Bone response to polymers based on poly-lactic acid and having different degradation times

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Authors studied two degradable and resorbable polymers derived from lactic acid: poly-L-Lactic acid (PLLA), with a relatively long time of degradation (longer than 6 months, PL10 Purac NL); poly-DL-Lactic acid (PDLLA), with a relatively short time of degradation (shorter than 6 months, PDL Purac NL). The animal species was the young adult New Zealand White rabbit. The *in-vivo* study was performed by implantation of small cylinders of 10×3 mm in size (length $\times$ diameter) in the distal metaepiphysis of the femur; 34 cylinders have been implanted. Retrievals of PLLA specimens took place at 3, 6, 9, 12 and 24 months; for PDLLA specimens at 1, 2, 4 months. Polarized light microscopy of undecalcified tissue sections was performed. The analysis for PLLA and PDLLA has shown a favorable response of bone tissue: alterations in the bone repair, growth and remodeling have not been observed. PLLA is persistent at the times studied; there is never a tight apposition between bone and PLLA implant and an intervening fibrous layer has often been observed. PDLLA is not persistent at the times studied and it degrades quite fast; bone repair of the empty implantation's hole occurs by bony growth from the endosteal trabeculae. The newly formed bone covers the hole's walls with an elongation parallel to them. For both polymers, whether the degradation is fast or slow, the material's substitution by newly formed bone never starts from the walls of the implantation hole. Only after the complete disappearance of the polymeric material newly formed bone begins to fill the hole.

1. Introduction

During the past 20 years polymers called biodegradables and bioabsorbables have been used in orthopedic surgery in order to produce implantable devices which do not require a second surgical operation for their removal [10, 12].

Nowadays there are several degradables polymers under laboratory and clinical testing for the eventual use in orthopaedic surgery, both alone or in combination with other materials [6]. Among early and now most tested polymers, there are those derived from lactic acid; they have been used for many years, in surgery, as material for absorbable sutures. Polymers derived from lactic acid have shown, actually, a better clinical response than those derived from glycolic acid, one of the first substance to be clinically tested and for which a number of adverse inflammatory reactions had been documented [4, 11].

In this work we will concentrate on the description of the *in-vivo* behavior, in bone, of polymers derived from lactic acid in order to show the general mechanism of this behavior.

First of all it is important to discuss about terminology. Even if there is not full agreement about the term ''degradable'', it is basically used to state that the polymer undergoes some hydrolytic transformations in the host environment after which it is reduced into smaller chains. The term ''resorbable'' is generally used to state that the polymer, and its sub-units, can be removed by cellular mechanisms of the host environment, either by enzymatic secretions or by phagocytosis. It is possible, but not definitive, that the sub-units will be metabolyzed too.

From these definitions it derives that a degradable polymer is not necessarily resorbable. Degradability is principally related to physical–chemical properties of the material while resorbability is principally related to the reactivity of host tissues.

The principal requirement for degradable polymers used in orthopaedic surgery is to maintain adequate mechanical properties long enough to allow fracture's healing and consolidation [5]. This time is generally considered to be 8 weeks but can have some variations ($\pm$ 4 weeks) according to the anatomical site and the

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type of fracture; moreover, delayed unions may occur. Consequently it can be useful to have the permanence of mechanical properties for a time variable from 8 to 12 months. Degradable polymeric materials which maintain mechanical properties for so long, have a very long time of degradation *in vivo*, generally more than three years [1], this is an unfavorable aspect because there are no evidences that, after such a long time, the space once occupied by the implant will be replaced by newly formed healthy bone. On the experience and knowledge acquired about metallic implants, where it is noticed that after removal the space is not filled before several years have passed (and sometimes never fully), it should be desiderable to obtain a polymeric material that, after accomplishing its task, degrades in a few months.

