Tissue restoration after resorption of polyglycolide and poly-laevo-lactic acid screws

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Bio-absorbable implants were developed to provide a convenient method of internal fixation in orthopaedic surgery which avoided the problems associated with retained metal. They have been in limited, but gradually expanding, clinical use for more than 15 years. The first field of application was with the fixation of small-fragment fractures, followed by the stabilisation of ligamentous structures in the shoulder and the knee. Mechanically, it is difficult to construct reliable absorbable implants larger than pins, screws, staples, anchors and small plates.

The first-generation devices, made of rapidly resorbable polymers such as polyglycolic acid (PGA), which has a degradation time of less than six months, were found to be associated with a relatively high incidence of inflammatory foreign-body reactions. Further development focused on suitable biodegradable macromolecular compounds with a longer degradation time. Poly-laevo-lactic acid (PLLA) became one of the most popular of these polymers.

Implants made of high-molecular-weight PLLA have been in clinical use for a decade, although their degradation time is not known. Internal fixation devices made of PLLA seldom elicit foreign-body reactions of clinical significance. However, if the degradation time of high-molecular-weight PLLA was shown to be very long, this would be a disadvantage.

In a long-term experimental study, we have determined the patterns of tissue restoration 36 and 54 months after implantation of polyglycolic acid and poly-laevo-lactic acid screws in the distal femur of the rabbit.

After 36 months in the polyglycolic acid group the specimens showed no remaining polymer and loose connective tissue occupied 80% of the screw track. Tissue restoration remained poor at 54 months, the amounts of trabecular bone and haematopoietic elements being significantly lower than those in the intact control group. The amount of trabecular bone within the screw track at 54 months in the polyglycolic acid group was less than in the empty drill holes (p = 0.04). In the poly-laevo-lactic acid group, polymeric material was present in abundance after 54 months, occupying 60% of the cross-section of the core area of the screw track.

When using absorbable internal fixation implants we should recognise that the degradation of the devices will probably not be accompanied by the restoration of normal trabecular bone.
50 mm long. According to the manufacturer, the molecular weight of the PGA was between 50,000 and 200,000 daltons. For PLLA, the viscometric mean molecular weight of the raw material was 700,000 daltons. After processing, the molecular weight of the PLLA was 50,000 daltons and the degree of crystallinity 50%. The density of the screws was 1.6 g/cm³.

We used 36 New Zealand white rabbits of both genders. They had closed epiphyseal lines and their mean weight was 3.7 kg (3.2 to 4.0). The animals were anaesthetised with a combination of 0.3 mg/kg of medetomidine and 30 mg/kg of ketamine administered subcutaneously. Under standard aseptic conditions, a lateral parapatellar incision was made in both knees in 18 animals and in the right knee only in another 18. The patella was displaced medially and the distal femur was exposed. A hole, 3.2 mm in diameter, was drilled in the cancellous bone by directing the drill-bit centrally through the intercondylar portion of the distal femur in a proximal direction towards the intramedullary canal (Fig. 1). The drill hole was then irrigated with saline solution and tapped. A PGA screw was inserted in 18 femora and a PLLA screw in another 18. No screw was inserted in the tapped drill hole in a further 18 femora, and the remaining 18 knees were left intact to show the normal tissue composition of the bone section for comparison. When a screw was inserted, the head of the screw was finally cut off to allow the full range of movement of the knee. The subcutaneous soft tissues and the skin were closed in layers. No post-operative immobilisation was used. The rabbits were free to move around and were fed with commercial laboratory animal fodder.

Throughout the study, the procedures were performed according to the regulations on animal experimentation of the institutions concerned and the legislation of Finland and the European Union.

**Tissue preparation and histomorphometric analysis.** One rabbit died early and had to be excluded from the study, leaving two groups of 17 and 18 animals respectively. Each was divided into groups of eight or nine which were killed by an overdose of sodium pentobarbital at 36 and 54 months respectively. Both femora were harvested. The distal third of each femur was fixed, in a series of ethanol immersions of increasing concentration (70% to 99%) and embedded in methylmethacrylate. The specimens were not decalcified.

