Guided Bone Augmentation around a Titanium Bone-Anchored Hearing Aid Implant in Canine Calvarium: An Initial Comparison of Two Barrier Membranes

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ABSTRACT

Background: The placement of a bone-anchored hearing aid (BAHA) implant in young children may be hampered by the presence of thin, poor-quality calvarial bone. The use of extraskeletal guided skull bone augmentation around the titanium implant is one potential solution.

Purpose: To compare the effectiveness of a collagen membrane BioSISt (Cook Biotech Inc., Lafayette, IN, USA) and a PGA/PLA barrier membrane, Osseoquest (W.L. Gore & Associates, Flagstaff, AZ, USA) in promoting extraskeletal bone formation, when combined with cancellous bone graft, around a titanium implant in the canine calvarium. The quality and quantity of bone tissue was compared.

Materials and Methods: A 4-mm titanium BAHA implant was placed in the cranial parietal bone of 11 dogs. The implant protruded from the bone surface by a measured distance. Two groups, each of three dogs, received an implant, cancellous bone graft, and either a BioSISt or Osseoquest membrane. Three dogs received implant and bone graft (positive controls), and two received an implant only (negative controls). Samples were retrieved at 3, 6, and 9 months after placement. Undecalcified histologic and histomorphometric assessments were made of the augmented bone thickness, and bone gain factors were calculated for each sample group.

Results: The process of osseointegration of the implants was ongoing and increased over time. Bone generation occurred with both test membranes and the early trabecular bone that formed, matured, and remodelled to compact bone at 9 months. BioSISt membrane samples showed superior quality and quantity of augmented bone compared with Osseoquest samples that exhibited thinner bone with persistent inflammation. Quantitatively, the BioSISt samples showed statistically greater new bone contact and bone area than both the positive and negative controls, whereas Osseoquest samples did not. The bone gain factor was statistically greater for BioSiSt samples when compared to the positive and negative controls whereas the Osseoquest samples were not.

Conclusions: In this study, the collagen BioSISt membrane promoted bone formation of superior quality and quantity compared with the polyglycolic/polylactic acid-based Osseoquest membrane and positive and negative controls over 9 months. Further investigation of the use of the collagen BioSISt membrane for cranial bone augmentation is warranted.

KEY WORDS: bone-anchored hearing aid, bone augmentation, cancellous bone graft, collagen membrane, polyglycolic acid/polylactic acid membrane, titanium implant

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The osseointegration techniques pioneered by Brånemark[1] and adapted for hearing rehabilitation have been in use in Europe since 1977.[2] This resulted in the bone-anchored hearing aid (BAHA), which is used in adults and children. Early hearing rehabilitation for children is critical in the development of speech and language.

The superior performance of the BAHA enhances speech and development of the child especially when fitted at an early age. The BAHA is more comfortable to wear and is aesthetically more acceptable than a standard bone conduction aid; however, implantation in young children under 5 years of age presents specific problems. The temporal bone is thin, often with less than 2 mm of bone available for implant placement at the surgical site.

These problems may be overcome by either delaying placement or allowing the implant to protrude from the bone and await bone growth. A third solution promotes guided bone augmentation (GBA) using a barrier membrane, for example, expanded-polytetrafluoroethylene (e-PTFE).

Dahlin and colleagues[3] in 1989 first described the extraoral use of GBA. The regenerative barrier membranes used for GBA may be nonabsorbable or absorbable. Nonabsorbable membranes must be removed and are susceptible to infection if exposed.[4] Absorbable membranes can be synthetic (eg, polyglycolic or polylactic acid [PLA]) or natural (eg, cellulose mesh or collagen, from skin, tendon, or intestine, etc.).[5] Synthetic membranes are reported to be less inflammatory with denaturation by hydrolysis in contrast with collagen products that are resorbed by inflammatory mechanisms.[4]

Bone may be generated either in a deficit within the skeletal envelope, or extraskeletally as in this study. Linde and colleagues[6] created new bone over rat cortical calvarium using e-PTFE domes, placed under the periosteum. Schmid and colleagues[7] augmented cranial cortical bone in rabbits using a titanium scaffold and the e-PTFE system. Dahlin and colleagues[3] augmented bone in rabbit tibias using a porous teflon membrane.

More recently, Lundgren[8] and Slotte[9] studied membrane-induced bone generation in the rabbit calvarium. Granström and Tjellström[10] augmented temporal bone around a BAHA implant in a series of children with inadequate bone depth, using the e-PTFE membrane.

The first aim of this study was to compare the ability of two membranes to support extraskeletal bone augmentation when combined with cancellous bone graft around a 4-mm titanium BAHA implant, placed in 2.0- to 2.5-mm thickness of canine cortical calvarial bone.

Second, comparisons of the quality and quantity of bone generated in association with each membrane at 3, 6, and 9 months after implant placement were performed as well as histological examinations of barrier membrane resorption.

