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# Filling of bone defects with porous hydroxyapatite reinforced with polylactide or polyglycolide fibres

P. YLINEN
*Department of Orthopaedics and Traumatology, Helsinki University Central Hospital, Helsinki, Finland and Biomaterials Laboratory, Tampere University of Technology, Tampere, Finland*

Hydroxyapatite has intrinsically poor mechanical resistance for loading bone replacements and its clinical applications are mainly limited to use as a filling device. In the present study, hydroxyapatite blocks were reinforced with resorbable polyglycolide (PGA) or polylactide (PDLLA) fibres so that most of the implant pores remained open assuming intimate contact with the host bone. The reinforced blocks, $2 \times 3 \times 4$ mm in size (Interpore 200), were implanted into the proximal and diaphyseal tibiae of rabbits in order to study the tissue and bone ingrowth into the implants. The samples were studied by histological, histomorphometrical, microradiological, and oxytetracycline fluorescence analyses. The results suggested that PGA or PDLLA fibre reinforcement does not hinder bony ingrowth into the hydroxyapatite implant. Maximal bone ingrowth was observed at 6 weeks but thereafter the amount of ingrowth remained constant up to the end of the 24-week follow-up period. Modest foreign body type reactions around the fibres were histologically seen and there was no difference between the two types fibres in relation to the bone ingrowth. With implants used in this study the bone ingrowth as measured with histomorphometry was $12.9 \pm 1.4\%$ in the cancellous implantation and $17.1 \pm 1.5\%$ in the cortical implantation . It seems that fibre reinforcement does not hinder bone ingrowth into the coralline hydroxyapatite implants and supports their further development as bone graft substitute in high loading conditions.

## 1. Introduction

Ceramics such as derivatives of calsiumphosphates, especially hydroxyapatite and tricalsiumphosphate, have been subjects of investigation as artificial substitutes for autogenous bone. Porous hydroxyapatite appears to be suitable for clinical practice and has already been used, for example, as an onlay graft in alveolar ridge augmentation and maxillofacial surgery [1–3], and as bone filler in cysts or bone defects such as tibial plateau fractures [4]. Hydroxyapatite coating has also been used in joint replacements [5, 6].

Hydroxyapatite (HA) is a material chemically similar to the inorganic matrix of living bone and it is entirely biocompatible [7]. The most important property of hydroxyapatite is its ability to bond to the host bone without any interposition of fibrous tissue or immunogenic side-effects [8–12]. Although hydroxyapatite in itself is osteogenically inactive, it allows new bone ingrowth into a porous structure.

Intimate and stable contact with the host bone is the main prerequisite for osteoconduction. Due to the brittleness of porous hydroxyapatite, it may not provide stable interfacial circumstances when implanted into sites where mechanical loading occurs. It seems that one of the most restrictive factors for new clinical applications of hydroxyapatite is its poor mechanical strength. Absorbable materials covering or impregnating the hydroxyapatite implants have been used in attempts to improve the tensile and fatigue properties of porous hydroxyapatite, however, some disturbance of new bone ingrowth into the implant was seen [13, 15, 16].

For this study a new method of strengthening the HA implants was developed [14]. In order to allow direct contact between HA and host bone from the moment of implantation, reinforcement of coralline HA blocks was carried out by wrapping their outer surface only partially with absorbable fibres. Polyglycolide and polylactide were chosen as raw material for reinforcing fibres, and implants reinforced with one or other of these fibres were manufactured. The aim of the present experimental study was to study and compare the osteoconductive properties, i.e. tissue and bone ingrowth into that kind of reinforced HA implant, and elucidate and compare the possible side-effects related to the two types of reinforcing fibres.

## 2. Materials and methods

### 2.1. Implants

Reinforced hydroxyapatite (HA) blocks were fabricated in the biomaterials laboratory, Tampere University of Technology, Finland. Commercially available

porous hydroxyapatite blocks with mean pore diameter of 200 μm were used as raw material (Interpore International, Irvin, CA, USA). They were sawn into small blocks $2 \times 3 \times 4$ mm in size, and 0.3 mm deep grooves were made on the outer larger surfaces. One groove was created lengthwise and the other crosswise in the centre of the surface (Fig. 1). Two kinds of blocks were made, wrapping reinforcing fibres of either poly-DL/L-lactide (PDLLA) or polyglycolide (PGA) (DEXON "S" 4-0) 0.3 mm in diameter into the prefabricated grooves of the blocks.