From the practical viewpoint, most authors consider that degradable implantable devices are suitable for traumatic pathologies [2, 3, 7, 8]. Reasons for this selection are: (a) the steady increase in occurrence of traumatic pathologies; (b) the increased preference for their surgical treatment instead of conservative treatment. The reason of this trend must be ascribed to trauma due to locomotion, in particular to motorcycles, and to sports at higher risk, like para-sailing. Besides, better surgical and anaesthesiologic techniques offer more indications for the surgical treatment of these pathologies, avoiding possible undesirable outcomes associated with the long time of immobilization in cast required by conservative treatment, like articular stiffness, muscular hypotrophy, possible insurgence of Sudeck's syndrome.

2. Materials and methods

We studied two degradable and absorbable polymers derived from lactic acid. Poly-L-Lactic acid (PLLA), with a relatively long time of degradation, longer than 6 months (PL10, Purac NL).

Poly-DL-Lactic acid (PDLLA), with a relatively short time of degradation, shorter than 6 months (PDL, Purac NL).

The animal species was the young adult New Zealand White rabbit. The *in vivo* study was performed by the implantation of small cylinders of 10×3 mm in size (length $\times$ diameter) in distal metaepiphysis of the femur; 34 cylinders have been implanted.

A sample retrieved on the same day of implantation (day 1), with the specimen retrieved immediately after the operation, was analyzed to validate the histological processing.

We wanted to assess the biological response at 3, 6, 9, 12 and 24 months from implantation of PLLA specimens, and at 1, 2, 4 months for PDLLA specimens, by histomorphometric analysis with polarized light microscopy.

The morphologic study was based on the analysis of repair, growth and remodeling of bone; it was not possible to perform a proper analysis of the interface because of possible presence of artifacts about the materials due to histologic procedures. The procedure of implantation consists of: trichotomy performed the day before operation; fasting for 24 h, without restrictions on water intake; recording of preoperation weight of the animal; administration of 40 mg/kg Ketamine + 2 mg/kg Xylazine. Gaseous anaesthesia with O_2 + isofluorane 1–2% 0.2 mL/min in spontaneous breathing. Physiologic solution was administred i.v. from the right ear.

In lateral position, after cleaning the area with Iodopovidone (Betadine, Asta Medica), the patella is medially sub-dislocated. A rectangular operating-field is prepared. On extended knee, a lateral parapatellar incision is performed. The capsule is reached by blunt dissection and arthrotomy is performed. The patella is medially dislocated and the knee is bent to access the intercondylar groove. By a sharp tip of stainless steel, the fit for a miller of 3 mm of diameter is prepared. A hole of about 15 mm in length is made by a hand drill and the cylinder is inserted. The patella is reduced and tissues are carefully sutured with absorbable thread (Dexon 2-0, Davis-Geck); non-absorbable Nylon 3-0 is used for the skin. Antibiotic therapy is administered (Rifocin 250 mg per day im).

After the sacrifice of the animal, the femur is retrieved. An anterior trasversal incision on the articular plane of the knee and a lateral longitudinal incision on the thigh are performed. By blunt dissection the bone is reached and the middle part of the femur is exposed. The femur is cut in its half by an alternative saw. Standard X-ray films of the samples are taken using the following parameters: 60 mA, 50 KV, 0.07 s of exposure. Samples are embedded in PMMA and cut in sections of 100 microns of thickness for the analysis by polarized light microscopy [9].

3. Results

3.1. Bone response to PLLA

The embedding and cutting procedures can produce alterations of the internal structure in the material and at the interface with bone, more probable as polymer with higher degradability is used. The sample retrieved on the same day of implantation (day 1) shows minimal alterations of the geometry and the presence of very well defined concentric rings (Fig. 1), perhaps related to the manufacturing process (internal tensions due to cooling after pressing).

