

Locally delivered TGF- β 1 and IGF-1 enhance the fixation of titanium implants

A study in dogs

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Background Osteogenic growth factors have been suggested to enhance the fixation of implants used in joint replacement. We examined the effect of locally delivered transforming growth factor- β 1 and insulin-like growth factor-1 in a biodegradable poly (D, L-lactide) coating.

Material and methods In a paired study using 9 dogs, unloaded titanium implants surrounded by a 1-mm gap were inserted into the proximal humerus. The growth factors were incorporated in a poly (D, L-lactide) coating at a 1% (w/w) ratio of TGF- β 1 and a 5% (w/w) ratio of IGF-1. Control implants were uncoated. After 4 weeks, the implants were evaluated by mechanical push-out test and by histomorphometry.

Results A twofold increase was seen in mechanical fixation (strength, stiffness, energy absorption) for the growth factor-treated implants ($p = 0.04$). Similar results were seen in histomorphometry, as bone ongrowth was 2.5 times higher ($p = 0.02$), and gap healing was 30–110% higher ($p = 0.04$) for the growth factor-treated implants than for the control implants. Ongrowth of fibrous tissue was eliminated by the treatment.

Interpretation TGF- β 1 and IGF-1, locally delivered in a biodegradable poly(D,L-lactide) coating, enhance the mechanical fixation and osseointegration of titanium implants in cancellous bone, and no fibrous tissue is produced in the growth factor treated implants.

Cementless total hip replacements (THR) require a rapid bony integration to ensure long-term fixation. This is even more important in the revision setting, where bone stock is diminished and healing potential reduced compared with primary surgery (Glassman et al. 1987, Engh et al. 1988, Engelbrecht et al. 1990, Nicolas et al. 1994, An et al. 2000).

One strategy for improving osseointegration is the administration of osteogenic/osteotropic growth factors. Applications employing one growth factor have been extensively investigated (Aspenberg et al. 1989, Thaller et al. 1993, Lind et al. 1996b, Zellin et al. 1998, Laffargue et al. 2000, Tielinen et al. 2001, Soballe et al. 2004). Applications combining two or more growth factors may be more favorable, due to their additive or synergistic effects on bone formation. Transforming growth factor beta 1 (TGF- β 1) and insulin-like growth factor 1 (IGF-1) are of special interest as they are highly expressed during bone growth (Dequeker et al. 1993, Nicolas et al. 1994, Lammens et al. 1998, Pfeilschifter et al. 1999, Eingartner et al. 1999, Kveiborg et al. 2001).

To minimize potential systemic side effects, the growth factors should be delivered locally. A controlled release system should prevent washing of growth factors off the implant by bleeding. Applications used in other studies for local growth factor delivery have included growth factor adsorption to calcium phosphates, mixtures of calcium

phosphates and collagen, collagen sponges, hydrogels, immobilization of growth factors on implant surfaces, and use of various polymers (Lee et al. 2000, Park et al. 2000, Vehof et al. 2002, Barboza et al. 2004, Rose et al. 2004, Ito et al. 2005). Lactide-based polymers such as poly (D, L-Lactide) (PDLLA) have been used clinically for decades as material for screws and plates, and PLLA has been used successfully as a drug carrier (Nicolas et al. 1994, An et al. 2000, Schmidmaier et al. 2001b, Rose et al. 2004). A few cases of inflammatory reactions have been seen, but only with bulky-design PLLA implants (Bostman 1998). The lactic acid released from the PLLA coating by hydrolysis is metabolized via the TCA cycle to carbon dioxide and water. The combination of TGF- β 1 and IGF-1 in PLLA has not been investigated previously in an implant model. We hypothesized that the combination of TGF- β 1 and IGF-1 in PLLA would enhance the osseointegration and mechanical fixation of unloaded cylindrical porous-coated titanium implants surrounded by a gap.

Material and methods

Implants and coating technique

We used cylindrical plasma-sprayed porous-coated implants made of titanium alloy (Ti-6Al-4V) with a nominal diameter of 6.0 mm and length of 10.0 mm (Biomet Inc., Warsaw, IN).

TGF- β 1 and IGF-1 (R&D Systems, UK) were dissolved in a poly (D, L-lactide) (PDLLA) - Resomer 203 (Boehringer Ingelheim GmbH, Germany) and ethyl acetate solution, resulting in a 1% (w/w) ratio of TGF- β 1 and 5% (w/w) ratio of IGF-1. The implants were dipped twice in the solution and air-dried, all under sterile conditions. Based on coating experiments, where the implants were weighed before and after coating, an estimated 140 μ g IGF-1 and 28 μ g TGF- β 1 would be incorporated into the PLLA coating of each implant.

