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Impact of surface contamination of implants with saliva during placement in augmented bone defects in sheep calvaria

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Abstract

Our aim was to try and find out whether contamination with saliva during insertion of dental implants affects osseointegration in bone that has been augmented with different grafts. Six bony defects were created in each of the calvaria of six sheep, and then augmented with three different materials (autogenous bone, bovine bone, and resorbable biphasic ceramic bone substitute) After five weeks of healing, three implants contaminated with saliva (contaminated group) and three not contaminated (uncontaminated group) were placed in the centre of the augmented areas. For histomorphometric analysis, bone implant contact, bone area fraction occupancy, bone and material area, and bony area were measured after a healing period of five weeks. There was a significant difference between the contaminated and uncontaminated groups ($p = 0.036$) for bone implant contact only in the augmented areas, but there were no significant differences in bone area fraction occupancy, bone and material area, and bony area. We conclude that contamination with saliva during placement of dental implants can significantly compromise bone implant contact in augmented areas, but had no significant effect on the formation of bone in areas more distant from the surface of the implant. We suggest that salivary contamination should be avoided during placement of dental implants in augmented areas.

Keywords: dental implants; osseointegration; surgery; contamination; saliva; bone augmentation

Introduction

Dental implants have become a reliable way to treat full and partial edentulism. Although the prognosis has been reported to be good,[1,2] recent publications have suggested that the degree of success may depend on the skill of the surgeon who places the implants.[3] However, the reasons for failure have been discussed in numerous reports and it has been suggested that multiple risk factors could be involved.[4] Albrektsson et al for example,[5] stated that surgical factors are important in regulating the fate of the implant.

When the concept of osseointegration was introduced,[6] it was proposed that an important factor was to operate under rigorously sterile conditions. The oral cavity contains more than 700 bacterial species,[7] and the implant may be contaminated during placement by contact with saliva, though contamination of endosteal metallic implants is not a phenomenon specific to dental implants. In orthopaedics there have been numerous studies about the failure of implants as a result of perioperative contamination. In particular, it has been proposed that early failure, estimated to 1% – 2%

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of all failed implants,[8,9] is strongly correlated with bacterial contamination,[10–12] despite the fact that rigorous efforts are made during such operations to create a sterile operating environment. For dental implants, early failure rates seem to be slightly higher than those of orthopaedic implants, but there is not sufficient evidence about whether operative contamination influences the success of the implant.[13]

For optimal results dental implants should be inserted into a sufficient amount of bone,[14] so when the volume is inadequate, the bone must be augmented before the implant is placed, and autogenous bone or different types of commercially-available grafting materials can be used.[15] Autogenous bone is the gold standard because it is osteogenic, osteoinductive, and osteoconductive.[16] As far as we know, there has been no study that has evaluated whether salivary contamination during insertion affects the osseointegration in augmented bone, or whether there are differences among sites augmented with different types of bone.

Our aim therefore was to try and find out whether salivary contamination during placement of implants affects osseointegration in bone that has been augmented with different type of grafts.

Material and methods

Design of the study

This animal study was approved by the ethics committee of the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France) (reference number: B940462) and is reported according to the ARRIVE guidelines for relevant items.[17] Six female Finnish Dorset crossbred sheep (age about 5 years and 4 months, weight about 63.1 kg) were housed together for one week preoperatively. All operations were done under general anaesthesia with ketamine (Imalgene 1000®, Merial) and diazepam (Valium, Roche) and with 2.5% isoflurane (Forane®/Forene®, Drägerverk AG) as inhalation anaesthesia. Morphine (0.1–0.5 mg/kg) was given intravenously every second hour during operation and subcutaneously for the three first postoperative days.

The study was designed as a controlled, experimental study. Three different bone augmentation materials were used: (A) autogenous bone, (B) bovine bone (Direct Oss™ anorganic bovine cancellous bone 1000–2000 μm, Implant Direct, Calabasas Hills) mixed with autogenous bone (50:50 in volume), and (C) a resorbable biphasic ceramic bone substitute consisting of 60% calcium sulphate and 40% calcium phosphate (hydroxyapatite) (CERAMENT™, bone void filler, Bonesupport AB).

