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Lumbar spine interbody fusion with reinforced hydroxyapatite implants

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Summary. Porous coralline and synthetic hydroxyapatite blocks reinforced with either self-reinforced polyactide or polyglycolide were implanted into 15 lumbar intervertebral disc spaces in five minipigs in order to determine whether they could provide an osteoconductive bridge for interbody fusion. Histological examination and radiological analysis with plain films, computed tomography and magnetic resonance imaging were carried out. The osteoconductive properties were promising; creeping bone formation could be observed, although no complete fusion had been achieved at 24 weeks.

Calcium phosphate ceramics have been widely investigated as bone substitutes, and have been shown to be biocompatible [7]. Hydroxyapatite (HA) especially, the most common form of mineral found in the bones, has been shown to have excellent biocompatibility when replacing or supplementing bone graft material [4]. Porous HA forms a strong bond with the host bone and provides a scaffold upon which creeping bone formation occurs [7]. So far, the clinical application of porous HA has been confined to its use as a filling material in bone cysts or defects such as those in metaphyseal fractures [2]. As a ceramic material HA is brittle and its use in locations were high mechanical strength is required has not been feasible. Dense HA with great mechanical strength can be fabricated, but it serves only as a spacer without osteoconductive properties [13].

Porous HA blocks have been earlier reinforced by applying an absorbable *dl*-polylactic acid (DL-PLA) coating to their surfaces [16]. This kind of coating does not permit instant intimate contact between host bone and HA. We reinforced porous HA implants with absorbable fibre bundles located in 0.5-mm deep grooves, so allowing the pores to remain open for tissue ingrowth. The study was performed on the lumbar spine of five minipigs by implanting the disc spaces with three different kinds of reinforced HA blocks. The aim was to achieve interbody fusion. Thorough radiological analysis with plain films, computed tomography (CT) and magnetic resonance (MR) imaging as well as a histological study were carried out.

Materials and methods

Implants

Synthetic porous hydroxyapatite blocks delivered by Patka [8] and coralline, porous hydroxyapatite blocks (Interpore 200, Interpore International, Irvin, CA, USA) were used as the raw materials. The 3 × 8 × 12 mm blocks were reinforced by winding absorbable fibre bundles on their outer surfaces. The fibres were placed in

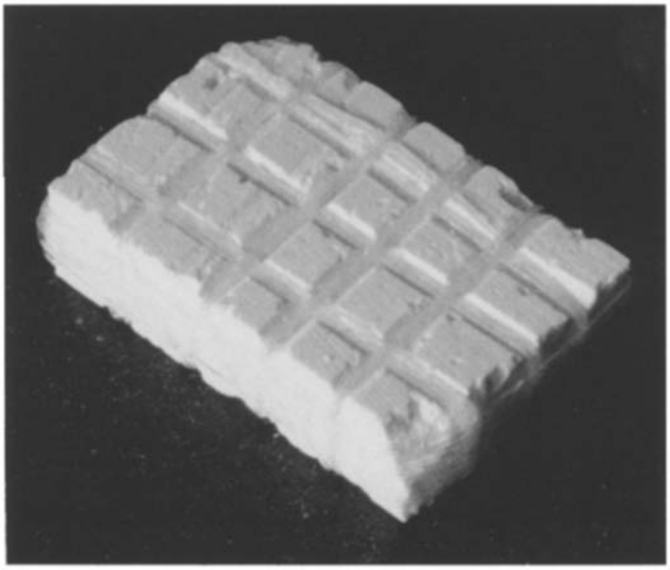

Fig. 1. Poly-*dl*/*l*-lactide reinforced coralline HA implant 3 × 8 × 12 mm in size

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0.5-mm-deep grooves 2 mm apart [17] (Fig. 1). With this method, most of the pores remained open to allow bone ingrowth. Three different blocks were used: poly-*dl/l*-lactide fibre-reinforced synthetic HA (Patka-PLA), poly-*dl/l*-lactide fibre-reinforced coralline HA (Interpore) (IP-PLA) and polyglycolide (Dexon) fibre-reinforced coralline HA (Interpore) (IP-PGA) blocks. The compression strength of the Patka-PLA blocks was 2.0 MPa (1.1 MPa without reinforcement), the compression modulus 77.3 MPa (57.0 MPa without reinforcement) and the impact strength 1.86 J/cm^2 (0.21 J/cm^2 without reinforcement); the corresponding figures for the IP-PLA blocks were: 0.7 (1.2) MPa, 128 (131) MPa and 1.95 (0.21) J/cm^2. Figures for the mechanical properties of the IP-PGA blocks were not available, but were estimated to closely resemble those of IP-PLA [17], Törmälä, unpublished data).