Statistical analysis. All values were reported as pooled means and SDs with the use of one animal as the experimental unit. The Mann-Whitney U test was used to detect differences. Statistical significance was set at p < 0.05.

**Results**

**PGA.** No remaining polymeric material was seen in the 18 specimens implanted with a screw made of PGA. No phagocytic or inflammatory cells were observed within the screw track or in its vicinity. Both the 36- and 54-month groups showed significantly lower amounts of haematopoietic tissue elements (p < 0.05) and trabecular bone (p < 0.01) than the intact specimens (Table I and Fig. 2). Instead, fat and loose connective tissue were the predominant components of tissue restoration. At the site of the

For hisological and histomorphometric analysis, sections 5 µm thick of the cancellous bone of the distal femur were cut with a microtome in the transverse plane 20 mm from the joint surface of the condyles, perpendicular to the long axis of the screw (Fig. 1). Two sections were cut from each specimen and stained using the Masson-Goldner trichrome method. The birefringence of PGA and PLLA on polarised light microscopy was used to assess the amount and distribution of polymeric material in the specimens. For contact microradiography, sections 80 µm thick were made with a saw microtome to provide images which were counterparts of the corresponding histological sections.

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original tissue-implant interface, a walling-off type of new bone with a mean thickness of 250 µm was seen. Few bone trabeculae had formed within the screw track (Fig. 3).

The amount of trabecular bone in the 54-month specimens was also lower (p = 0.04) than in the empty drill holes (Fig. 2). The variation in the total volume of new bone between specimens from different animals was relatively high, but no statistically-significant differences in the fractional osteoid surface were seen between the groups. In the 54-month specimens, the mean osteoid surface fraction as determined histologically and microradiographically was 0.14 (± 0.05) in the PGA group, 0.15 (± 0.04) in the empty drill hole group and 0.13 (± 0.04) in the intact control group.

### Table I. Mean (±S.D) fractional cross-sectional occurrence of tissue components and implant polymer in the histological specimens

<table>
<thead>
<tr>
<th></th>
<th>Trabecular bone</th>
<th>Haematopoietic tissue</th>
<th>Connective tissue and fat</th>
<th>Polymeric material</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>PGA</em> screw</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 months (n = 9)</td>
<td>0.02 (0.01)</td>
<td>0.18 (0.07)</td>
<td>0.80 (0.24)</td>
<td>0</td>
</tr>
<tr>
<td>54 months (n = 9)</td>
<td>0.03 (0.02)</td>
<td>0.21 (0.09)</td>
<td>0.76 (0.21)</td>
<td>0</td>
</tr>
<tr>
<td><strong>PLLA† screw</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central (n = 9)</td>
<td>0</td>
<td>0</td>
<td>0.29 (0.10)</td>
<td>0.71 (0.22)</td>
</tr>
<tr>
<td>Peripheral (n = 9)</td>
<td>0.02 (0.01)</td>
<td>0.05 (0.03)</td>
<td>0.67 (0.19)</td>
<td>0.26 (0.10)</td>
</tr>
<tr>
<td>54 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central (n = 8)</td>
<td>0.05 (0.02)</td>
<td>0.02 (0.01)</td>
<td>0.33 (0.12)</td>
<td>0.60 (0.21)</td>
</tr>
<tr>
<td>Peripheral (n = 8)</td>
<td>0.04 (0.02)</td>
<td>0.07 (0.04)</td>
<td>0.70 (0.20)</td>
<td>0.19 (0.09)</td>
</tr>
<tr>
<td>Empty drill hole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 months (n = 9)</td>
<td>0.13 (0.07)</td>
<td>0.26 (0.10)</td>
<td>0.61 (0.20)</td>
<td>-</td>
</tr>
<tr>
<td>54 months (n = 8)</td>
<td>0.15 (0.08)</td>
<td>0.28 (0.12)</td>
<td>0.57 (0.18)</td>
<td>-</td>
</tr>
<tr>
<td>Intact specimen (n = 8)</td>
<td>0.29 (0.09)</td>
<td>0.57 (0.13)</td>
<td>0.14 (0.04)</td>
<td>-</td>
</tr>
</tbody>
</table>