MATERIALS AND METHODS

Both membranes used were absorbable. Osseoquest (W.L. Gore & Associates, Flagstaff, AZ, USA) was composed of polyglycolic acid (PGA), polylactic acid (PLA), and trimethylene carbonate, and had a three-layer structure of two random fiber matrixes on either side of a cell-occlusive film. Osseoquest's manufacturers claim that barrier function is maintained for 6 months and substantially resorbed by 12 to 14 months.[11]

The second membrane was a porcine small intestinal submucosal collagen membrane, Vet BioSISt, also marketed in the USA, Europe, and Australia for human surgical use as SIS® (Cook Biotech Inc., Lafayette, IN, USA). In its preparation, the small intestinal mucosal layer and the external muscle layers were removed mechanically, leaving the stratum compactum layer of the tunica muscularis mucosa, and the tunica submucosa. The membrane was then rendered cell free under hypotonic conditions leaving an extracellular matrix.[12] The stratum compactum consists of dense organized connective tissue and a less dense submucosal layer.

The BioSISt was 100-μm thick and contained collagen types I, III, V, and naturally occurring growth factors, glycosaminoglycans, proteoglycans, and fibronectin, according to the manufacturer. In the canine, it has been used for vascular grafts, wound treatment, cruciate ligament repair,[13] bladder augmentation,[14] and abdominal wall repair[15]. Human use includes wound treatment, bladder augmentation, hernia, and paravaginal repairs.[16] A four-layer preparation of BioSISt was used in this study.

In six dogs, one of the two test membranes was placed over a BAHA implant, around which half a cubic centimeter of autogenous cancellous bone graft had been packed. A positive control group ($n = 3$) received bone graft and implant alone, and a negative control group ($n = 2$) received a titanium implant only.

The quantity and quality of bone tissue produced over 9 months were compared for all four groups on undecalcified histological stained sections prepared by the Donath Exakt technique.[17]

The Canine Models

Eleven cross-bred dogs from the same litter were raised under identical conditions from birth. All dogs were treated regularly for intestinal worms (Drontal, Bayer, Pymble, New South Wales (NSW), Australia), vaccinated against distemper, hepatitis, parvovirus and *Bordetella* (Protec 4, Fort Dodge, Baulkham Hills, NSW Australia), and given a *Dirofilaria immitis* preventative (Pro-Heart, Fort Dodge). At 6 months of age, all animals were desexed.

Anesthesia

At 8 months of age, the dogs were anesthetized for radiographic examination and implant placement. Premedication of methadone 0.5-mg/kg subcutaneous (Parnell Labs, Mascot, NSW Australia), acetyl promazine 0.05 mg/kg subcutaneous (Promex 2, Apex Labs, Somersby, NSW Australia), and atropine 0.02 mg/kg (Apex Labs) was given.

Anesthesia was induced with thiopentone sodium 2.5% intravenously to effect 10 to 12 mg/kg (Mavlab, Brisbane, Queensland, Australia), and maintained with oxygen, halothane (Laser Animal Health, Salisbury, Queensland, Australia) and nitrous oxide. Isotonic saline was given intravenously at 10 mL/kg/h throughout these surgical procedures.

Subsequent anesthetics for craniotomy and sample retrieval used a similar premedication; in addition, phenobarbitone 4 mg/kg (Sigma Co. Ltd, Croydon, Victoria, Australia) was given subcutaneously to prevent possible seizures. Anesthesia was induced with propofol given intravenously at 3 to 4 mg/kg (Abbott, Botany, NSW Australia), and maintained with isoflurane (Abbott) and oxygen, using positive pressure ventilation.

A fentanyl infusion (Janssen-Cilag, North Ryde, NSW) was given for intraoperative analgesia, at a loading dose of 5 µgm/kg and maintenance of 6 to 10 µgm/kg/h. Bupenorphine 1 µg/kg (Reckitt & Coleman, Hull, UK) was used 8-hourly postoperatively. Intravenous cephazolin 22 mg/kg intravenous (Lilly, West Ryde, New South Wales, Australia) was given 30 minutes before and 2-hourly during surgery. Isotonic saline was given at 3 mL/kg/h during the craniotomies.

Measurement of Cranial Bone Depth

The initial depth of left parietal cranial bone was measured in a transverse (axial) plane by computed tomography (Com.T.) (Toshiba X Speed, Toshiba Medical, North Ryde, NSW, Australia). This depth varied from 2.02 to 2.57 mm between dogs. The point of CT measurement was marked by a transdermal injection of 2% methylene blue (David Bull Laboratories, Melbourne, Victoria, Australia) on the periosteum.

