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The effect of adding an equine bone matrix protein lyophilisate on fixation and osseointegration of HA-coated Ti implants

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Introduction

Insufficient bone stock or segmental bone loss reduces load distribution and initial stable fixation of a joint replacement, which is critical for its long-term survival¹. This can be a challenge in revision arthroplasties in particular, where the bone bed is often insufficient. Even in primary uncemented joint replacements as little as 10–20% of the implant surface may be in direct contact with the surrounding bone shortly after implantation². In current clinical practice, large bone defects can be managed with bone grafts, bulky implants, or by bypassing bone defects with implants that rely on alternative fixation sites in the bone. For smaller defects these measures are often uncalled for, and it may be sufficient to stimulate the patient's own bone to bridge partial defects in the bone-implant interface.

Colloss E (Ossacur AG, Germany) is a lyophilized complex of the extracellular matrix proteins of equine bone. The device has a cotton-like structure, takes up practically no volume when mixed with other substances, and has no mechanical strength of its own. It is therefore not expected to be of benefit by itself in closing large defects around implants in need of mechanical support. It is FDA-approved and used clinically as an osteoinductive bone void filler and to augment fracture healing. The main constituents of Colloss E are collagen type I chains in combination with TGFβ-1, BMP-2, BMP-7, VEGF, IGF-1, TGFβ-2, and BMP-3 and possibly other, not yet determined, growth factors³. Colloss E has a proliferative effect on immature bone cells and a differentiative effect on more mature osteoblasts in vitro⁴. It is osteoinductive vivo⁵ and seems to stimulate enchondral bone ossification via upregulation of cartilage-related genes⁶. In canine experiments, the device has proven useful in augmenting implants grafted with allograft⁷ and ceramic bone graft substitutes⁸.

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Joan Bechtold: design, surgery, manuscript review

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The rationale for combining the bone protein lyophilisate with hydroxyapatite-coated (HA-coated) implants was to stimulate bridging of defects in the bone-implant interface that may be present immediately after surgery in primary as well as revision joint replacements⁹. We chose HA coated implants because of their strong history of excellent fixation and longevity (experimental and clinical), and to provide a challenging setting for demonstrating further improvement. Whereas HA is osteoconductive and perhaps even stimulatory of bone growth on the surface on which it is deposited, the osteogenic growth factors within the collagen I matrix proteins of the device could be able to stimulate bone formation in the void between the implant surface and the surrounding bone. We therefore hypothesized that the addition of Colloss E would increase fixation of HA-coated implants. For experimental purposes, we defined this as increasing the mechanical fixation, increasing the new bone formation, and reducing the presence of fibrous tissue.

Materials and Methods

Experimental design (Figure 1)

The experiment was conducted in a paired design, where each dog received a treated implant and an untreated control implant in the proximal tibia. The placement of the control and the intervention implant was alternated systematically between the left and the right side with random start. All implants were surrounded by a 2 mm coaxial defect. Nine dogs were included. The observation time was 4 weeks.

Implants

We used 18 HA-coated porous titanium alloy (Ti-6Al-4V) implants for the experiment, manufactured by Biomet Inc. (Warsaw, IN, USA). The implants were cylindrical with a height of 10 mm and 6 mm diameter. The porous Ti coating was plasma sprayed, giving a mean pore diameter of 480 μ m and a mean porosity of 44% as specified by the manufacturer. The implants were then additionally given a 50 μ m thick HA-coating by plasma-spray technique. A footplate of 10 mm diameter was attached on both ends of each implant. When inserted into a 10 mm drill hole, the footplates secured implant centricity and provided a uniform 2 mm defect around it (Figure 1).

Animals

Nine skeletally mature, 13–16 month old American Hounds with a mean weight of 21.8 kg (range 19.5–22.8 kg) were included into the study. The dogs were bred for scientific purposes, and the experiment was approved by the Institutional Animal Care and Use Committee (Minneapolis Medical Research Foundation).

Surgical procedure

With the dogs under general anaesthesia and with sterile technique, a skin incision was made with cautery on the anteromedial face of the proximal tibia just below the joint line. The periosteum was elevated with a rongeur and pushed aside. A 2.5 mm guide wire was inserted perpendicular to the anteromedial cortical surface 10 mm below the joint line. A cannulated drill ($\varnothing$ 10 mm) was used to drill a 12 mm deep cylindrical cavity over the guide wire at a speed of maximum two rotations per second. The cavity was irrigated with 10 ml saline for removal of loose bone chips. The implant with premounted footplates in both ends – with or without 20 mg Colloss E on the implant surface – was inserted into the drill hole securing uniform central placement. Finally, the soft tissues including the periosteum were closed in layers. The procedure was repeated for the opposite side. All 18 implants were operated on by the same surgeon (JB). Pre- and postoperatively, the dogs were given one gram of dicloxacillin intravenously as antibiotic prophylaxis. All animals were allowed

unlimited activity. After four weeks observation time, the dogs were sedated and euthanized with an overdose of hypersaturated barbiturate.