* PGA, polyglycolic acid
† PLLA, poly-laevo-lactic acid
‡ within 2.0 mm of the original centre of the screw track
§ within a zone 2.0 to 4.5 mm from the centre

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**Fig. 2**

Bar chart showing the occurrence of the tissue elements and polymeric material in the 54-month specimens (PGA, polyglycolic acid; PLLA, poly-laevo-lactic acid).

**Fig. 3**

Photomicrograph showing that at three years after implantation of a PGA screw, the polymer had completely disappeared and become replaced by loose connective tissue and fat. The normal trabecular bone architecture of the distal femur has not been restored (*, centre of the screw track; Masson-Goldner trichrome, x6).
specimens (Table I). The remaining polymer was not evenly distributed within the implant track. Tissue replacement had begun in the periphery of the screw, while the core area of the polymer in the centre persisted. In several specimens this had resulted in a secondary front of new bone around the remaining polymer which walled off the more peripheral parts of the screw track (Fig. 5). The mean osteoid surface fraction of the secondary front of new bone within the screw track was 0.20 (SD 0.05) which was significantly higher (p = 0.03) than that of the new bone formed at the original tissue-implant interface 0.14 (SD 0.03). At 54 months, the amount of trabecular bone was significantly lower (p = 0.01) and the amount of connective tissue significantly higher (p = 0.01) in the PLLA-implanted femora than in the intact control specimens (Fig. 2).

A zone of phagocytic cells, most mononuclear, with ingested intracellular polymeric material was often seen in the most peripheral parts of the screw track, close to the original tissue-implant boundary (Fig. 6). Loose connective tissue was found to be the main tissue element replacing the polymer in the PLLA specimens (Table I). Haematopoietic tissue was scarce within the screw track. There were no signs of bacterial infection or tumorous conditions in any of the animals. The only inflammatory cells seen in the PLLA-implanted specimens were sporadic lymphocytes and they were few in number.

Discussion

The two principal findings of our study were the poor restoration of the normal tissue composition after degradation of PGA screws and the very slow degradation process of PLLA screws. Because of the long depolymerisation and degradation time of PLLA within bone, the ultimate pattern of tissue replacement after degradation of the PLLA screws could not be determined in this study.

PGA belongs to the fast-degrading bio-absorbable polymers. Intraosseously implanted PGA screws have been shown to disappear completely from the tissues within six months.23,24 During the early years of the development of bio-absorbable internal fixation devices, it was considered that these implants might possess some osteostimulatory potential and promote bone healing. In this long-term study, the final amount of trabecular bone was less in the
PGA-implanted specimens than in the empty tapped drill holes. Moreover, the scarce osteoid formation in the 36-month specimens indicated that the tissue restoration process had already ceased at that time. In a recent study on defects in rabbit calvarial bone, PGA membranes were found to retard osteogenesis.\textsuperscript{55} In our study the screw track was relatively large in proportion to the recipient bone section and tissue restoration might have been more favourable with smaller implants.

The life span of test animals limit studies on the tissue behaviour of slow-degrading polymers such as PLLA. It must also be recognised that fixation devices made by different manufacturers constitute a heterogenous group of implants. The specific physicochemical details of each PLLA implant have their influence on the process of degradation.\textsuperscript{26-30} Consequently, no definite conclusions concerning the depolymerisation of PLLA devices can be made from the findings of a study on one type of implant.