Operative procedure

Three adjoining lumbar intervertebral disc spaces (IVDSs) (L4–7) in each of five minipigs (age 10–15 months, mean weight 43.3 kg) were operated on through laparotomy, i.e., 15 IVDSs in all. All the disc material was evacuated, and the cartilaginous endplates were chiselled until the bloody spongy bone was reached. The three different reinforced HA implants were inserted into the pigs according to a code known only by the surgeon (PY). The anterior longitudinal ligament was preserved in order to prevent anterior protrusion of the implant. The minipigs were followed up on for 24 weeks. Plain films in vivo were obtained immediately postoperatively and at 6 weeks. After slaughtering of the minipigs at 24 weeks, the lumbar spines were retrieved en bloc and a thorough radiological analysis with plain films, CT and MR imaging was performed.

Radiological studies

The results were evaluated blindly and independently by three senior radiologists with the aid of a Peak magnification loupe × 7 (accuracy 0.1 mm) as follows: *ossification* of the implant was scored: 0 = 0% (none), 1 = 1%–25% (slight); 2 = 26%–50% (moderate); 3 = 51%–75% (plenty); and 4 = 76%–100% (complete). Its *fragmentation, residual height and volume* were scored in the same manner. The same kind of scoring was also used in assessing *the narrowing of the IVDSs, the roughness, fragmentation and osteolysis of the endplates, the vertebral body epiphyseolysis, thickening and ossification of the anterior longitudinal ligament, anterior and posterior spondylosis and spinal stenosis. The migration of the implant* was determined as the distance of the implant midpoint from the IVDS midpoint as coronal and sagittal coordinates. *The total operative block kyphosis* was determined as the angle between the endplates of the uppermost and the lowermost lumbar vertebral bodies operated upon.

After slaughtering of the minipigs, contiguous sagittal CT images were taken of the retrieved lumbar spine block (Philips Tomoscan 60/TX, slice thickness 2 mm). CT evaluation included the scoring of reactive vertebral sclerosis (0–4).

MR studies of the spinal block were performed with a superconductive system (Magnetom 42SP, Siemens AG) operating at 1.0 T. Contiguous 3 mm T1-weighted sagittal images (T1WI) were obtained with SE 500/20 ms. Sagittal proton density and T2-weighted images (T2WI) were obtained with SE 2500/22 and 90 ms, with two acquisitions. The imaging matrix was 256 × 256. The evaluation of the MR studies comprised the scoring (0–4) of *the narrowing of the IVDS, the roughness of the endplates, thickening of the anterior longitudinal ligament, and the IVDS signal intensity on T2WI and the signal intensity of the vertebral bodies on T1WI.*

Histological studies

The samples of the lumbar spine blocks of each IVDS with adjoining vertebral body halves were prepared by immersing in ethanol solutions at increasing concentrations, whereafter they were embedded in methylmethacrylate. The 5-µm thick sections were cut sagittally in the midline. They were stained by the Masson-Goldner method [5]. Both the bone and the connective tissue ingrowth into the implant were scored semiquantitively (0–4) as in the radiological part of the study. Additionally, microradiographs were obtained from 80-µm-thick sections.

Statistics

The comparisons between the three kind of implants, between the operated and nonoperated IVDSs, and between all the vertebrae were performed by using the Kruskal-Wallis test. The imaging modalities were compared using the Wilcoxon signed-rank test. The correlations were calculated by the Spearman rank correlation test.

Results

One minipig (A) suffered transient paraparesis. Otherwise the minipigs recovered uneventfully and resumed normal gait and activity.

Radiological studies

In one minipig (E), the intervertebral disc spaces L5–S1 instead of the L4–7 as planned, were implanted. The initial positioning of the implants was precise; the mean lateral deviation from the intervertebral midpoint was 2 mm, the range being 0–6 mm, and the mean anterior deviation 1 mm (range 0–2.5 mm). No implant was inserted too far posteriorly (Fig. 2a).