## 2.2. Operative procedure

Twenty-three adult rabbits weighing 3100–4200 g (mean 3620 g) were operated on. Atropin (Atropin 1 mg/ml, Orion, Finland) 0.5 ml/kg and diazepam (Diapam 5 mg/ml, Orion, Finland) 0.3 ml/kg were injected subcutaneously as premedication. Anesthesia was accomplished with subcutaneous medetomidine (Domitor 1 mg/ml, Lääkefarmos, Finland) 0.3 ml/kg and ketamine (Ketalar 50 mg/kg, Parke-Davis, Spain) 0.5 ml/kg [17]. As infection prophylaxis procaine penicillin (Procapen 300 000 I.U./ml, Orion, Finland) was given (150 000 I.U.) subcutaneously.

Both hind limbs in twenty-one rabbits and one hind limb in two rabbits were shaved and scrubbed with antimicrobial solvent (Neo-Amisept). The proximal tibia was exposed through a medial incision, starting at the joint level and extending 3 cm distally. The medial collateral ligament was identified, and the level of implant application was prepared to be between the joint level and distal insertion of the ligament, i.e. 3–5 mm below the joint level. Anterior to the medial collateral ligament a drill hole, 2.0 mm in diameter and 4.0 mm in length, was drilled transversely to the tibial shaft using physiologic saline (NaCl) for flushing. The drill hole was then extended so that the metaphyseal defect created was $2 \times 3 \times 4$ mm in size. Finally, the defect was copiously irrigated with saline to remove all debris before implant insertion. A similar defect was created through a lateral incision into the cortical bone region of the middle part of the tibia.

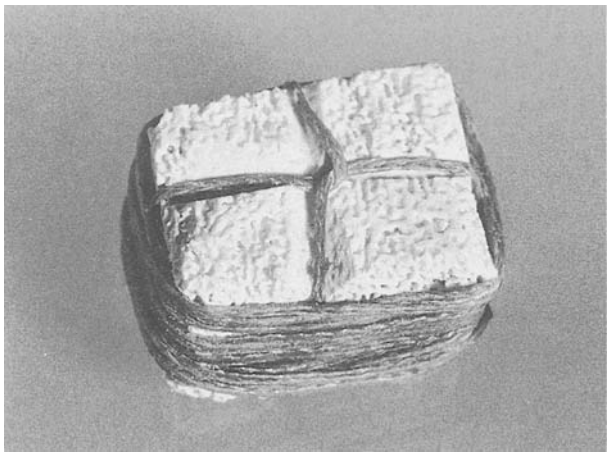

*Figure 1* Porous hydroxyapatite block $2 \times 3 \times 4$ mm in size reinforced with polyglycolide (PGA, Dexon "S" 4-0) fibres.

The reinforced HA blocks were plugged in so that the implants were flush with the tibial bone cortex. The fascia and cutis were sutured with interrupted 3-0 Dexon sutures. In twenty-one rabbits both hind legs were operated on the right tibia implanted with poly-DL/L-lactide (PDLLA) reinforced HA and the left tibia with ploy glycolide (PGA) reinforced HA. As a pilot study, only one hind leg was operated on in two rabbits using PGA reinforced implants in one rabbit and PDLLA reinforced implants in the other rabbit, and these were also included in the study. The follow-up times were 6, 12, 16 and 24 weeks. Oxytetracycline, 50 mg/kg (Terramycin 100 mg/ml, Pfizer, Belgium) was given intramuscularly two to three days before sacrifice at 6, 12 and 16 weeks.

## 2.3. Tissue sampling techniques

The rabbits were sacrificed and the operated tibiae retrieved. Radiographs in standard lateral projections were taken (Fig. 2). The proximal and middle parts of the tibia, including the implants, were sawn for sampling. The samples were fixed in a series of ethanol immersions with rising concentrations and embedded in methylmetacrylate. Transverse sections were cut beginning cranially until the implant was seen. The 5-μm-thick sections for histologic and histomorphometric studies were sawn at two section levels, a surface section at the depth of the first display of the