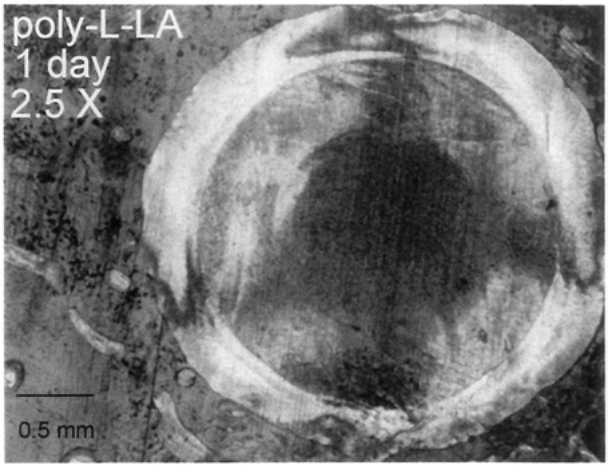

Figure 1 Transverse section in polarized light microscopy (magnification 2.5 $\times$) of a PPLA cylinder the same day of the implantation (day 1). The sample shows concentric rings perhaps related to the manufacturing process.

Analysis of the implanted specimens has presented the same results at 3, 6, 9, 12 and 24 months showing the morphologic integrity of the specimens at all times (Figs 2–4). Microscopic analysis shows a normal growth and physiologic remodeling over all the section and close to the implant (Fig. 5), where there is, often, a thin annular rim of bone. Anyway, bone growth is never characterized by a tight apposition with the implant; an intervening fibrous layer is often present.

3.2. Bone response to PDLLA

The study has shown the disappearance of the specimens, in their morphologic integrity, at every retrieval time (Fig. 6). Only the implantation's hole and some material debris are visible (Fig. 7).

It is very interesting to note that the presence of the debris seems not to alter the bone repair at the implant site. In some cases it has been possible to find smaller repairing trabeculae in the middle of the hole, close to larger pre-existent peripheric trabeculae and material debris (Fig. 8).

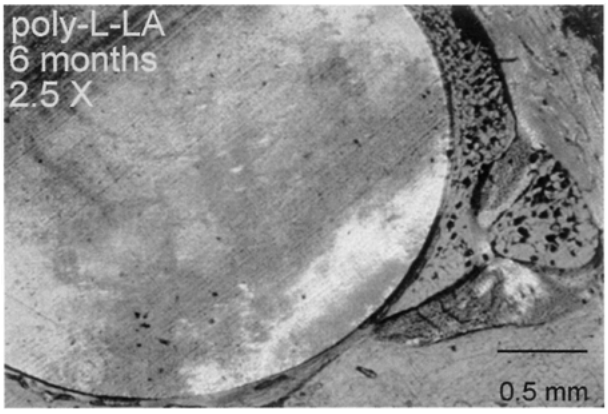

Figure 2 Transverse section in polarized light microscopy (magnification 2.5 ×) at 6 months from implantation: trabecular bone grows in proximity of an integer PLLA cylinder.

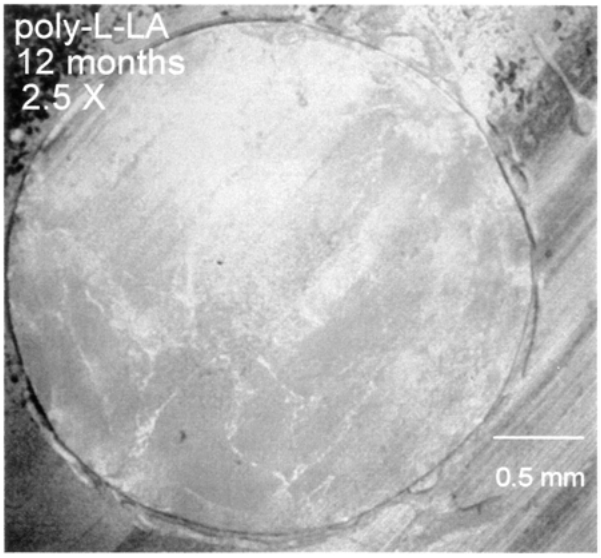

Figure 3 Transverse section in polarized light microscopy (magnification 2.5 ×) at 12 months from implantation: the full morphological integrity of the PLLA cylinder is evident; trabecular bone grows around the implant.