During the first 6 weeks, but no later, five implants in two minipigs (B and C) *migrated* in the sagittal plane; two Patka-PLA 1.5 mm and 3.0 mm anteriorly, one IP-PLA 3.0 mm anteriorly, one IP-PGA 6.0 mm anteriorly, and another IP-PGA 2.5 mm posteriorly.

At 24 weeks on plain films, one IVDS with a Patka-PLA implant showed *ossification* by more than 50% (score 3); all the other IVDSs with Patka-PLA as well as the other types of implants scored less (Table 1). On three IVDSs, one with a Patka-PLA and two with IP-PGA implants, there was no visible ossification at all. The ossification was marginal, surrounding the implants (Fig. 2c). CT demonstrated ossification vaguely, only in one IVDS with an IP-PLA implant (score 1) and in another IVDS with an IP-PGA implant (score 2). Subsequently the plain film and CT results did not correlate significantly with each other. *Ossification*, visible on plain films, proved to have a negative correlative trend to implant *migration* ($\rho = -0.44$; $P < 0.10$) and a positive correlative trend to *the residual height* of the implant $\rho = 0.45$, $P < 0.09$).

On plain films, the mean *residual heights* of the three kinds of implants resembled each other: those of the Patka-PLA implants were 68% ± 27%, those of the IP-PLA 70% ± 27%, and those of the IP-PGA 64% ± 26%. In four minipigs, fragmentation of all three kinds of implants was fulminant (11 implants scored 4 and one 3). In one minipig the implants were only slightly *fragmented* (all three different implants scored 1). Nevertheless, these implants too had lost up to 51%–71% of their orig-