Specimen preparation

The proximal tibiae were frozen and stored at -20°C immediately after retrieval. After thawing for specimen preparation the outermost 0.5 mm of the implant-bone specimen was cut off and discarded. The rest of the implant with surrounding bone was divided into two sections perpendicular to the long axis of the implant with a water-cooled diamond band saw (Exact Apparatebau, Nordenstedt, Germany). The outermost section was cut to a thickness of 3.5 mm and stored at -20°C until mechanical testing. The innermost section was cut to a thickness of 5.5 mm and prepared for histomorphometry. These specimens were dehydrated in graded ethanol (70–100%) containing basic fuchsin, and embedded in methylmethacrylate (Technovit 7200 VCL; Exact Apparatebau, Nordenstedt, Germany). Using vertical sectioning technique^{10, 11}, each specimen was cut into four 30 μm thick histological sections with a microtome (KDG-95, MeProTech, Heerhugowaard, Holland). Finally, these were surface counterstained with 2% light green for 2 minutes, rinsed and mounted on glass. This preparation provided red staining of non-calcified tissue and green staining of calcified tissue. The tissues were classified based on their morphological characteristics¹².

Mechanical testing

Thawed specimens were tested to failure by axial push-out test on an MTS Bionics Test Machine (MTS, Eden Prairie, MN, USA) using a 2.5 kN load cell. Testing was performed blinded and in one session. The transversely cut specimens were placed with the cortical side facing up on a metal support jig with the implant ($\varnothing$ 6 mm) centered over a 7.4 mm opening and under a cylindrical test probe of 5 mm diameter. A preload of 2 N defined the contact position for the start of the test. The implants were then pushed out of the surrounding tissue in the direction of the implant axis at a displacement rate of 5 mm/min. Load versus implant displacement data was continuously recorded. Maximum shear strength (Strength; Pa) was determined from the maximum force applied until failure of the bone-implant interface. Maximum shear stiffness (Stiffness; Pa/mm) was obtained from the slope of the linear section of the load versus displacement curve. Total energy absorption (Energy; J/m²) was calculated as the area under the load displacement curve until failure. All mechanical parameters were normalized by the cylindrical surface area of the transverse implant section tested.

Histological evaluation

Blinded histomorphometry was performed using the stereological software C.A.S.T. Grid (Olympus Denmark AS, Ballerup, Denmark). With the aid of the software, two regions of interest were defined: an implant-near Zone 1 from the implant surface and 0.5 mm into the coaxial defect, and a Zone 2 covering the rest of the defect (0.5–2 mm from the implant surface). In these zones, volume fractions of bone, fibrous tissue, and marrow space were quantified by point-counting technique¹³. On the implant surface, the area fractions of the same tissues were quantified by line-interception technique¹⁰. These techniques provide highly reliable results with negligible bias¹⁴. Bone was surface-stained green, and therefore easy to distinguish from the other tissues. Fibrous tissue was identified by its presence of clearly visible fibril fibre complexes and low cell density. The fibrous tissue largely appeared oriented, dense and well-organized, but also as a loosely, not clearly oriented, interconnected fibrous network. Marrow space consisted of fat vacuoles and surrounding blood cells.

Statistical analysis

Both the mechanical and histological datasets were evaluated non-parametrically, as normal distribution could not be assumed for all parameters, in most cases because of values close to zero. The datasets were evaluated with Wilcoxon signed-rank test. For all datasets, differences between medians were considered statistically significant for p-values <0.05 . Statistical analysis was performed using Intercooled STATA 9.0 software (StataCorp LP, College Station, TX, USA).

Results

Observations on animals

All nine dogs were fully weight bearing within three days after surgery and completed the four weeks observation period without signs of infection or other complications.

Mechanical tests

The group treated with the lyophilized bone matrix proteins had better mechanical fixation than the control group, statistically significant in the parameters Strength ($p=0.038$) and Energy absorption ($p=0.021$), but not in Stiffness ($p=0.11$) (Table 1).