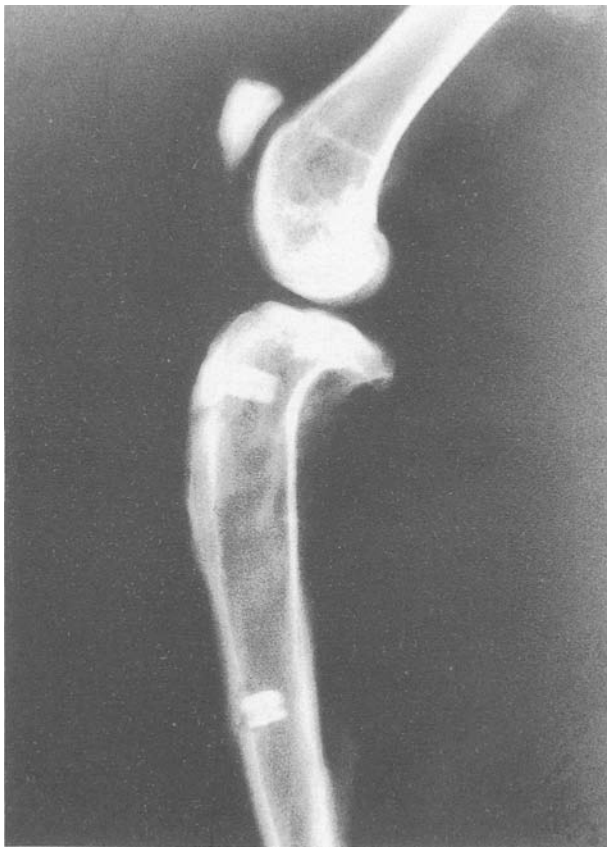

*Figure 2* Radiograph of the tibiae in a rabbit followed for 24 weeks showing the implantation with PDLLA-reinforced blocks. The blocks are radiologically well visible and show no adverse reactions in the bone–implant interface.

implant and a deep section at 1 mm depth, using a microtome Jung Polycut S. Between the histologic sections an 80-μm-thick section was taken with a saw microtome Leitz 1600 for oxytetracycline (OTC) labelling and microradiographic studies.

### 2.4. Histomorphometric studies

Histomorphometric studies were performed with a computer-aided image analyser (Kontron Videoplan Image Processing System, Kontron, Eching/Munich, Germany). A colour camera (Saticon DK-81K, Hitachi Denshi Ltd, Japan) was connected with a C-adaptor (1:1) to a Leitz microscope (Leitz Wezlar, Ernst Leitz Co., Germany), and the microscopic field was displayed on the screen of the computer. Using a PLF 6.3/0.20 objective the magnification on the screen was 250. Distance calibration was used to obtain the results in square millimetres.

Using measuring frame and digitizing board two fields of 0.57 $mm^2$ in size were analysed in all samples, i.e. an area of 1.14 $mm^2$ in all (TOTAL). The first measuring field uniformly faced the cortical edge of the implant and a second field the medullary edge of the implant (Fig. 3a). Initial tissue response with invading host cells and capillaries formed the reactive connective tissue which pervaded the empty spaces of HA and began to deposit new bone. Histomorphometrically the tissue proportions of connective tissue (CONNECT), and new bone (NBONE), were measured. Further calculations for the hydroxyapatite (HA = TOTAL − (CONNECT + NBONE)) and percentage tissue proportions of connective tissue (% CONNECT) and new bone (%NBONE) as well as the proportion of hydroxyapatite substratum (%HA) were performed using Microsoft[R] Excel Worksheets. The results were calculated at different periods of the follow-up for the PGA- and PDLLA-reinforced implants.

### 2.5. Histologic and microradiographic studies

All 5-μm-thick sections were stained with Masson modification of Goldner stain [18] for histologic studies. Two sections from both types of implantations at different follow-up times were stained with Gomori reticulin stain [19]. The samples were evaluated for the development of bone and the development and distribution of connective tissue, and also for the amount of inflammatory cells and giant cells. In microradiography the Faxitron cabinet X-ray system (Hewlett-Packard 43855 A) and Kodak Spectroscopic plates type 649-0 were used. The tube voltage was 21 KV, the exposure time 15 min, and the film distance the shortest possible.

### 2.6. OTC-fluorescence

The intensity of OTC-fluorescence was scored semiquantitatively. First, the number of fluorescing areas or spots was calculated for all the OTC-samples using a 6.3 × objective. The number of fluorescing spots varied from zero to 75 and the range of variation was divided into seven categories. Based on this category a fluorescing index from grade 0 to grade VI was given to all samples. The fluorescing indexes were then compared between the different follow-up groups.

### 2.7. Statistics

The strength of association between variables in the histomorphometric results was analysed by computing the sample (Pearson) correlation coefficient, $r$. The significance in relation to the follow-up period, the different implants and implantations as well as the two section levels and measuring fields were further evaluated by Student's $t$-test. In comparing the OTC-fluorescence indexes the Student's $t$-test was also used. The level of significance was set at $p < 0.01$. The results are presented as sample mean ± standard error of mean values (mean ± SEM).