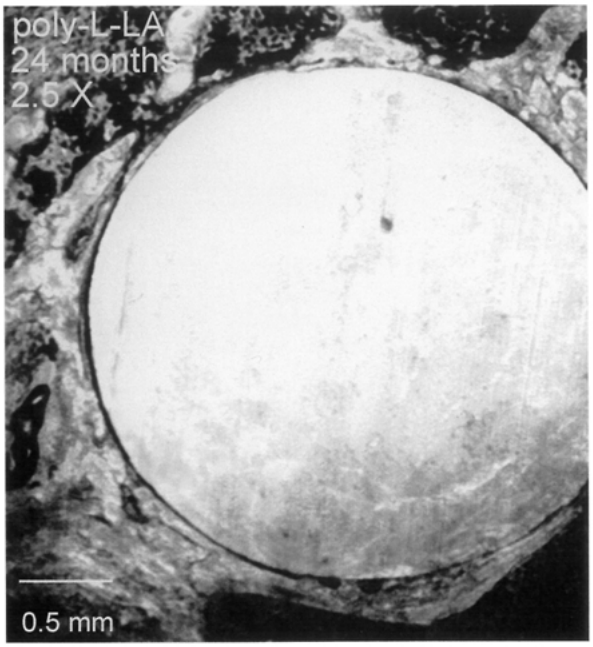

Figure 4 Transverse section in polarized light microscopy (magnification 2.5 ×) at 24 months from implantation: a well defined annular rim of bone surrounds the implant and the full morphological integrity of the PLLA cylinder is maintained.

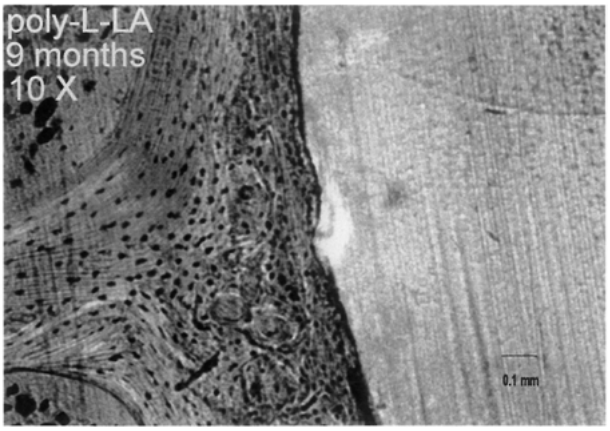

Figure 5 Transverse section in polarized light microscopy (magnification 10 ×) showing healthy trabecular bone growing close to the PLLA implant surface (on the right) at 9 months from implantation.

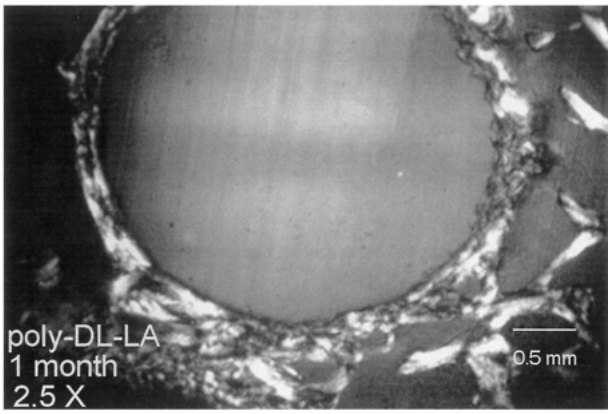

Figure 6 Transverse section in polarized light microscopy (magnification 2.5 ×) at 1 months from implantation of a PDLLA cylinder: a hole is present inside a well-defined trabecular bone area.