Implant Placement

A 4-mm commercially pure titanium BAHA implant (designed by Nobel Biocare [Göteborg, Sweden] and now marketed by Cochlear Ltd, Sydney, Australia) was inserted in the cranial bone at the site of thickness measurement. A caudally based cresentic skin flap 24-mm diameter was raised, and a similar flap of cervicoscutularis and cervicoauricularis muscles. The marked periosteal site was located and the periosteum elevated, and a circular piece 20 mm in diameter was removed.

Using the DEC Brånemark Drilling System (Nobel Biocare AB, Göteborg, Sweden), the 4-mm fixtures were placed according to the Brånemark method.[18] The implant height protruding above the bone was measured medially and laterally using a graded periodontal probe and recorded photographically (Figure 1).

All dogs received an implant and were randomly allocated into one of four groups. In group 1, (three dogs) 0.5 cc of autogenous cancellous bone graft from the proximal humerus was packed around the implant and covered with a circular disk of BioSISt membrane 20 mm in diameter. The membrane was secured by the cover screw and sutured to the periosteum with a con-

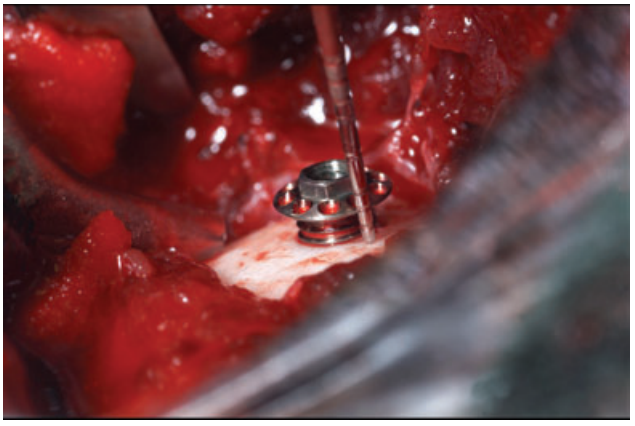

Figure 1 Intraoperative distance between the implant flange and parietal bone measured with a periodontal probe.

tinuous 4/0 polydioxanone (PDS) suture (Johnson & Johnson, Broadway, NSW, Australia).

In group 2, three dogs received a similar quantity of bone graft and a 20-mm disk of Osseoquest membrane similarly placed.

In group 3 (positive controls), three dogs received bone graft and fixture after periosteal removal.

In group 4 (negative controls), two dogs received only an implant after periosteal removal. The implant flange to bone distance had been measured intraoperatively using a graded periosteal elevator.

Anatomical wound closure was performed, and surgical sites were dressed for 10 days postoperatively.

Surgical recovery was uneventful except that two dogs with Osseoquest membranes formed seromas, 1 to 2 cm in diameter, at the surgical site 9 days postoperatively. These resolved without treatment.

At 3, 6, and 9 months after implant placement, the dogs from each group underwent a craniotomy to retrieve the implant and a 20-mm disk of surrounding bone. The surgical approach was similar to implant placement, and craniotomies were performed using a 20-mm bone trephine and then a cranial burr (Stryker Drilling Systems, Kalamazoo, MI), with constant cooling from isotonic saline. The dura and endosteum were found to be intact in all cases, so no implant base was visible, except in the 9-month positive control sample, a small area of the implant base was visible through the endosteum.

Barrier membrane integrity was maintained during removal, and the marked samples were immersed in 4% neutral buffered formaldehyde pH 7.03. The closure of wounds was as previously described, and healing was uneventful.

The Sample Processing

Undecalcified cut and ground sections were processed according to the internal guidelines at the laboratories of Biomaterials/Handicap Research (Institute of Surgical Sciences, University of Göteborg, Sweden) and according to the Donath Technique,[17] resulting in histologically stained sections of final thickness of 10 to 15 µm.[19] The process involved refixation of the samples for 1 week after arrival at the laboratories. Dehydration was carried out in graded series of ethanol (70–100%) followed by infiltration in dilute and pure resin. All these steps were carried out in a vacuum and in stirring conditions. Finally, the samples were embedded in light cure resin (Technovit® 7200 VLC, Heraeus Kulzer GmbH & Co., Wehrheim, Germany). The cured blocks were divided in a mediolateral plane through the center of the implant, and the surface plane was ground parallel and plexiglass was glued on the surface. One thick central section was prepared from each sample and ground to a thickness of 100 µm using the Exakt sawing and grinding equipment (Exakt Apparatebau GmbH & Co., Norderstedt, Germany). This thick section was microradiographed and then ground to a final thickness of 10 to 15 µm followed by histological staining in 1% toluidine blue mixed with 1% pyronin G.

The ground sections were observed in a light microscope with respect to quality and quantity of tissue surrounding the implants, and the microradiographs were generally examined. The presence of membrane remnants, cells, and tissues seen at the flange, the implant neck, within the threads, and in the apical/base region were assessed. Comparison was made between the 3-, 6-, and 9-month test samples, and the positive and negative controls.