## 3. Results

One rabbit with bilateral operation suffered a postoperative fracture in tibial diaphysis and was sacrificed two days after the operation. Another diaphyseal tibial fracture, which was healed in a good position, was seen next to the implant while retrieving the tibia at 6 weeks and the implant was included in the study. The postoperative course of all the other rabbits was uneventful. Macroscopically the sites of implantation had healed normally. Radiologically the implants were visible throughout the 24 h week follow-up and showed no signs of resorption. Implant fractures were not seen, and radiologically osseointegration appeared good.

A total of 168 histologic, 84 microradiographic, and 64 OTC samples was available for analysis.

### 3.1. Histologic and microradiographic findings

Hydroxyapatite was unstained, showing up as void spaces bordering the ingrowing tissues. Reticulin fibres could be visualized in the Gomori stain, especially around the block. Histologically all porous spaces were filled with ingrowing connective tissue, and no apparent empty spaces were found.

Reactions towards reinforcing fibres were microscopically observed from 6 weeks up to the end of the follow-up (Fig. 3a,b). In the case of PGA, foreign-body-type giant cells and foam cells clustered in the vicinity of the PGA threads, especially at the end of the blocks where more than one thread were wrapped (Fig. 3a). Occasional foreign body granulomas were seen. Areas of tissue reaction around the PDLLA fibres were smaller in size and showed fibrous-type connective tissue formation (Fig. 3b); there also were several multinucleated giant cells. Birefringent material representing both PGA and PDLLA threads was seen till the end of the follow-up. Both types of reinforced HA blocks were partially surrounded by a thin fibrous tissue layer, especially at the sites of tissue reactions related to the reinforcing fibres (Fig. 3b).

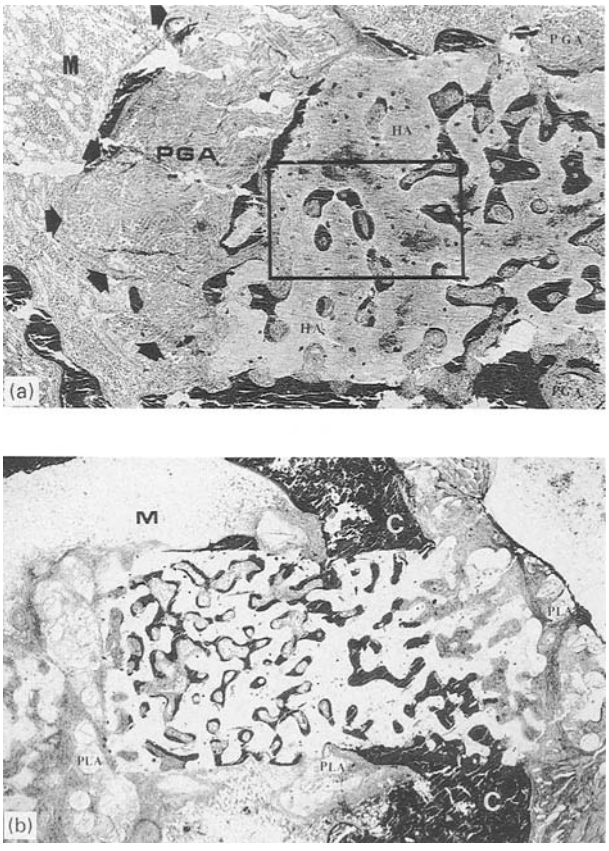


*Figure 3* Micrographs of the histologic samples after 6 weeks and 24 weeks *in vivo* in the proximal tibia of a rabbit. Original magnification 40, Masson-Goldner stain. (a) PGA-reinforced implant, 6 weeks. The degradation of polyglycolide has prompted a fibrous reaction (arrows), showing, however, abundant bone response inside the implant (dark coloured areas). The measuring field facing the medulla is schematically presented on the medullary end of the implant. M = medullary canal. (b) PDLLA-reinforced HA-block, 24 weeks. Reinforcing PDLLA-fibres have created a fibrous reaction around the implant, still showing abundant bone response throughout the implant. Area of most intense bone ingrowth is in the cortical side of the implant; it follows the delineation of bone cortex. Transverse sections of PDLLA fibres are clearly seen (PLA).