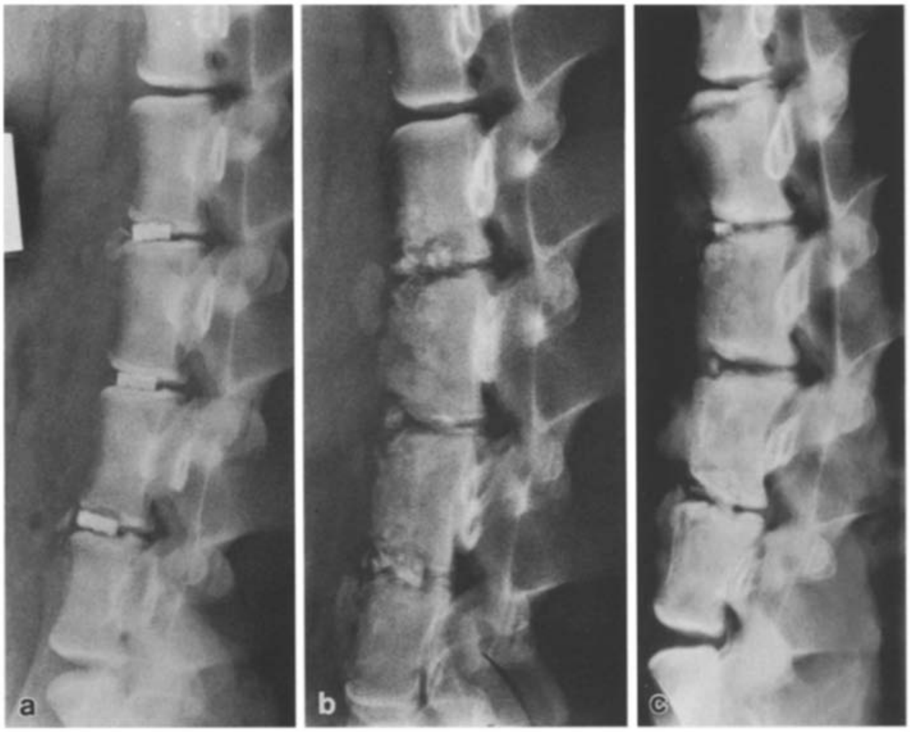


Fig. 2. **a** Lumbar spine of minipig A after insertion of the three different kinds of hydroxyapatite implants into the L4–7 intervertebral spaces. **b** Plain film of minipig A at 6 weeks. Implant ossification is not visible. The residual height of the cranial IP-PGA implant is 86%, that of the middle Patka-PLA 66%, and the caudal IP-PLA implant has retained its initial height. All the implants are fragmented, all the vertebral endplates are rough and osteolytic, and the cranial and caudal ones are slightly fragmented. **c** Plain film of minipig A at 24 weeks, revealing marginal osteoconductive bridging alongside the implant at the cranial IP-PGA implant as well as at the caudal IP-PLA implant, more pronounced at the latter. The implants have continued to lose height and volume, and fragmentation is fulminant: extensive anterior longitudinal ligamentous calcification and thickening, posterior spondylosis at the L3–6 levels, encroaching slightly on the sagittal spinal canal. Compared to the first postoperative radiograph, there is a uniform 10° increase in lumbar kyphosis. Note also the complete epiphyseolysis at the L4 cranial endplate

Table 1. Ossification of intervertebral disc spaces visualized on plain films and on histological sections at 24 weeks[a]

Minipig	Patka-PLA		IP-PLA		IP-PGA	
	Plain	Histol	Plain	Histol	Plain	Histol
A	0	0 (3)[b]	2	2 (3)	1	1 (3)
B	2	0 (3)	2	0 (3)	0	1 (3)
C	2	0 (3)	2	0 (3)	1	1 (2)
D	3	0 (3)	1	1 (1)	1**	1 (3)
E	1	0 (3)	1*	1 (1)	0	3 (1)
Sum of scores (max 20)	8	0	8	4	3	7

[a] For scoring system, see Materials and methods

[b] Figures in parentheses are scores for amount of connective tissue ingrowth into implants

*, ** Only intervertebral disc spaces in which CT also showed ossification: * score 1, ** score 2

inal height. The universal implant fragmentation was also visible on CT, but was scored statistically significantly lower ($P < 0.002$) than on the conventional radiographs. The *volumes* of the implants were seen to be heavily reduced on the plain films: four Patka-PLA, three IP-PLA and three IP-PGA implants scored 1 (< 25% residual volume); the CT scores were no better.

All the *IVDSs operated on* were clearly *narrowed*. The plain films revealed over 50% narrowing in 3/5 of each implant type. In MR imaging the figures for over 50% narrowing on T1WI were: Patka-PLA 1/5, IP-PLA 2/5 and IP-PGA 1/5. The results according to MR and plain films did not differ significantly. The uniform decrease of *IVDS signal intensity* on T2WI in MR correlated statistically highly significantly with its narrowing on plain films $\rho = 0.77$, $P < 0.0003$).

The increase in *total kyphosis* was determined in those four minipigs in which the L4–7 IVDSs had been operated. The mean initial kyphosis on the operative area was 10.5° (range 2°–17°), the mean increase was 8.8° (range 4°–13°) and the mean resulting kyphosis 19.3° (range 15°–21°). The increase in kyphosis occurred uniformly and very similarly in all operated IVDSs (Fig. 2c). There were no changes towards either kyphosis or lordosis in the nonoperated IVDSs in the nearby region.

The plain films demonstrated all the operated *endplates as being rough* and *osteolytic* from the 6th week on (Fig. 2b). At 24 weeks on the conventional radiographs 60% of the operated endplates had marked *roughness* (scores 3 or 4; Fig. 2c), on CT only 40% ($P < 0.03$), while on MR, as on the conventional radiographs, 60% of the operated endplates had marked *roughness*, but the endplates of one IVDS implanted with Patka-PLA and of another with IP-PLA looked smooth. There were no significant differences between the three kinds of implants. Plain films as well as CT showed *osteolysis* involving over 25% (scores > 1) of the endplate area in half of the operated IVDSs. There were no significant differences between the different implants. The plain films demonstrated *fragmented endplates* on 12 of the 15 operated IVDSs. Neither plain films nor CT showed any significant differences between the different implants.