Histological observations

By histomorphometry, the treated implants had increased new bone formation on the implant surface ($p=0.008$) as well as in the defect around the implants ($p<0.05$) (Figures 2 and 3), and the presence of fibrous tissue was eliminated ($p<0.02$) (Figures 2 and 4). The histological observations were consistent with the mechanical findings, showing a positive correlation between mechanical fixation and new bone formation, and an inverse correlation between mechanical fixation and fibrous tissue encapsulation (Table 2)

Discussion

The purpose of the study was to investigate the effect of adding an osteoinductive signal to HA-coated implants to stimulate osseointegration and bridging of voids present around uncemented implants immediately after surgery. These periprosthetic defects are thought to compromise the initial mechanical stability of the implants, promote fibrous encapsulation, and contribute to later failure of the prosthetic components. In this experiment we used a non-loaded gap model, where the whole implant was surrounded by a periprosthetic defect. The gap model is designed to study early fixation and osseointegration of an uncemented implant component. It isolates the contribution to implant fixation from tissues formed after surgery and provides highly reproducible conditions. However, it does not model clinically relevant influences such as direct load, joint fluid, or particulate debris.

We found that topical application of the Colloss E device around the HA-coated implants increased the mechanical fixation compared to the untreated control HA-implants. Addition of the device improved implant osseointegration and new bone formation, and eliminated the presence of fibrous tissue on and around the implant.

The most important biological factors for mechanical implant fixation in this study was new bone formation on the implant surface as well as in the immediate implant vicinity. It is notable that this could be further improved by the addition of an osteoinductive signal, as HA-coated porous surfaces are already considered a highly successful substrate for achieving bone ongrowth¹⁵. The osteoconductivity of an HA coating can ensure bone formation even before the defect around an implant is bridged in a process described as bidirectional bone growth¹⁶. However; the source of osteogenic precursor cells is the drill

hole border, and this study shows that the addition of the lyophilized bone matrix proteins stimulates the bridging of defects between the implant and the drill hole border, as well as the direct bone implant ongrowth.

The reduction of fibrous tissue formation seemed to be an almost as important factor for implant fixation as new bone formation. Fibrous tissue encapsulation may be detrimental to implant fixation, which is thought to be a strong contributor to later failure of prosthetic components. Many factors regulate the presence of fibrous tissue around the implant, such as implant micromotion and particulate wear debris in the bone-implant interface. Under controlled circumstances where these factors had been eliminated, the addition of the lyophilized bone matrix proteins was able to prevent fibrous tissue formation on and around the implant completely. We mainly attribute this directed tissue differentiation to the presence of osteogenic growth factors as seen in previous studies with the combination of TGF- β 1 and IGF-1¹⁷, and BMP-2¹². However, it may also have been influenced by the presence of vascular growth factors contained in the device, such as VEGF-1, allowing more metabolically active and higher differentiated tissue to form due to improved vascular supply⁷.

This could be considered after the authors review and comment in the discussion section the possible literature findings on whether other proteins (or blends) such as collagen I has been compared to Colloss E in a similar set up? In other words, I find it important the authors discuss the uniqueness of the blend and possible advantages and drawbacks compared to other approaches such as HA-granula, Ti-granula, collagen I, hyaluronic acid, local bisphosphonates, etc.

The focus of this experiment was to promote early implant fixation and reduce the risk of early implant subsidence, which is known to be related to late loosening. The observed features of more new bone formation and reduced fibrous tissue in the treated group may express a progressed stage of early healing, rather than long-term permanent differences between the groups. Since the experiment only considers one time point in the healing process, it is beyond the limitations of the study to definitely attribute causality.

One concern in using biological material from another species (xenograft) is the fear of an excessive immunological response. Our histological preparation of the implant-tissue interface necessarily precluded an analysis of inflammatory response, but we saw no sign of bone growth inhibition in implants treated with the Colloss E device. If there was in fact a relative inhibition of bone growth caused by a foreign-body related inflammatory response in our study, then it seemed to have been outweighed by the benefit of the osteogenic stimulus.

Previous experiments with both bovine and equine bone matrix protein lyophilisates have also shown good bone regenerating properties in areas varying from augmenting the bioactivity of bone grafts⁷ and bone graft substitutes¹⁸ in canines,¹⁹ to spinal fusion models in pigs²⁰. One ovine study showed a reduced implant fixation with the use of Colloss E²¹. The encouraging results may be attributed to its range of osteogenic growth factors (BMP-2, BMP-7, TGF- β 1, and IGF-1) in low concentrations and with a delayed release from the Collagen I matrix proteins³. This distinguishes the device from the human recombinant growth factors, which are only available as mono-therapy treatments. Although BMPs are generally thought to induce ectopic bone through endochondral ossification²², we saw no evidence of this in the present study. The gap healing in both the treated and the control groups rather seemed to follow an intramembraneous pathway. This is consistent with previous findings of TGF- β in augmenting implant fixation²³, and with the fact that TGF- β 1 seems to be the most predominant growth factor in the Colloss E device²⁴.