Animals and surgical procedure

A controlled animal study was carried out. The study was approved by the local committee for animal care and use. Implants were inserted into cancellous bone sites in the proximal humerus under general anesthesia, using sterile technique.

Unloaded implants with a circumferential 1.0-mm gap were inserted bilaterally in 9 skeletally mature mongrel dogs (25–27 kg). The study design was paired with insertion of growth factor-coated implants on one side, and identical control implants without PLLA or growth factors on the contralateral side.

The implantation site was exposed through a lateral approach, going through a cleavage in the deltoid muscle. The periosteum was removed only at the site of drilling, just distal to the minor tubercle. A guide wire was inserted, and then an 8.0-mm cannulated drill was used to prepare the hole to receive the implant. Drilling was performed at approximately 2 rotations per second, to prevent thermal trauma to the bone. The implantation site was lavaged using isotonic saline prior to insertion of the implant. Bottom and top washers, 8.0 mm in diameter, were attached to stabilize the implants and prevent soft tissue ingrowth, and the implants were inserted. After insertion, the overlying soft tissue was closed in layers. During surgery, 750 mg of Cefuroxime was administered intravenously. After 4 weeks of observation, the animals were killed and the specimens harvested. Cultures were taken from all implantation sites.

Specimen preparation

Pending preparation, the proximal humerus was stored at -20°C . The bone-implant specimens were cut on a water-cooled diamond band saw (Exact Appartebau, Germany) leaving two transverse sections. The outermost section of approximately 3.5 mm was used for mechanical testing. The remaining section was sectioned for histomorphometric analysis.

Mechanical testing

Implants were tested to failure in shear by a push-out test on an Instron Universal Test Machine (Model 4302; Instron, UK). The specimens were placed on a metal support jig with a 7.4-mm circular opening. A preload of 2 N was applied, to ensure contact with the implant. The displacement rate was 5.0 mm/min. Maximum shear strength (MPa), apparent shear stiffness (MPa/mm), and energy to failure (J/m^2) were calculated from the recorded load-displacement data.

Table 1. Results from push-out test presented as median and range

	Control	TGF- β 1 + IGF-1 in PDLLA
Energy to failure (J/m ²) (range)	621 (7–1528)	1244 ^a (567–2448)
Apparent shear stiffness (MPa/mm)	14 (2–26)	29 (17–48) ^a

^a ($p = 0.04$).

Histological evaluation

The specimens were dehydrated in graded ethanol (70–100%) containing 0.4% basic fuchsin, and embedded in methylmethacrylate. After sectioning, they were counterstained with 2% light green. The preparation method allows distinction between mineralized bone, fibrous tissue and bone marrow (Gotfredsen et al. 1989). The embedded specimens with the implant in situ were randomly rotated around the vertical axis of the implant. In the central part of implants, 4 serial sections of 15–20 μ m were produced using a Leiden microtome (Leiden, Holland) (Overgaard et al. 2000, Schmidmaier et al. 2001b). Histomorphometry was performed in a blinded fashion using an image analysis system (CAST-Grid; Olympus, Denmark). Quantification of tissue was performed using stereological principles (Gundersen et al. 1988).

Tissue ongrowth was defined as tissue in direct contact with the implant surface, and was determined using the cycloid intercepting technique. The number of intersections with tissue in contact with the implant surface was counted in successive adjacent fields at the tissue-implant interface. The gaps were divided into inner (0–480 μ m) and outer (480–960 μ m) zones and tissue volumes in the two zones were determined by the point-counting technique.

Statistics

Statistical analysis was performed using Intercooled Stata version 8.0 software (StataCorp, College station, TX). The difference between pairs did not follow a normal distribution, as indicated by the qq-plot. Thus, a paired non-parametric analysis was applied (Wilcoxon matched-pairs signed-ranks test). All results are presented as median and range. P-values less than 0.05 were considered significant.

Table 2. Histomorphometric results. Tissue ongrowth results presented as median and range

Surface percentage of:	Control	TGF- β 1 + IGF-1 in PDLLA
Bone	8.1 (0.7–13)	20 (4.7–28) ^a
Fibrous tissue	28 (0.5–62)	0 (0–0.7) ^b

^a ($p = 0.02$); ^b ($p = 0.01$).