A computer-based histomorphometric technique was used to make measurements in the regions of interest on cut and ground sections, directly in the eyepiece of a Leitz Aristoplan Light microscope connected to a Microvid® unit (Ernst Leitz GmbH, Wetzlar, Germany) and a personal computer.[20]

The computer-based quantifications involved:

1. The mean percentage of total bone to implant contact.
2. The mean percentage of new bone to implant contact formed on previously exposed threads. The number of threads originally exposed had been measured at surgery, and the final bone coverage of the threads was measured on the sections.
3. The mean percentage of total bone area (TBA).
4. The mean percentage of new bone area (NBA) within previously exposed threads.

Second, a bone gain factor was calculated for specimens from each group. The original distance between the implant flange and bone surface had been measured at implant placement by the use of a graduated periodontal probe and high definition photography. The final distance was calculated from the histological specimens (Figure 2), as CT examination, which was also used to estimate bone quantity, can be prone to error.[21]

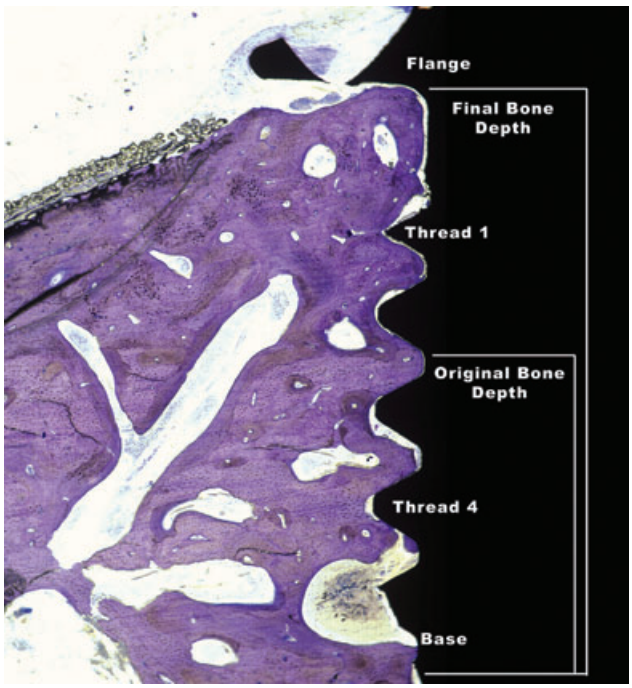

Figure 2 A histological sample showing labelled implant threads and bone gain. Six-month BioSISt sample. A histological central sectional overview of the section. Medially lamellar bone and secondary osteons were present to the flange. Vascular channels ran from the cortical plate into new generated bone (staining, 1% toluidine blue and 1% pyronin G; magnification, distance of 0.6 mm between the thread peaks).

The original and final bone depths were calculated for the medial and lateral sides of each implant. A gain factor (final bone depth/original bone depth) was developed for each implant and each group of sample types, $n = 3$ for both the BioSISt, Osseoquest, and positive controls, and $n = 2$ for the negative controls.

Statistical Analysis

The values of mean percentage +/– standard error (SE) for total and new bone/implant contact, and TBA and NBA were combined for all samples in each group where $n = 3$ for BioSISt, Osseoquest and positive controls, and $n = 2$ for the negative controls.

Statistical analysis was performed using computer software (Jandel Sigmastat Statistical software Version 2.0 Jandel Corporation, Silicone Valley, CA).

Pairwise comparisons between groups were made using *t*–tests if normality or equal variance tests were passed, and Mann-Whitney rank sum tests were used if normality or equal variance tests were failed. Graphs were drawn using GraphPad Prism 4 software (Graphpad Software, San Diego, CA).

When a bone gain factor had been calculated for each group of samples, BioSISt, Osseoquest, and positive and negative controls, a Shapiro-Wilk W test was used to check for nonnormality between samples. Comparison of these multiple groups was made using one-way analysis of variance.

Then a Tukey–Kramer multiple pairwise comparison test was used to show any significant mean difference between the bone gain factors of the groups.

RESULTS

Gross and Histomorphometric Examination

At sample retrieval, three of the 6-month and all of the 9-month samples showed minor dural adhesions at the implant site over a diameter of 4 mm. These adhesions were easily separated, and the dura remained intact.

Gross inspection of the ground sections revealed that all implants were undergoing osseointegration; however, the degree of bone generation and the maturity of the bone formed varied.