This could be considered after the authors review and comment in the discussion section the possible literature findings on whether other proteins (or blends) such as collagen I has been compared to Colloss E in a similar set up? In other words, I find it important the authors discuss the uniqueness of the blend and possible advantages and drawbacks compared to other approaches such as HA-granula, Ti-granula, collagen I, hyaluronic acid, local bisphosphonates, etc.

In the present study, the Colloss E device was compared to an empty defect. Since the device consists of the collagenous and non-collagenous proteins of bone, our experiment cannot conclude on which components of the material that gave the observed effect. Collagen by itself is not osteoinductive^{25,26}, but bovine Collagen Type I was found to promote healing of implants similar to the ones employed in this experiment²⁷.

Conclusion

The results suggest that a lyophilisate of equine bone matrix proteins as in the formulation of the medical device Colloss E may augment early implant fixation and thereby reduce the risk of long-term failure. It stimulates bridging of periprosthetic gaps and seems to be a particularly powerful agent to reduce the risk of fibrous tissue encapsulation. This may also be useful in revision arthroplasty with bone loss, although the device should be supplemented with a mechanically stable filler material in larger load-bearing defects. Some caution should be taken due to its xenograft nature and adverse results in one preclinical experiment. Human use of the device with load-bearing implants should therefore be documented in protocolled clinical studies initially.

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Figure 1.

Implantation sites Schematic drawing of the implants inserted into the anteromedial face of the proximal tibiae. The implants are centered in the oversized drill hole by the footplates providing a 2 mm gap around the implant. The groups were alternated systematically between the right and the left knee with random Start.

Figure 2. Representative histological sections of the two groups

The displayed sections are from the two implants inserted in the same animal; HA-coated Ti implants surrounded by a 2 mm concentric defect. The sections are cut parallel to the long axis of the implant. The footplate seen on the bottom of both implants indicates the drill hole border. CONTROL (left): Fibrous tissue encapsulation (70% vs. group median 85%), little bone-implant contact (7% vs. group median 7%), and little new bone formation in the defect between the implant and surrounding bone (5% vs. group median 7%).

COLLOSS E (right): No fibrous tissue (0% vs. group median 0%), more bone-implant contact (39% vs. group median 39%), and more new bone formation in the defect between the implant and surrounding bone (25% vs. group median 16%).

Figure 3. Histomorphometry new bone

Fractions of new bone on implant surface (area fractions), in the gap Zone 1 (0–0.5 mm) volume fractions), and in the gap Zone 2 (0.5–2 mm) (volume fractions). Paired implants interconnected. 293x261mm (300 × 300 DPI)

Figure 4. Histomorphometry fibrous tissue

Fractions of fibrous tissue on implant surface (area fractions), in the gap Zone 1 (0–0.5 mm) (volume fractions), and in the gap Zone 2 (0.5–2 mm) (volume fractions). Paired implants interconnected.

Table 1
Mechanical push-out test

Parameters of biomechanical implant fixation from normalized force-displacement curves [median (*iqr*)].

	Strength [MPa]	Stiffness [MPa/mm]	Energy [kJ/m ²]
CONTROL	0.04 (0.03–0.5)	0.45 (0.2–2.6)	0.01 (0.01–0.12)
COLLOSS E	2.6 (2.1–3.8)	16 (10–19)	0.65 (0.49–0.73)
Wilcoxon	p=0.038	p=0.11	p=0.021

Table 2
Association between mechanics and histology

Spearman's rho and Bonferroni-corrected p-values for the association between mechanical fixation (Strength, Stiffness, Energy) and tissue fractions (new bone and fibrous tissue) on implant surface and in gap.

Zone	Tissue	Correlation to mechanical fixation (Spearman's rho)		
		Strength	Stiffness	Energy
Zone 1 (0–0.5 mm) area fractions (ongrowth)	New Bone	0.79 (p<0.001)	0.77 (p=0.002)	0.77 (p=0.002)
	Fibrous Tissue	–0.77 (p=0.002)	–0.77 (p=0.002)	–0.80 (p<0.001)
Zone 1 (0–0.5 mm) volume fractions (ingrowth)	New Bone	0.91 (p<0.001)	0.92 (p<0.001)	0.91 (p<0.001)
	Fibrous Tissue	–0.78 (p=0.001)	–0.78 (p=0.001)	–0.79 (p<0.001)
Zone 2 (0.5–2 mm) volume fractions (ingrowth)	New Bone	0.64 (p=0.036)	0.66 (p=0.025)	0.64 (p=0.036)
	Fibrous Tissue	–0.69 (p=0.014)	–0.69 (p=0.015)	–0.70 (p=0.012)