All imaging modalities demonstrated four complete *epiphyseolyses* (score 4) in three minipigs: in one animal on the cranial ends of the L3 and L4 vertebral bodies, in the other two on the cranial ends of the L3 vertebral bodies (Figs. 2c, 3). Also, all methods showed well marked

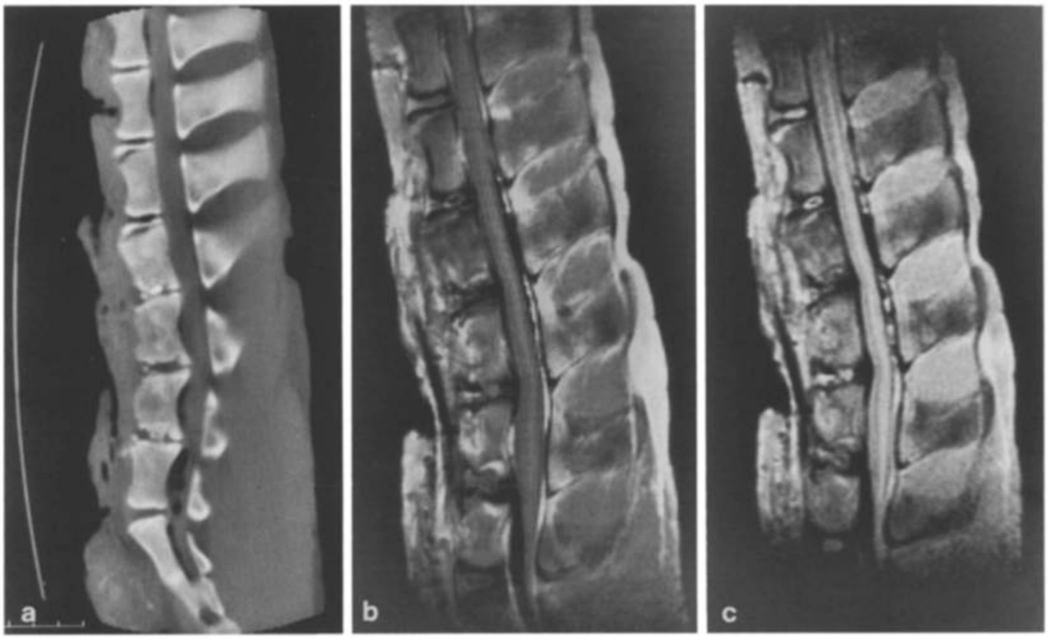


Fig. 3. a Sagittal CT cut of the lumbar spine block of minipig A at 24 weeks. In addition to the changes visible in the plain film, the L3 cranial epiphyseolysis is also in the field. Note the reactive vertebral body sclerotic changes, most clearly visible at the cranial part of the L4 vertebral body, and the intraspinal air surrounding the thecal sac. **b** Sagittal MR T1WI (SE 500/20 ms, the L2 vertebra is at the top end) of minipig A at 24 weeks, demonstrating clearly increased signal intensity of the operated L4–7 vertebral bodies and also at the caudal end of the L3 vertebral body, reflecting reactive bone marrow changes (fatty conversion). The cranial epiphyseolyses on L3 and L4 are visible as high signal intensity bands. The abundant anterior longitudinal ligamentous thickening and calcification are clearly visualized. **c** Sagittal MR proton density image (SE 2500/22 ms, the L2 vertebra is at the top end) with a myelographic effect. The thecal sac is compromised anteriorly at the L5–7 IVDSs by the low-signal posterior longitudinal ligament and osteophytes. The thecal sac looks slightly flabby, as the normal physiological pressure of the cerebrospinal fluid is absent

anterior longitudinal ligamentous ossification on the operated vertebrae. Both plain films and MR demonstrated similar but significantly smaller (MR $P < 0.01$, plain films $P < 0.008$) changes in the anterior longitudinal ligaments of the nonoperated nearby vertebrae L3 and S1.

There was slight to moderate (score 1–2) *anterior horizontal osteophytosis* on the three operated IVDSs. There were also anterior osteophytes on the non-operated IVDSs in the nearby region, but the differences between these non-operated and the operated ones were not significant. Nevertheless, in all operated IVDSs, and also, in three minipigs, on non-operated IVDSs nearby, there was slight to moderate (score 1–2) *posterior osteophytosis*. These findings were statistically highly significantly more common on plain films than as detected by CT ($P < 0.0003$). There were no significant differences either between the three implant types or between the operated and nearby non-operated IVDSs.

Subsequently, owing to posterior osteophytes, slight spinal stenosis was demonstrated by all imaging modalities (Figs. 2c, 3). The difference between plain films and MR was not significant, but the plain films scored highly significantly more ($P < 0.003$) spinal stenosis than CT, which had a trend ($P < 0.09$) towards less spinal stenosis than MR.