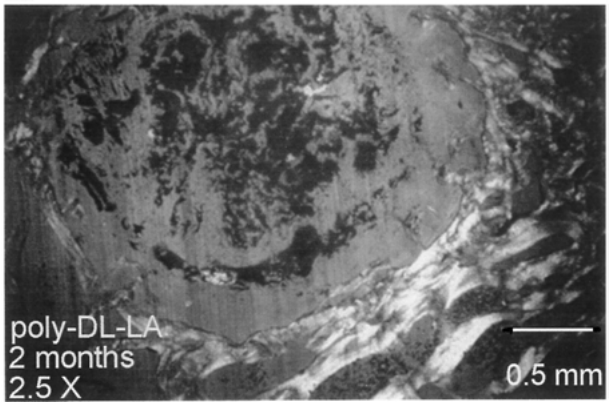

Figure 7 Transverse section in polarized light microscopy (magnification 2.5 ×) at 2 months from implantation of a PDLLA cylinder: a hole is present inside a well defined trabecular bone area; debris of the degraded PDLLA cylinder are present in the hole.

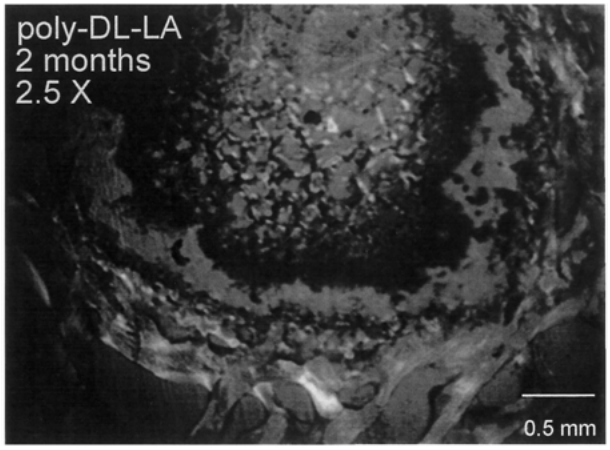

Figure 8 Transverse section in polarized light microscopy (magnification 2.5 ×) at 2 months from implantation of a PDLLA cylinder: a hole is present inside a well-defined trabecular bone area; debris of the degraded PDLLA cylinder are present in the hole and tiny new trabeculae grow in the center.

Bone repair of the implantation's hole occurs by growth starting from the endosteal trabeculae. The newly formed bone covers the hole's walls with an elongation parallel to them and growth at the periphery of the hole is nearly at right angle with the old cortical bone.

There are no evidences for bone growth starting in continuity with the hole's walls.

4. Discussion

The analysis for PLLA has shown a favorable bone response because alterations in the bone repair, growth and remodeling have not been observed. The material is quite persistent at all the times studied. An intervening fibrous layer has often been observed between bone and material.

The analysis for PDLLA has shown a favorable bone response because alterations in the bone repair, growth and remodeling have not been observed. The material is not persistent at the times studied. Bone repair of the empty implantation's hole occurs by growth starting from the endosteal trabeculae; the newly formed bone covers the hole's walls with an elongation which is parallel to them.

Whether the degradation is fast or slow, the material's substitution by newly formed bone never starts in continuity with the walls of the implantation's hole and new bone tends to fill it only after the complete disappearance of the polymeric material. There is never a tight apposition between bone and implant and there is often only a thin rim of non-calcified tissue that, anyway, should not be confused with a frank fibrotic reaction. Since PLLA and PDLLA materials degrade *in vivo* with different velocities, it is possible to use them in orthopaedic surgery for differentiated applications, according to the mechanical properties required. Another option could be the association of both in a single implant design.

Anyway, the use of these materials should be peculiar in those cases where it is advantageous to avoid a second surgical operation for implant retrieval.

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