DL)LA 70/30 fixation screws.

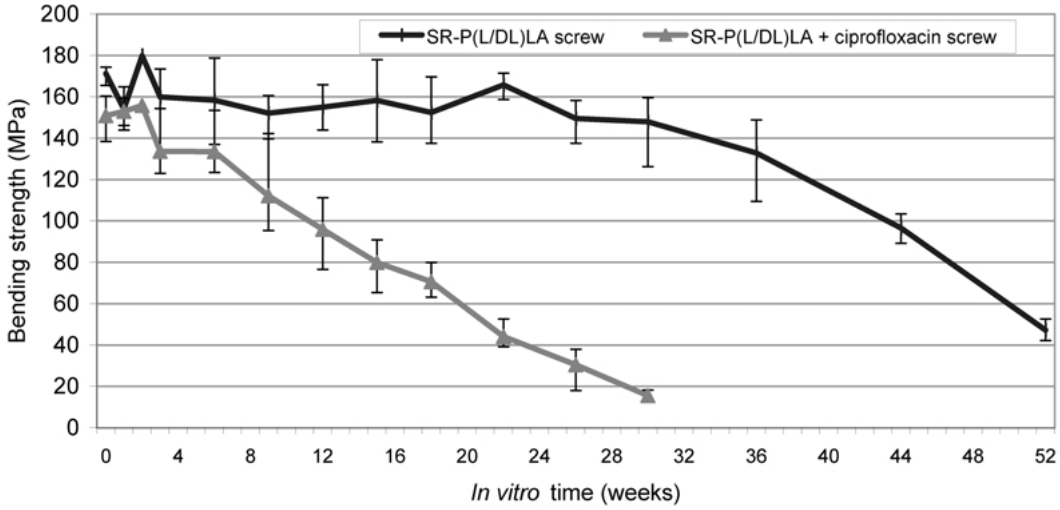

Figure 5 In vitro bending strength retention of studied neat and ciprofloxacin-containing bioabsorbable self-reinforced gamma sterilized P(L/DL)LA 70/30 fixation screws.

be used clinically for osteofixation and infection prophylaxis. The reported results have to be verified with *in vivo* experiments.

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