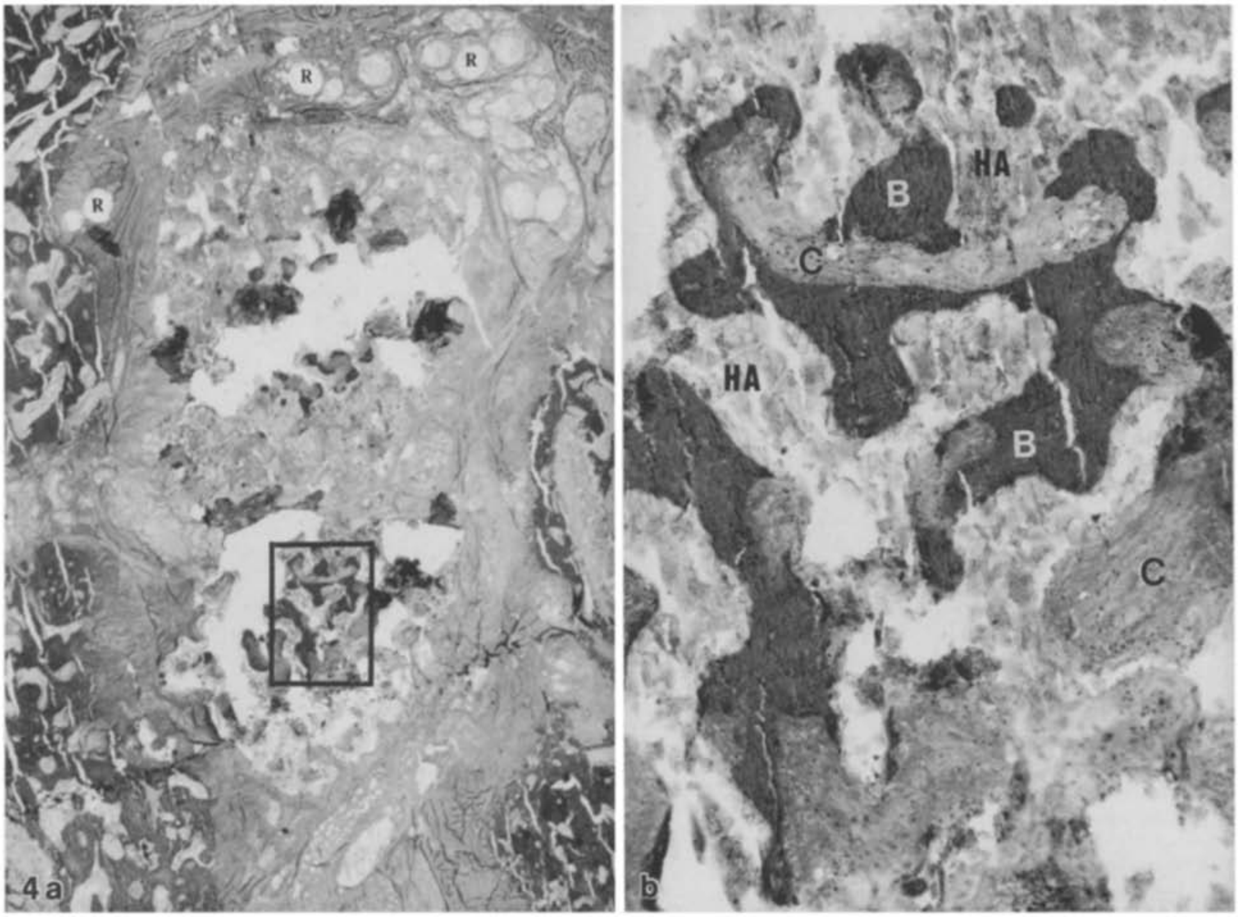
Out of the 20 vertebrae operated on, 17 showed *increased signal intensity* on T1WI on MRI. In addition, two non-operated vertebrae cranially revealed a similar increase in signal intensity on T1WI (Fig. 3b). A special CT finding was *vertebral body sclerosis*, observed in three operated vertebral bodies (score 2 once, score 1 twice; Fig. 3a), which in MR showed an increased signal intensity on T1WI.

Histological studies

There was some calcified new bone ingrowth into all the IP-PGA and into three IP-PLA implants (Table 1; Figs. 4, 5). Calcified bone was formed by connective tissue protrusions which lined with osteoblasts towards HA. However, there were no solid continuous bony bridges over the whole IVDSs from one vertebral body to the other.