Operative technique

The parietal region was shaved and iodine solution applied for disinfection. After a midline incision down to the pericranium, the calvarial bone was exposed with a periosteal elevator. Six bone defects about 8 mm in diameter were created in each animal with a round bur. Each defect was hemispherical in shape and about 4 mm deep, and was drilled at 1500 rpm under abundant irrigation with sterile saline to avoid stacking of bone and overheating. Autogenous bone for groups A and B was harvested from the calvaria with a cortical bone collector (Safescraper® Twist curve, Geistlich). Two defects were randomly filled with the three types of augmentation material, and the flap was closed in two layers using a resorbable suture (Vicryl™, Ethicon) for the subcutaneous layer and a non-resorbable suture (Ethilon®, Ethicon) for the cutaneous layer.

Non-absorbable sutures were removed after seven days. Meloxicam (Metacam®, Boehringer Ingelheim Vetmedica) 0.4 mg/kg was given postoperatively for three days for pain relief. Benzylpenicillin 114 mg, dihydrostreptomycin 164 mg and procaine 13 mg (Peni Dhs Coophavet, M.C. Santé Animale) was given postoperatively for five days.

Five weeks after the first operation (Fig. 1), the calvarial bone was exposed again. Six dental implants (Brånemark system® Mk III RP implant with turned surface, Nobel Biocare AG) 3.75 mm in diameter and 7 mm long were used for each sheep. The sites of the implants were prepared at the centre of each augmented area and the implants were inserted according to the manufacturer's instructions. One defect in each augmentation group had one implant inserted that had previously been impregnated for 15 seconds with fresh human saliva from one donor with periodontitis (contaminated group), and the other defect had one non-contaminated implant. Management of wounds and postoperative treatment were as described for the first operation.

Preparation of samples

After a further healing period of five weeks, all animals were killed with an intravenous injection of a combination of embutramide 4000 mg, mebezonium 538.4 mg and tetracaine 87.8 mg (T61, Intervet International, Unterschleißheim). The blocks of calvarial bone that contained the implants were retrieved (Fig. 1).

Histological preparation and histomorphometric analysis

The samples of bone were fixed in 4% formalin for three days before being dehydrated in ascending concentrations of ethanol and embedded in light-curing methylmethacrylate (Technoivit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany) for undecalcified ground section procedures. The embedded samples were cut along the longitudinal implant axis of the implant using a diamond saw cutting machine (EXAKT 300, EXAKT Advanced Technologies GmbH, Norderstedt), to generate one central section, and ground down to a final section thickness of 40 μm (EXAKT 400CS,