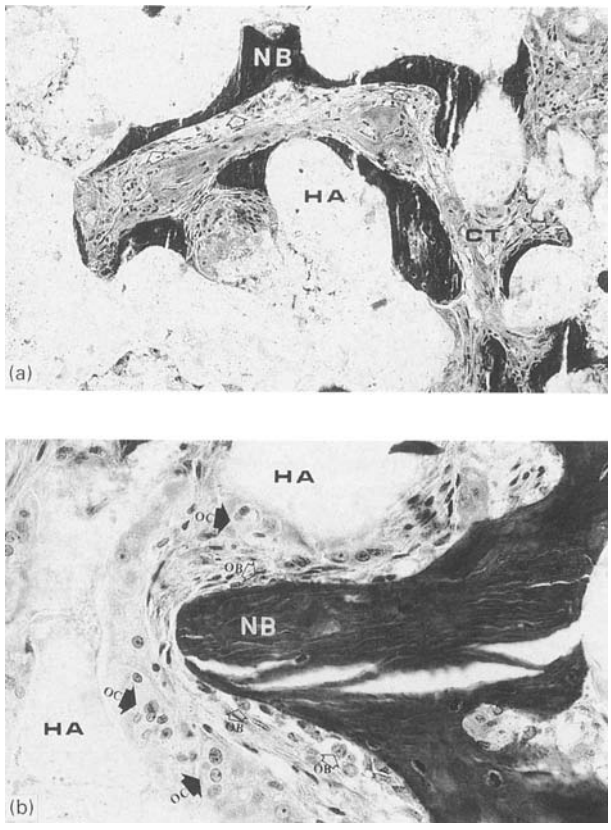


*Figure 4* Micrograph of histologic section in the middle of an implant section at 6 weeks representing new bone-forming process inside the HA implant. (a) Calcified new bone is created by differentiated osteoblasts (open arrows). The new bone is formed without any adverse reactions in the interface between HA and the connective tissue. HA = hydroxyapatite substratum, CT = connective tissue, NB = new bone (original magnification 250, Masson-Goldner stain). (b) Connective tissue protrusion with osteoclasts and osteoblasts grown into the porous structures of HA showing bone-forming and calcifying process towards HA substratum. The "cutter head" with osteoclasts probably tries to resorb HA substratum. OB = osteoblast, OC = osteoclast, HA = hydroxyapatite, CT = connective tissue, NB = newly formed bone (original magnification 400, Masson-Goldner stain).

Degradates of PGA and PDLLA fibres were seen until 24 weeks.

In spite of reactions around the absorbable fibres, the bony response inside the implants was abundant already at 6 weeks (Fig. 3a), and histologically no difference between the two implants was seen. In some places the implants were directly bonded to the host bone, at the same time showing a far advanced bony response in the proximity. Histologically it seemed that the ingrowth of connective tissue and bone started at the cortical end of the implants and at sites where intimate contact was best accomplished.

Starting at 6 weeks the connective tissue within the porosities of HA was structurally and intercellularly loose and its vascular component was remarkable. New bone was being formed and calcified along the margin of the connective tissue protrusions towards the HA (Fig. 4a). On the interfaces between connective tissue and HA or newly formed bone there was a deposition of osteoblasts and osteoclasts, obviously connected with the bone forming and remodelling process (Fig. 4b). Infrequently, HA fragments were seen inside the connective tissue protrusions surrounded by multinucleated osteoclast type cells.

At a later stage, connective tissue with a pronounced vascular component started to include fat cells and, later on at 16 and 24 weeks, marrow cells, while at the same time the occurrence of vascular components was decreasing (Fig. 5). Inactive osteoblasts or "lining cells" without an osteoid layer corresponded with the interrupted bone forming process. Commonly, the connective tissue in the 16- and 24-week samples showed a more fibrous pattern with small, inactive nuclei and intense collagen staining. In areas with abundant new bone the bone matrix was sparsely populated by osteocytes, and the amount of connective tissue was only marginal. In microradiographies three implant fractures were registered. Direct apposition of the host bone to the implant was distinct at least at one point in every case (Fig. 6). However, within the areas around the PGA or PDLLA reinforcing fibres no immediate contact with the host bone was achieved and these areas were seen as radiopaque.

*Figure 5* Micrograph of the medullary part of the implant section at 24 weeks. Fat (f) and marrow cells (m) occur increasingly, while layers of inactive osteoblasts or "lining cells" (open arrows) are seen along the bony trabeculae (original magnification 250, Masson-Goldner stain).

## 3.2. Histomorphometric findings

Combining the data of all measurements for correlation analysis, only small variations of mean values measured at different periods of the follow-up were observed in the area fractions of new bone and connective tissue (Table I). The mean area fractions of bone did not correlate with the duration of *in vivo* implantation for the surface sectioned ($r = 0.056$) or the deep sectioned levels ($r = 0.196$), $p > 0.05$. Likewise, the means of connective tissue ingrowth did not correlate with the follow-up time in either section level, $p < 0.05$. Moreover, correlations of tissue ingrowths with the reinforcing fibres (PGA versus PDLLA) were non-significant in both section levels, $p > 0.05$. Consequently, in the combined material, the variations between implants and variations over the course of the follow-up were non-significant.