In our study, nearly half of the PLLA material implanted was left within the screw track after a follow-up of 54 months. This has been noted in previous experimental observations.\textsuperscript{31,32} As for the spatial pattern of the degradation process, an interesting finding was the occurrence of a secondary front of new bone within the screw track around the core area of the PLLA screw. In the specimens at 54 months, formation of osteoid was more active in the secondary front of new bone than in the front at the original tissue-implant boundary. Such a walling-off front of new bone may lengthen the process of degradation and absorption further by interfering with the centripetal tissue replacement within the cavity of the implant.

Invasion of tissue from the periphery towards the centre of the PLLA screw was a constant finding. There was no evidence of migration of PLLA particles outside the implant track as has been reported for PGA during its degradation.\textsuperscript{33} The hydrophobicity of PLLA due to its methyl groups could explain this. In a previous study, transmission electron microscopy showed that PLLA degrades by disintegrating into polygonal particles, 10 \( \times \) 20 \( \mu \)m in size on average, which are then digested by mononuclear phagocytic cells.\textsuperscript{34} Clinically, remnants of PLLA implants have been retrieved several years after use in internal fixation.\textsuperscript{17,18,35-37} Since systematic histological surveys on the process of degradation are not feasible in patients, attempts have been made to study the disappearance of the slowly degrading implants by MRI.\textsuperscript{38-40} Such indirect methods can only give approximate estimates of the degradation time, but the reports indicate that intraosseous PLLA can be seen in the tissues by imaging for at least five years.

Analysis of the biocompatibility of the polymers was not an objective of our study, but no inflammatory reactions were seen. Both PGA and PLLA did not produce an inflammatory response or a foreign-body reaction. Considerable foreign-body inflammatory responses have been seen clinically, but are rare in animal experiments. The clinical reactions to PGA implants occur during the first months after implantation,\textsuperscript{9} whereas those to PLLA are seen as late as four years after the original operation.\textsuperscript{35,41} Thus, the probable cause of the foreign-body reactions is an overload of the clearing capacity of the tissues.

In clinical studies, the degradation of bio-absorbable implants has sometimes been found radiographically to be accompanied by osteolytic changes in the vicinity of the implant tracks. This was first observed for PGA,\textsuperscript{42,43} but has subsequently been seen with PLLA.\textsuperscript{44,45} Recently, a clinical trial was stopped when loss of cancellous bone was observed around a suture anchor for shoulder stabilisation made of one of the newly-developed stereo-isomeric copolymers of polylactide, poly-L/DL (70/30)-lactide.\textsuperscript{46} The long-term significance of the osteolytic changes seen when using polylactide implants is so far unclear, but those occurring around a PGA screw at the ankle have been found to be associated with the development of osteoarthritis.\textsuperscript{47} Experimental studies have been unable to reveal the exact pathogenesis of the osteolytic lesions. In our study, no signs of osteolysis were seen at the implant tracks.

Our findings have some clinical implications. Firstly, since the poor tissue restoration probably applies to all internal fixation devices made of PGA or PLLA, certain precautions are necessary in the clinical use of bio-absorbable devices. To maintain the load-bearing capacity of the bone concerned and to minimise the risk of subsequent bone failure, large implants in proportion to the recipient bone should be avoided. Secondly, it is questionable whether devices made of high-molecular-weight PLLA with a degradation time of more than five years can be regarded as being bio-absorbable from a clinical point of view. A very long degradation time is a disadvantage in those clinical situations in which subcutaneous prominence or intra-articular breakage of an implant may cause problems or discomfort.\textsuperscript{19,35,36,41,48,49} Devices made of polymers with degradation time of medium length, such as glycolide-lactide copolymers and stereo-isomeric copolymers of polylactide (pol-L/DL-lactic acid), may not have the specific problems associated with the fast- and slow-degrading polymers. However, the preliminary results are controversial.\textsuperscript{46,50,51} More clinical experience of these implants is required. Since there may be some significant species-related differences in the potential restoration of tissue, information on the tissue replacement of bio-absorbable implants in patients should also be collected by retrieving tissue specimens whenever feasible.

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References