Quantitative Results

In eight of the 11 samples, more bone was formed on the medial side of the implant than on the lateral side. The

TABLE 1 Calculation of a Bone Gain Factor for Each Sample Group

Bone Gain Factor	BioSISt	Osseoquest	Positive Control	Negative Control
Medial	2.3	2.3	0.72	0.82
Medial	1.74	0.86	1.45	0.87
Medial	1.85	1.7	1.22	
Lateral	1.93	1.25	0.82	0.92
Lateral	1.42	1.17	0.87	0.91
Lateral	1.35	0.83	1.22	
Overall bone gain factor	1.77	1.36	1.05	0.88

Bone gain factor = final bone height/original bone height.

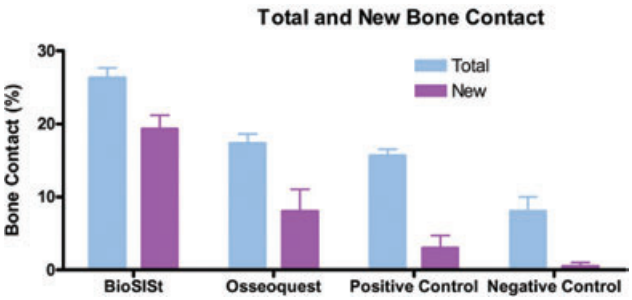

Figure 3 Mean percentage +/– standard error, total and new bone/implant contact pooling samples of each type, over 9 months; BioSiSt, $n = 3$, Osseoquest, $n = 3$, positive control, $n = 3$, negative control, $n = 2$. BioSISt samples showed significantly greater total bone contact ($p = .003$) and new bone contact ($p = .003$) compared with the positive control, and also with the negative control, total bone contact ($p = .004$), and new bone contact ($p = .004$). Osseoquest and positive control samples only showed significantly greater total bone contact ($p = .026$) compared with the negative control.

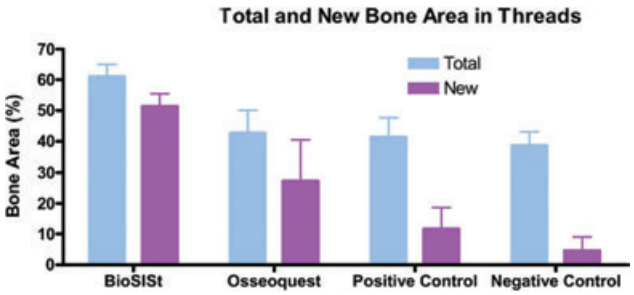

Figure 4 Mean percentage +/– standard error, total and new bone area (NBA) in the threads for all samples of each type over 9 months; BioSISt, $n = 3$, Osseoquest, $n = 3$, positive control, $n = 3$, negative control, $n = 2$. BioSISt samples showed significantly greater NBA in threads compared with the positive control ($p = .008$) and negative control ($p = .005$). BioSISt showed significantly greater total bone area than the negative control ($p = .036$) but not the positive control ($p = .059$). Osseoquest samples did not show any significant differences.

samples with membranes showed a greater quantity of bone generation than the positive and negative controls.

The values of mean percentage +/– SE for total and new bone/implant contact, and total bone area (TBA) and new bone area (NBA) were combined for all samples in each group.

Pairwise comparison was made between groups using *t*-tests if normality or equal variance tests were passed, and Mann-Whitney rank sum tests if normality or equal variance tests were failed.

The sections from membrane samples showed a greater quantity of bone generation than the positive and negative control. The BioSISt samples showed significantly greater NBA, total bone contact, and new bone contact, when compared with the positive and negative controls. The Osseoquest and positive control samples showed significantly greater total bone/implant contact compared with the negative controls only. Figures 3 and 4 illustrate the histomorphometrical results.

The bone gain factors for each group ($n = 3$ for the samples with membranes and the positive control group, and $n = 2$ for the negative control group) are shown in Table 1. A Shapiro-Wilk W test showed no evidence of nonnormality between samples. One-way analysis of variance ($p = .0084$) showed significant differences within the groups, and the Tukey–Kramer multiple pairwise comparisons test showed a significant mean difference between the bone gain factor for BioSiSt and the positive ($p = .0234$) and negative ($p = .0116$) controls. This was not the case for Osseoquest samples (Table 2).

Qualitative Histologic Results

The histological results for each group of samples are summarized in Table 3. The BioSISt test samples showed the greatest overall bone generation. The early bone formed was trabecular, and this underwent extensive remodelling over time. At 3 months, the trabecular bone

TABLE 2 Tukey–Kramer Multiple Comparisons of the Mean Differences Between Bone Growth Factors

Comparison	Mean Difference (95% CI)	$\|L/SE(L)\|$	
BioSISt versus negative control	0.893333 (0.178806–1.60786)	4.9972	$p = .0116$
BioSISt versus positive control	0.723333 (0.084241–1.362426)	4.523834	$p = .0234$
Osseoquest versus positive control	0.48 (–0.234527–1.194527)	2.685063	$p = .2634$
BioSISt versus Osseoquest	0.413333 (–0.225759–1.052426)	2.585048	$p = .2931$
Osseoquest versus positive control	0.31 (–0.329092–0.949092)	1.938786	$p = .5324$
Positive control versus negative control	0.17 (–0.544527–0.884527)	0.95096	$p = .9061$

Critical value (Studentized range) = 3.996978; $|q^*| = 2.826352$.
Pooled SD = 0.391658.
SE = standard error.
CI = confidence interval.