No new bony ingrowth into the synthetic Patka-PLA implants was seen. All the Patka-PLA implants were surrounded by dense connective tissue with collagen fibers and a few fibrocytes (Table 1; Fig. 4). The connective tissue around and inside the Patka-PLA implants was more thickly grown and stained more intensively than that of the IP-PGA and IP-PLA implants.

There were no significant correlations between the histological and plain film results relating to ossification. However, the histological and CT results did correlate significantly in one respect: that neither demonstrated any ossification at all in the Patka-PLA implants.



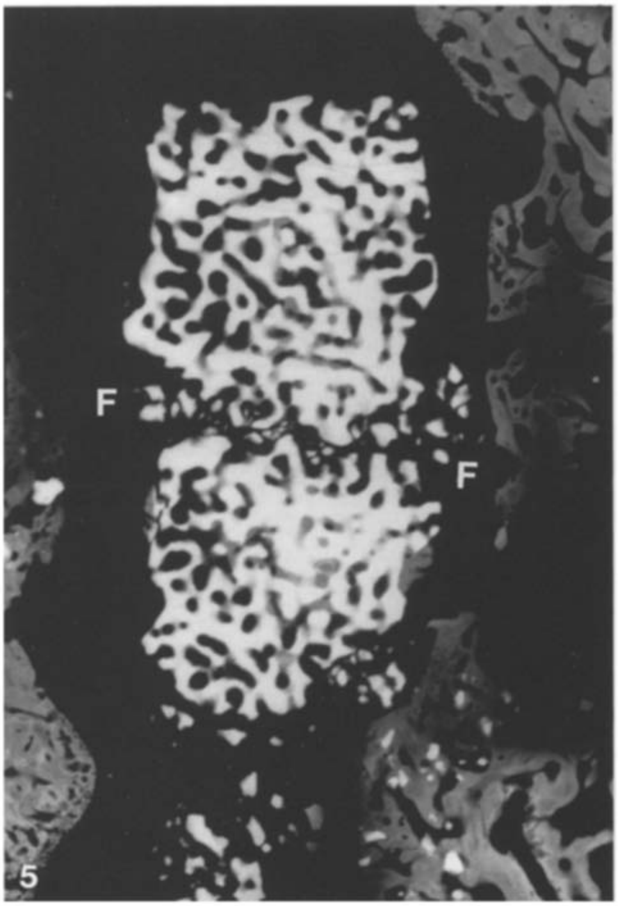


Fig. 4a, b. Histological sagittal section of the IVDS of minipig A. The disc material was replaced with IP-PLA. Goldner-Masson stain. **a** Islets of ossification are seen inside the HA block, but no continuous bony bridge between the vertebral bodies is visible. *R*, cross-section of the reinforcing PLA fibres. Original magnification × 16. **b** Bony ingrowth. The rectangle from **a** at original magnification × 100. *B*, New bone formed by the connective tissue protrusions with osteoblasts; *C*, connective tissue; *HA*, hydroxyapatite

Fig. 5. Microradiograph of the same IVDS as in Fig. 4. The sagittal section is 80 µm thick. The calcified new tissue ingrowth into the implant pores has a grey tone. *F–F* indicates a fracture of the implant

Discussion

This is the first time, to our knowledge, that lumbar spine interbody fusion has been tried with reinforced hydroxyapatite implants. The osteoconductive properties of the reinforced hydroxyapatite implants used in the present study were promising, and came out in favour of the IP-PGA and IP-PLA implants (Table 1). Vague creeping ossification into the implants could be observed histologically (Fig. 4) and microradiographically (Fig. 5). However, the macroscopic radiological picture differed greatly from the histological one, as the radiologically visible new bone formation was mainly marginal, alongside the implants (Fig. 2c).

The plain films no doubt exaggerated the ossification of the IVDSs, firstly because it was confined to the periphery surrounding the implants, and secondly because it was also ligamentous. The CT results with thin cuts obtained through the implants were in markedly better agreement with the histological picture than were the plain films (Table 1).