There was, however, a difference in comparing the tissue area fractions between cancellous and cortical implantation. The mean new bone ingrowth into the hydroxyapatite blocks implanted cortically was 17.1%, compared to 12.9% in the cancellous implantation, $p < 0.01$ (Fig. 7). A consistent, but negative, correlation was found in relation to mean connective tissue ingrowth, which was smaller in the cortical (24.1%) than cancellous implantation (27.8%), though a statistical significance was found only in the deep sectioned samples ($p < 0.01$). In the cortical implantation the new bone ingrowth was greater in

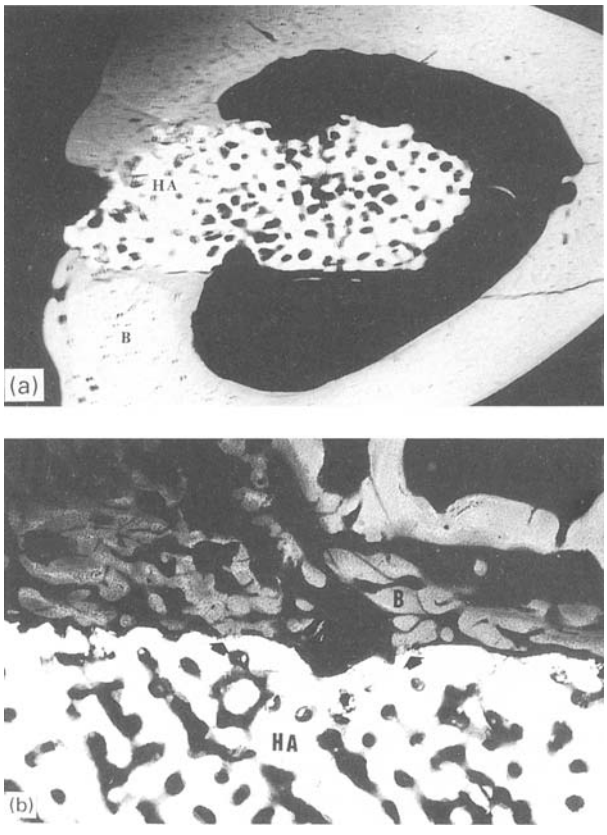


*Figure 6* Microradiographs where direct appositional contacts of the host bone to the implant are seen (arrows). HA = hydroxyapatite block, B = host bone. (a) Cortical implantation at 12 weeks (original magnification 16). (b) Cancellous implantation at 6 weeks (original magnification 40). Black colored areas between implant and host bone signify interposition of fibrous tissue prompted by the reinforcing fibres. (c) Higher magnification at the bone–implant interface where macroscopic bone ingrowth, i.e. osteoconductivity with direct appositional contact, is clearly visible (6 weeks, original magnification 100).

TABLE I Results of histomorphometrical measurements for polyglycolide (PGA) and poly-DL/L-Lactide (PDLLA) reinforced HA implants in cancellous and cortical bone region. The mean area fractions of new bone (%NEW BONE), connective tissue (%CONNECT) and hydroxypatite (%HA) are presented (mean ± standard error of mean).

| Follow-up period | | 6 weeks | | 12 weeks | | 16 weeks | | 24 weeks | |
|---|---|---|---|---|---|---|---|---|---|
| Implantation | | Cancellous | Cortical | Cancellous | Cortical | Cancellous | Cortical | Cancellous | Cortical |
| %NEW BONE | PGA | 19.3 ± 2.8 | 13.8 ± 2.0 | 11.7 ± 0.8 | 19.4 ± 1.6 | 10.8 ± 1.7 | 13.7 ± 1.2 | 13.7 ± 1.3 | 12.5 ± 1.6 |
| | PDLLA | 13.7 ± 1.5 | 16.6 ± 1.0 | 8.1 ± 1.0 | 23.4 ± 2.3 | 13.1 ± 2.2 | 17.2 ± 1.7 | 12.7 ± 1.9 | 20.1 ± 1.1 |
| %CONNECT | PGA | 37.6 ± 4.2 | 23.9 ± 2.7 | 23.5 ± 2.2 | 21.8 ± 1.4 | 26.2 ± 2.0 | 23.9 ± 2.1 | 25.1 ± 1.6 | 25.6 ± 2.1 |
| | PDLLA | 24.6 ± 2.4 | 24.9 ± 2.0 | 28.6 ± 2.8 | 20.3 ± 2.1 | 26.3 ± 2.0 | 30.8 ± 2.3 | 30.2 ± 2.3 | 21.7 ± 1.7 |
| %HA | PGA | 43.1 ± 6.1 | 63.0 ± 1.9 | 64.9 ± 2.1 | 58.8 ± 2.1 | 63.0 ± 2.4 | 62.4 ± 2.5 | 61.3 ± 0.7 | 61.8 ± 1.8 |
| | PDLLA | 61.7 ± 2.5 | 58.5 ± 1.8 | 63.2 ± 2.4 | 56.3 ± 3.6 | 60.6 ± 1.4 | 52.0 ± 2.0 | 57.1 ± 1.4 | 58.2 ± 1.3 |