Results

All dogs completed the study without complications. Culture swabs from implantation sites were all negative. At the time of necropsy, there were no clinical signs of infection or inflammation.

Mechanical fixation

8 of 9 treated implants had higher values in strength, stiffness, and energy than their controls ($p = 0.04$) (Table 1; Figure 1). There was a marked difference in the variance of the controls compared to the treated implants for all three parameters. The effect of the growth factors on fixation was relatively larger in animals in which the controls were poorly fixated.

Histomorphometry

There was a 2.5 fold median increase in bone ongrowth ($p = 0.02$), a 30% median increase in inner zone gap healing ($p = 0.04$), and a 112% median increase in outer zone gap healing ($p = 0.02$) (Table 3; Figure 2). There was practically no fibrous tissue on the treated implants (ongrowth, $p = 0.01$; inner zone gap healing, $p = 0.01$). In the control implants there was a significant difference between the healing of the inner and outer zones ($p = 0.02$), while there was no difference between the healing of the inner and outer zones in the growth factor-treated implants ($p = 1$) (Table 3).

Discussion

The formation of gaps around cementless implants is unavoidable. Some authors have reported that as little as 10–20% of a press-fit inserted prosthesis is in contact with the bone (Noble et al. 1988,

Table 3. Histomorphometric results. Gap healing presented as median and range. Data on gap healing are presented as percentage of total count in the gap

	Gap inner zone (0–480 μ m)		Gap outer zone (480–960 μ m)	
	Control	TGF- β 1 + IGF-1	Control	TGF β 1 + IGF-1
Bone	23 (7–49)	30 (24–42) ^a	17 (6–47)	36 (18–48) ^b
Fibrous tissue	3 (0–11) ^c	0 (0–0)	0	0

^a (p = 0.04); ^b (p = 0.02); ^c (p = 0.01).

Maximum shear strength (MPa)

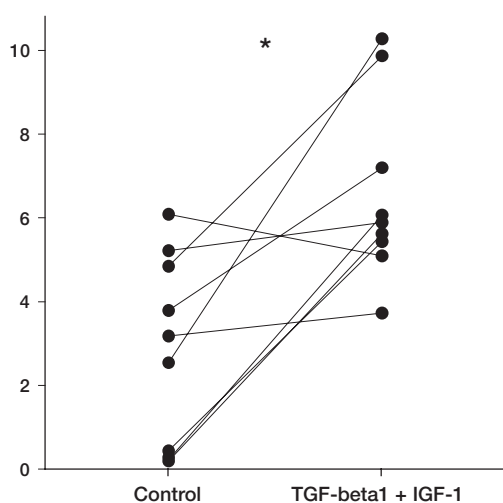


Figure 1. Plot of the paired data for maximum shear strength. Lines connect the data pairs corresponding to each dog. The graphs for energy to failure and apparent shear stiffness were similar. *(p = 0.04).

Schimmel and Huiskes 1988, Geesink 2002). Our previous work with gap implants has consistently shown abundant ongrowth of fibrous tissue, inferior bony ongrowth, and poor mechanical fixation (Soballe et al. 1990, Elmengaard et al. 2005). In previous studies, the titanium implant surface has been shown to be similar to that of commercially available prostheses with a plasma spray titanium coating (Soballe et al. 1992, 1993). We hypothesized that the combination of TGF- β 1 and IGF-1 in PDLLA would enhance the osseointegration and mechanical fixation of unloaded cylindrical porous-coated titanium implants surrounded by a gap.

We found a significant effect of the combination of growth factors on all mechanical parameters.

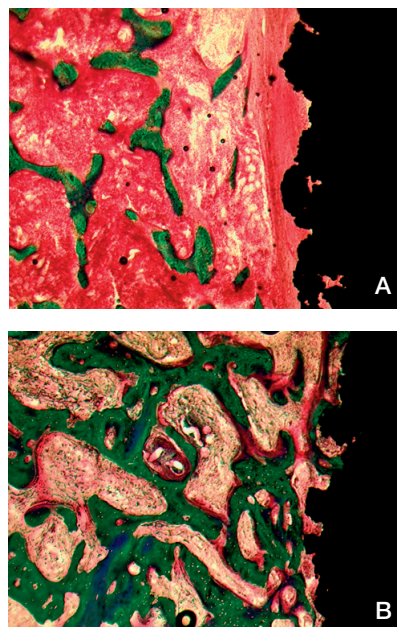


Figure 2. A. Illustration of an uncoated control implant. Adjacent to the implant (black) there is a thick rim of fibrous tissue (red, arranged longitudinally), and there is very little bone (green) in the gap. B. Illustration of a growth factor-treated implant. There is abundant bone (green) on the implant surface, and no fibrous tissue.