The present results agree well with others found in the cervical spine in some earlier reports, where varying degrees of osseous fusion of absorbable tricalcium phosphate as well as hydroxyapatite implants in canine cervical disc spaces were found but at the same time extensive implant fractures were demonstrated. The authors [3, 14] concluded that the main reason for failure was mechanical, namely that the HA ceramic was too brittle. Indeed, in earlier studies anterior cervical disc excision combined with interbody fusion with autogenous bone grafts [18], allografts [12], bovine bone [15] or synthetic dense non-resorptive hydroxyapatite spacers [13] has led to fusion in the great majority of cases.

The reinforced implants we used had a shear strength four times and a compression strength 1.5 times greater than those of the plain hydroxyapatite blocks. Nevertheless, all but one were fragmented as early as during the first 6 weeks. In addition, four implants migrated slightly. There was also a negative correlative trend between implant ossification and migration during the first 6 weeks, and a positive correlative trend between ossification and residual height at 24 weeks. These findings support the idea of insufficient stabilization. New bone formation for spine fusion requires not only intimate contact between the implant and the host bone, but also perfect stabilization ([10, 11], Patka, personal communication). Spondylosis and the abundant anterior longitudinal ligamentous thickening and calcification, resulting in slight spinal stenosis as a by-product, are reactive changes towards increased stability, but may not have been effective enough, as there was progressive kyphosis (Figs. 2, 3). From the orthopaedic point of view, artificial replacement of a destroyed disc requires additional stabilization to regain enough rigidity to prevent spondylosis, ligamentous thickening and kyphosis. Obviously, stability is crucial for ossification of the HA implant as well.

Neither the porous hydroxyapatite material nor the absorbable fibre composites covering the outer surface of the HA blocks can be blamed for the incomplete fusion in the present study. Porous hydroxyapatite implants in the distal femur of rabbits [4] and bone grafts used as substitutes in canine proximal tibial metaphyseal defects [6] have been shown to be ossified. In an earlier study of ours [19], the same kinds of implants reinforced with PGA and PLA fibres were placed into metaphyseal defects of rabbits' tibiae, and good and uniform ossification was revealed throughout the implants.

We would expect the rather marked fragmentation and osteolysis of the endplates in the operated IVDSs to be a purely mechanical phenomen. It seems unlikely that the mainly centrifugally arranged arteriolar supply of the vertebral body would have been significantly compromised by surgery. Any slight transient osteolytic "acid attack" [1] should be negligible, since this kind of effect was not seen histologically in metaphyseal defects replaced by PLA and PGA reinforced implants [19].

As there were no signs of infection, the four complete epiphyseolyses (Figs. 2c, 3) must have also a biomechanical aetiology. The pigs had to compensate for the progressive iatrogenic lower lumbar kyphosis with subsequent increased thoracolumbar lordosis; this produced anterior distraction, forcing anterior opening of the epiphyseal plates.

The diagnosis and grading of spinal stenosis on MR images was based on the compromise of the hyperintense thecal sac in the proton density (Fig. 3C) and T2WI. Cerebrospinal fluid pulsations and pressure were absent, thus diminishing the physiological distention of the thecal sac. The thecal sac was also surrounded by large amounts of intraspinal air for its full length in all of the spine specimens (Fig. 3a). The zones of signal void produced by the postmortem air may have mimicked spinal stenosis on the MR images. Consequently, the in vitro MR findings of the present study relating to spinal stenosis may be considered unreliable and the CT results should be preferred.

With CT, increased sclerosis was seen in only three vertebral bodies (Fig. 3a), which also had reactive bone marrow MR changes (Fig. 3b). These signs are interrelated, as they have both been found in association with disc degeneration [9], iatrogenically evoked in the present study as well. However, these changes are not correlated in time: the MR signal is an earlier reflection of marrow elements, whereas the increased bony sclerosis noticed in CT can be considered a much later reactive phenomenon, very probably associated with the altered loading stresses caused by the increased kyphosis. Reactive sclerosis was indeed found in those minipigs with the greatest increases in kyphosis in the operative region.

To conclude: the results were promising in that osteoconductive ossification was visible, but disappointing in that there were implant migration and loss of implant height showing negative correlative trends with ossification. Additional stabilization in conjunction with interbody fusion seems to be essential. CT should be preferred to plain films as the method of choice to assess the ossification of the IVDSs, as it better discerns the ossification of the implants from that of the ligaments.

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