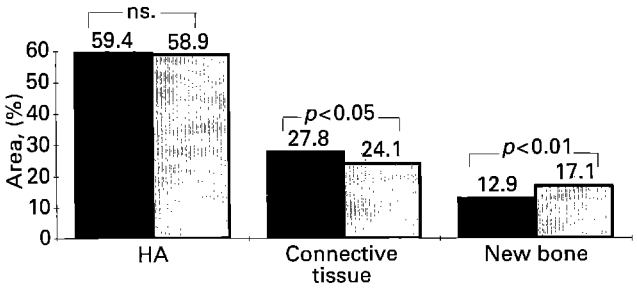


*Figure 7* End results of tissue ingrowth expressed as a percentage of the histomorphometric measuring area in cortical (□) and cancellous (■) implantation after showing that the follow-up period and the type of reinforcing fibres had no significant influence on them. The figures are means of the means obtained to both PGA- and PDLLA-reinforced implants after different periods of follow-up. More new bone has ingrown into the HA blocks implanted in the cortical area than in the cancellous area. Connective tissue response emerges vice versa.

the PDLLA-reinforced implants than the PGA-reinforced implants ($p < 0.001$), whereas in the cancellous implantation the bone ingrowth was somewhat greater in the PGA-reinforced implants, the difference being, however, non-significant ($p < 0.1$).

Excepting the site of implantation, tissue ingrowth was also dependent on the place of measurement, i.e. the specific field which the analysis was focused on. The new bone area fraction was greater when measured at the end of the implant facing cortically than when measured at the medullary end. This could be seen in both implantations (cancellous versus cortical) and section levels (surface versus deep), $p < 0.001$. An equal, but negative, correlation was found with respect to the connective tissue ingrowth, $p < 0.001$.

Since histologically no porous spaces without tissue ingrowth were seen, all void spaces were considered hydroxyapatite substratum. No differences were found in the percentage amounts of hydroxyapatite between implants or implantations. The mean percentage area fractions of HA implants itself were 59.4 $\pm$ 2.5% and 58.9 $\pm$ 0.9% in the cancellous and cortical implantations, respectively (Fig. 7).

### 3.3. OTC-fluorescence findings

The average fluorescence indexes were scored as grade IV at 6 weeks, grade II at 12 weeks and grade I at 16 weeks. Hence, OTC-fluorescence at 6 weeks was greater than that at 12 weeks and fluorescence was greater at 12 weeks than at 16 weeks ($p < 0.001$ and $p < 0.01$, respectively, Student's $t$-test).

## 4. Discussion

The successful use of coralline hydroxyapatite in cancellous bone defects has been thoroughly reported in several experimental studies [1, 8–11, 20–22], and bone morphogenesis has been shown even extraskeletally in intramuscular implantation [23]. In clinical practice hydroxyapatite has been used as a bone graft substitute in tibial plateau fractures [4]. Due to poor mechanical strength, the feasibility of porous scaffolding HA implants has been limited to non or low weight-bearing conditions. So far, only a dense form of hydroxyapatite has been used in human patients, as a spacer in strenuous loading conditions as in the cervical spine [24].

Creating mechanically stronger coralline implants which would still preserve their osteoconductivity has been a challenge to material engineers. Reinforcement of brittle coralline HA would facilitate the handling of the implants and make artificial bone bridging possible in different load-bearing conditions. Tencer *et al.* [15, 16] reinforced porous HA, both internally and externally, by creating a composite with biodegradable DL-polylactide (PDLA). In their work PDLA was impregnated into the porosities of HA implants. In the mechanical testing the PDLA-coated HA implants had compressive strength on average 3.76 times as strong as uncoated implants. Iwano *et al.* [13] prepared collagen-coated porous HA blocks and improved the compression strength of native HA by 4.3 times. Histologically and histomorphometrically, however, a delay of bone ingrowth and the appearance of tissue reactions with multinucleated giant cells were observed in both types of coated implants. Moreover, in the implants with thicker PDLA coatings the bone ingrowth was shown to be more inhibited [16].