TABLE 3 Qualitative Histological Assessment of the Cranial Bone and Implant Samples

Sample	Membrane	Tissue under Flange	Tissue in Threads	Tissue in Apical Region	Original Cortex/New Bone Interface	Inflammatory Cells	Bone Height Apex up to Flange Lat/Med
BioSISt 3 months	Vascularized collagen and few bone chips and macrophages	Trabecular bone and a few bone chips	Trabecular bone, good bone-implant contact, small areas soft tissue, BVs at interface	Large areas marrow cavity + resorption/ remodelling	Interface visible	Few macroP and PMNG at neck	First T/flange
BioSiSt 6 months	Membrane remnants under cover screw, macrophages	Maturing lamellar bone, Haversian S, inflammatory cells	New lamellar bone, maturing Haversian S, pockets of woven bone	Soft tissue capsule with macroP.	Barely visible and BV crossing it	MacroP at apex and under membrane, few plasma cells, PMNG at threads	First T/flange
BioSiSt 9 months	Thick collagen elements, BVs, macrophages	Mature bone, Haversian systems, no inflammation	Mature cortical bone with pockets remodelling at first threads, good bone-implant contact	Marrow spaces + trabecular bone	Not visible	Two macroP in trabeculae at implant apex	First T/flange
Osseoquest 3 months	Vascularized mesh, bone graft chips, macrophages, giant cells polymorphs ++ inflammation	Lamellar bone (med), fibrous tissue (lat), trabecular bone islands	Osteoid, trabecular bone, remodelling to lamellar bone; good bone-implant contact in places present; giant C macroP present	Marked inflammatory cell response, little bone-implant contact	Very visible	Many inflammatory cells at the membrane, flange and apex	Second T/flange
Osseoquest 6 months	Membrane (lat) side less vascular than at 3 months, more inflammation	Sheets fibrous CT, +++ macroP, giant Cs, PMNGs	Thin integrated cortical bone, small amount new lamellar bone (med), osteoclast activity (lat)	Little bone contact, inflammatory cells present	Not visible	More cells than at 3 months seen at membrane under flange and macroP at the threads	Fourth T/third T
Osseoquest 9 months	Membrane visible on the medial side of implant, +++ inflammatory cells	Connective tissue, inflammatory cells, BVs; thin cortical bone (med)	Thin mature cortical bone large marrow cavities; few macroP, giant C; good bone-implant contact; bone resorption (lat)	Large marrow cavities and blood vessels	Not visible	++++ cells >6 months at membrane and lateral side of implant	Fourth T/first T
Positive control 3 months	None Areas of BG above the flange	Maturing bone/osteoid (med) fibrous CT (lat)	Maturing bone osteoid rims Oblast/Oclast activity good bone-implant contact	Little bone-implant contact but cell-rich vascular marrow	Clearly shown	Few macroP and fibroblasts around BG	↓Third T/first T

TABLE 3 ***Continued***

Sample	Membrane	Tissue under Flange	Tissue in Threads	Tissue in Apical Region	Original Cortex/New Bone Interface	Inflammatory Cells	Bone Height Apex up to Flange Lat/Med
Positive control 6 months	Fibrous CT and BG remnants muscle, BVs	Fibrous CT, BG remnants, few macroP	Mature cortical bone no active bone remodelling or formation	Organized CT capsule	Visible	Few macrophages under flange, giant Cs into upper threads	Third T/third T
Positive control 9 months	None, fibrous CT	Above the mature bone, fibrous T, macroP	Mature cortical bone good contact	Bone fractured away on sample removal	Not visible	Very few macroP	Second T/first T
Negative control 6 months	None, fibrous CT, macroP	Fibrous CT, macroP, muscle suture	Dense cortical bone, no new bone growth; good bone-implant contact	Poor bone contact but large marrow cavities	No	Few macroP	↓Third T/↓third T
Negative control 9 months	None	Fibrous tissue	Dense cortical bone, no new bone growth	Poor bone contact but thick bone below	No	Few seen	↓Third T/↓third T

BG = bone graft; BV = blood vessel; CT = connective tissue; lat = lateral; macroP = macrophage; med = medial; Oblast = osteoblast; Oclast = osteoclast; OI = osseointegration; PMNG = polymorphonuclear granulocytes; T = implant thread.

of the BioSISt sample was less mature than in the Osseoquest sample, where corticalization was occurring. This Osseoquest sample did show a considerable inflammatory response. The positive control showed less new bone formation but greater bone maturation. In all the test specimens at 3 months, it was possible to see the cement line boundaries between original cortical bone and the new bone.