This was in accordance with the histomorphometric results. We saw a greater effect at the implant-tissue interface than in the surrounding gap. Bone ongrowth was generally 2–3 times higher for treated implants, while ongrowth of fibrous tissue was eliminated. In the gap, the major effect was a higher bone ingrowth for the treated implants. We used a combination of TGF- β 1 and IGF-1, as a synergetic or additive effect on fracture healing has been demonstrated when combining the two growth factors as opposed to administration of either growth factor alone (Schmidmaier et al. 2003).

Previous studies using a similar implant model in the dog have reported varied results with regard to the effect of TGF- β 1 on implant fixation. TGF- β 1 adsorbed to a hydroxyapatite coating only increased bone ongrowth by one-third, while no improvement was seen in gap healing and mechanical fixation compared to the control implants (Lind et al. 1996a). When TGF- β 1 was adsorbed to a tricalcium phosphate coating, bone ongrowth and gap healing was increased by 50% (Lind et al. 1996b).

For IGF-1, positive effects have been shown on obliteration of rabbit femoral defects and rat calvarial defects (Thaller et al. 1993, Laffargue et al. 2000). In a titanium chamber in a rabbit tibia, IGF-1 had the effect of reducing the turnover rate of minerals, and did not change the amount of bone mineral (Aspenberg et al. 1989). The combination of TGF- β 1 and IGF-1 has been investigated in a rat fracture model and a sheep spinal fusion model (Kandziora et al. 2003, Schmidmaier et al. 2003). In both models, the combination enhances healing and mechanical strength. In a long-term study in a rat fracture model, this combination of growth factors in PDLLA accelerated fracture healing and the recovery of mechanical strength, while the long-term results for the treated group and the control group were equal. The authors conclude that this strongly indicates that this growth factor treatment does not alter the natural healing process, but only accelerates it (Schmidmaier et al. 2004).

We used a PDLLA coating to deliver growth factors into the gap locally. Several studies have demonstrated that PDLLA is a suitable delivery vehicle for growth factors in both fracture healing and spinal fusion studies (Schmidmaier et al. 2001a,b). In a saline dilution model using the same coating process, PDLLA released approximately half of the growth factors within 48 h and another 25% within 6 weeks (Schmidmaier et al. 2001b). Although the *in vivo* release pattern may be accelerated, we can expect that the growth factors were indeed released to the surrounding gap.

This study did not include control implants with a PDLLA coating; thus, we cannot conclude that the positive effects were due to the growth factors alone. However, we do not expect the PDLLA coating to have had a bone stimulating effect in our model, as preliminary results from another experi-

mental study performed at our institution have indicated that the PDLLA coating does not alter the bony integration of similar titanium implants compared to uncoated samples. This contrasts with the rat model, where a stimulating effect has been observed.

IGF-1 and TGF- β 1 were administered in 5% and 1% (w/w) ratios of the PDLLA coating, respectively. These w/w ratios have worked in several animal models (Kandziora et al. 2003). Our method of administration is more advantageous than the adsorption of a certain amount of growth factor to a ceramic surface, as the dose is automatically adjusted for the size of implant, and an even distribution of growth factors is easier to obtain.

The possibility of adverse effects of the PDLLA coating must also be taken into consideration. In the present study there was sporadic presence of giant cells in all PDLLA-coated implants. Our thick histological sections did not permit quantification of cells; we can only state that they existed. The long-term consequence of the presence of these giant cells is unknown. However, the finding of giant cells on implanted polymers is common (Paivarinta et al. 1993, Coonts et al. 1998, Wildemann et al. 2005).

In conclusion, the combination of TGF- β 1 and IGF-1 in PDLLA showed some very promising results in the present study—as all the parameters examined were improved by the treatment. The finding of more bone and a dramatic reduction in the amount of fibrous tissue on the treated implants provides far better conditions for the continued osseointegration of the implant. The combination of TGF- β 1 and IGF-1 in PDLLA appears to be so powerful that it should be compared with hydroxyapatite, a surface coating that is already widely used for clinical purposes.

Contributions of authors

AL protocol, surgery, specimen preparation and analysis, and writing of manuscript. BE protocol, surgery and revision of manuscript. GS protocol and revision of manuscript. KS protocol and revision of manuscript.

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