With the present method, which utilizes absorbable fibre coating on prefabricated grooves, the main surface of the implant may achieve direct contact with the host bone. Theoretically, this was thought to be an advantage for ingrowth. Histologically, bone ingrowth was more abundant in locations having direct contact between bone and implant. Also, notable mechanical improvements have been achieved with this reinforcing method. With implants larger than those used in this study the impact strength was significantly improved, to ten times better than that with non-reinforced implants [14]. Shear strength and bending strength were 3.1 and 4.9 times better, respectively. The average compression strength, however, showed to be slightly superior only when compression was over 20% as compared to non-reinforced implants [14]. Accordingly, the reinforcing method used in the HA-blocks in the present study has been shown to improve tensile and fatigue properties, and to maintain longer the compactness of implants.

Histologically, in this study both the PGA and PDLLA reinforcing fibres caused foreign-body-type tissue reactions with an occurrence of multinucleated giant cells, but the reactions were focal. According to the statistical analysis, correlations between the area fractions of new bone and connective tissue ingrowth with duration of implants *in vivo* were non-significant. Consequently, the maximal amount of new bone response inside the porosities of HA implants was exceeded already at 6 weeks. Findings in OTC-fluorescence were in accordance with this, revealing an abrupt decline in fluorescence after the 6-week period. Probably the limit of the maximal amount of new bone which can be ingrown is achieved relatively early and is dependent on the implants and the site of implantation.

The mean area fractions of new bone in this study were 12.9% in cancellous and 17.1% in cortical implantation. The average area fractions for the implant itself were 59.4 and 58.9% respectively. In the literature the figures for bone ingrowth vary from 13% [22] to 45% [16] and seem to depend on the pore size and the porosity of the implant. Iwano *et al.* [13] had a mean 32.1% bone ingrowth at 12 weeks, in both coated and uncoated HA implants with a mean 280 μm pore size. In the study of Tencer *et al.* [16] the bone fraction exceeded 45% after 24 weeks in implants with 600 μm pore size (Interpore 500), both uncoated or with a thin PDLA coating. The area fractions of the implant itself were lower, i.e. the porosity was higher than in the present study. It seems, therefore, that the higher pore size and porosity, the more bone ingrowth can be expected. Probably, the implants with 200 μm pore size exceed their bone ingrowth capacity earlier and the bone amount remains constant thereafter.

This study showed evidence of spatial distribution in bone ingrowth. Histomorphometric measuring fields facing the cortex had a more intense bone ingrowth than the fields in the medullary side (Fig. 3b). Similarly, bone ingrowth was more abundant in implants in the cortical region than in those in the cancellous region. This finding has not been shown previously. Tencer *et al.*, having a nearly identical operative procedure, concluded that the location of the implant did not affect the resultant growth of bone into it [16]. Also in the study of Holmes *et al.* bone was homogeneously distributed in both the implants and the normal specimens [22]. This study, however, strongly suggests that the bone response is dependent on the mechanical loading on the implant. The bone ingrowth probably follows Wolff's law, showing an adaptive pattern according to the load transmitted through the implant. New bone was also ingrown into the area of medullary canal, where autogenous bone graft without HA scaffold would have been resorbed. HA showed, however, no evidence of resorption during the 24-week follow-up.

In summary, according to the literature the amount of the bone ingrowth is dependent on pore size and porosity of the HA implant. In implants with pore size of 200 μm the maximal bone ingrowth is reached relatively early and thereafter it remains stable. Bone ingrowth seems to be dependent on the site of implantation and is probably regulated by the stresses in the implant according to the statistical differences in bone ingrowth in this study. While the amount of ingrowing bone can be increased by increasing the pore size or porosity of the HA, reinforcement of implants becomes more crucial when considering widening the clinical applications. The fibre reinforcement used in this study seemed not to disturb the normal bone response, and both polyglycolide (PGA) and polylactide (PDLLA) fibres did equally well. With this method the tensile and fatigue properties of HA are shown to be improved [14], but the compressive strength may still be insufficient under high loading conditions. Owing to this, further development is needed, e.g. by replacing the threads with PDLLA or PGA embedded in the grooves of HA.

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