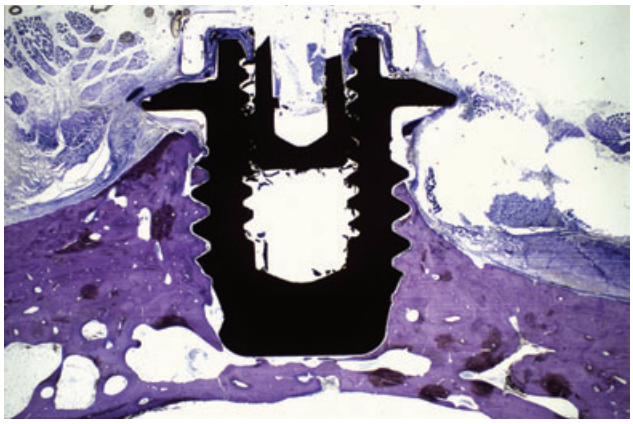

Figure 5 Nine-month BioSISt and bone graft sample. A histologic central overview section showing mature bone with Haversian systems and pockets of remodelling lamellar bone. The implant base is within a marrow cavity. The thick collagen membrane has been incorporated at the bone surface (staining, 1% toluidine blue and 1% pyronin G; magnification, distance of 0.6 mm between the thread peaks).

Figure 6 Nine-month Osseoquest and bone graft sample. A histologic central sectional overview showing thin mature bone present to the first thread medially, with remnants of the membrane. Laterally, some bone resorption has taken place and a thin capsular layer and inflammation are present (staining, 1% toluidine blue and pyronin G; magnification, distance of 0.6 mm between the thread peaks).

At 6 months, the BioSISt sample showed the greatest quantity of new bone generation. The bone was maturing and few cells were present. Vascular channels passed between the old cortical bone and the new remodelling augmented bone. In comparison, the Osseoquest samples showed greater cellular activity and inflammatory response particularly near the membrane. There was a small amount of thin new cortical bone growing laterally and some resorption medially. The positive and negative control samples showed little new bone formation and even resorption in some areas.

At 9 months, the BioSISt sample (Figure 5) showed the greatest amount of mature bone with pockets of remodelling and few cells. The Osseoquest sample (Figure 6) showed additional new thin cortical bone formation with large marrow cavities, lateral bone resorption, and inflammation. Inflammation was seen near the Osseoquest membrane.

The positive control showed some new mature bone formation especially medially, with little cellular activity. The negative control showed little new bone or remodelling at any stage.

The Comparative Histology of the Membranes

The membranes were examined on histological stained sections in the light microscope and with the aid of polarizing filters.

At 3 months, the BioSISt membrane was still present and was being vascularized showing many fibroblasts and sparse macrophages. By 6 and 9 months, some membrane remnants were under the cover screw, with few cells, and no inflammation was present. The BioSISt membrane had become incorporated into the tissues.

At 3 months, the Osseoquest membrane was a mesh of vascularized fibers supporting a few macrophages, giant cells, and fibrous tissue. At this time, bone graft was still present. At 6 months, the membrane was still visible with the presence of less vascularity than before, but with more inflammatory cells. At 9 months, the Osseoquest sample still showed membrane remnants with inflammatory cells and macrophages.

From histological examination and measurement of bone/implant contact and bone area in the threads, it was shown that bone/tissue integration was occurring in all samples at varying rates, and increased with the duration of implantation.[20]

Extraskeletal bone augmentation had occurred with both membrane types. These samples showed degrees of bone augmentation that varied consistently in quantity and quality with the membrane used. Considering the membranes, the quality (maturity) and quantity of bone produced improved as the time duration from implantation increased. The trabecular bone that formed in early samples remodelled later, and the division between original cortical bone and new trabecular bone disappeared later. At 3 months, the BioSISt sample was trabecular when compared to the 3-month Osseoquest sample.

However, at 6 and 9 months, the BioSISt samples showed superior quality and quantity of bone, and while Osseoquest samples showed progressive increase in bone formation, it was thin cortical bone and was associated with a significant degree of long-term inflammatory response and lateral bone resorption.

The negative controls showed no bone augmentation over time. This would suggest that disturbance of the periosteum alone did not promote marked osteoneogenesis in these young dogs. Bone augmentation in the positive control did increase in the 3- and 9-month samples, but the quantity produced was small.

DISCUSSION

Although the numbers in each group were small in this study, the biological variability between individuals was reduced by the fact that the animals were full siblings. The value of the study was thus enhanced by the genetic closeness of the dogs.

In eight of the 11 samples, more bone was formed on the medial side of each implant. Auricular muscle action or the natural curvature of the cranium may have caused uneven implant loading laterally. In one human patient, abnormal BAHA implant loading was reported to cause a unilateral reduction in bone augmentation.[22] Alternatively, muscle movement may have caused micromovement at the interface with the barrier membrane and may have reduced bone formation.[8]

In all samples, the greatest amount of augmented bone formed was found to be in contact with the titanium implant surface. This has also been found in other studies.[8] No marked increase in bone thickness developed ventral to the implants. One study[23] reported that in a human BAHA patient, where a 4-mm implant perforated the endosteum, compact bone had grown ventral to the fixture a year after placement. This was not

seen in the present canine 9-month study. Minor dural adhesions were encountered at the site of implant placement in seven specimens, even though only one implant had perforated the endosteal surface of the parietal bone.

In the present study, early trabecular bone formation remodelled to cortical bone. While augmented bone in canine mandibular defects matured and remodelled to cortical and cancellous bone,[24] extraskeletal bone generation in the rabbit calvarium was found to still be trabecular after 3 months.[25] One study in the rat found that while this extra bone might increase in thickness by appositional growth over time, it was unlikely to remodel to cortical bone.[26] However, a later study in rats found that cortical extraskeletal mandibular bone augmented under polytetrafluoroethylene domes over a year did remain stable with minimal resorption occurring.[27] In the present study, extraskeletal cranial bone did remodel to cortical bone, and the early demarcation between the original cortical bone and generated bone was lost. The 3-month test samples showed local resorption canals between the cortical plate and the trabecular bone, as seen in other studies.[9,28] Vascularization and remodelling at the interface of the cortical plate and generated bone resulted in bone morphology similar to the cortical bone.

A study in guided tissue generation in the temporal bone in children has shown the presence of mature bone under the membrane, 4 months after implant placement.[10] The duration of augmentation and functional stimuli on the bone may influence the bone maturation.

In the present study, implants were not loaded with hearing aids, and it is possible that doing so may have altered the pattern of bone deposition in accordance with Wolfs law.[29] Conversely, resorption of bone after implant loading has also been recorded.[30]

Also, while the cortical bone surface was disturbed by the removal of the periosteum, no decortication or perforation of the cortical plate was performed. Work in the rat maintained that these procedures promoted bone formation,[31] while other studies found that decortication[8] or cortical perforations[9] did not enhance bone formation. It is also of interest to note that the removal of periosteum alone in the negative control subjects did not stimulate new bone formation in young dogs at 8 months of age.

Considering the properties of the two biocompatible membranes tested, the passage of nutrients and growth factors may have occurred through either membrane, and their roughened porous outer layers facilitated tissue integration, giving a high degree of direct bone contact.[8]

The ability of the barrier materials to maintain space through membrane stiffness was assisted by the presence of blood clot and bone graft[8,32,33] under the membranes in all samples except the controls. Peripheral membrane sealing was good, because it was sutured to the edge of the removed periosteum. In all cases a circular portion of the periosteum was removed to prevent a local osteogenic effect.

PGA/PLA membranes like Osseoquest are resorbed by hydrolysis[5] rather than by cell-mediated inflammation, but in this study, this membrane consistently appeared to cause more cellular reaction than the collagen BioSISt. Local foreign body reactions to poly DL lactide polymers, and their breakdown products have been reported to stimulate inflammatory cells and fibrosis, and to interfere with canine periodontal alveolar bone formation.[34] An inflammatory reaction to PGA was not found in one 3-month study of bone augmentation around mandibular deficits in the dog comparing a PGA and a collagen membrane.[35] Differences in mechanical, biological properties and surface structure of membranes and bone graft, and varying bone maturation rates under different membranes may account for the findings.[36]

Fibers from the Osseoquest membrane were still present in the tissues after 9 months, but the membranes mechanical integrity at this time is not known.

The BioSISt collagen membrane was incorporated by the tissues over time,[13] and this was complete by 9 months post placement. The membrane was found to produce little inflammation and is reported to resist bacterial challenge.[13] The haemostatic and chemotactic properties of collagen membranes,[37] and in the case of BioSISt, the naturally occurring growth factors Transforming Growth Factor-β, Fibroblast Growth Factor-2, Vascular Endothelial Growth Factor, glycosaminoglycans, proteoglycans, and fibronectin may have promoted bone formation in this study,[16] especially when used together with cancellous bone graft.

CONCLUSIONS

In this study, it was possible to generate bone in the extraskeletal envelope around osseointegrated BAHA implants using either a PGA/PLA or a collagen test membrane.

In the number of specimens tested, the use of a collagen small intestinal submucosal membrane BioSiSt compared favorably in the consistent quality and quantity of bone produced around titanium implants, and in its biocompatibility when compared with the synthetic PGA/PLA membrane Osseoquest. Further investigations of the use of the collagen BioSISt or SIS membrane for bone augmentation are warranted.

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