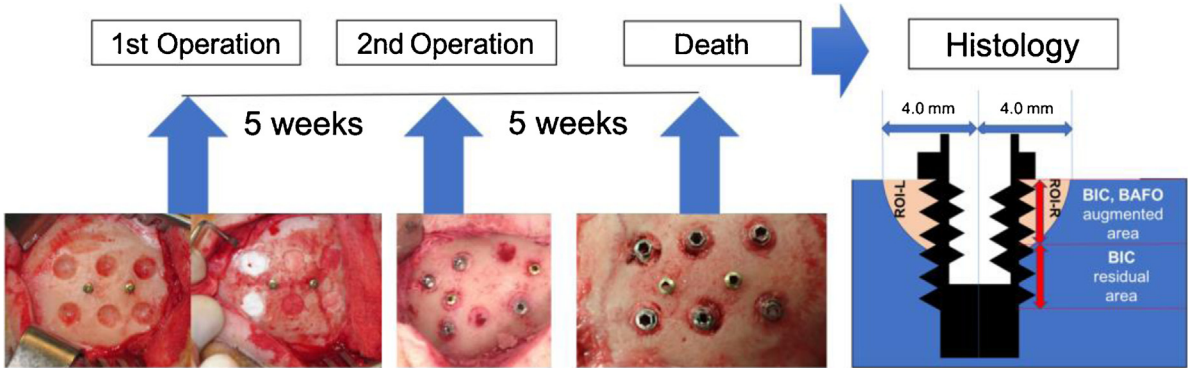

Fig. 1. Flow design of study, and diagram of the regions of interest (ROI) for histomorphometric analysis for calculation of bone to implant contact (BIC), bone area fraction occupancy (BAFO), bone and material area (BMA), and bony area (BA).

EXAKT Advanced Technologies GmbH). After staining with toluidine blue and pyronin G, all sections (n = 36) were photographed using light microscopy with a digital imaging system (BZ-9000, Keyence).

A histomorphometric analysis was made using two regions of interest (ROI) on each side of the implant, ROI (left) and ROI (right). Each ROI corresponded to the augmented area excluding the implant area. The data were analysed with a software for image analysis (Image J v.1.43u, National Institutes of Health) using the following variables (Fig. 1).

Bone implant contact (%)
Augmented area: The mean percentage of total bone implant contact in the left and right ROI.
Residual area: The mean percentage of total bone implant contact in residual bone (left and right side of lower part of ROI, same height as each ROI)

Bone area fraction occupancy (%)
Percentage of the area inside the implant threads in augmented area occupied by bone.

Bone and material area (%)
Measured within the augmented area.

Bony area (%)
Measured within the augmented area.

Statistical analysis

We used descriptive analysis for bone implant contact augmented area, bone implant contact residual bone, bone and material area, and bony area. Normal distribution and equal variances were not assumed because of the small numbers of samples (n = 6). The Friedman test and the Wilcoxon signed-rank test were used, and probabilities of less than 0.05 were accepted as significant. The data were analysed using two-way ANOVA with repeated measurements on two factors about groups A–C (group A = autogenous bone; group B = bovine bone; and group C = resorbable biphasic ceramic bone substitute) and subgroups of contamination (contaminated or not). We used IBM SPSS Statistics for Mac (version 23.0 IBM Corp).

Results

There were no complications, infections, or other clinical concerns during the operations.

Histological observations

All implants osseointegrated successfully in various amounts of new bone and residual bone (Fig. 2A–C).

Histomorphometric analysis

Bone implant contact (%)
The mean values in the augmented area and residual area of the contaminated and uncontaminated subgroups are shown in Table 1. The bone implant contact in the augmented area of Groups A and B did not differ significantly between contaminated and uncontaminated implants, but in Group C the bone implant contact of the augmented area of contaminated implants was significantly lower than that in the augmented area of uncontaminated implants ($p = 0.046$). In the residual area, there were no differences between the contaminated and uncontaminated subgroups for Groups A, B, and C.

Bone area fraction occupancy (%)
The mean values in the ROI are shown in Table 1, and there were no significant differences between the contaminated and uncontaminated subgroups.

Bone and material area (%)
The mean values are shown in Table 2, and there were no significant differences among the groups ($p = 0.836$).

Fig. 2. Histological specimens (toluidine blue and pyronin G stain, original magnification x 20).

Table 1
Mean value of bone implant contact (%) in the augmented and residual areas and bone area fraction occupancy (%) in the augmented areas of the experimental groups.

Group	Bone implant contact (%)						Bone area fraction occupancy (%)		
	Augmented area			Residual area			Augmented area		
	No contamination	Contamination	p value	No contamination	Contamination	p value	No contamination	Contamination	p value
A	32.15	15.66	0.249	48.08	37.20	0.60	51.89	38.54	0.60
B	36.88	20.72	0.173	45.41	53.64	0.463	52.33	42.62	0.463
C	13.58	5.45	0.046	50.05	28.06	0.173	30.71	16.29	0.173
Overall p value*			0.036			0.429			0.196

Group A = autogenous bone; group B = bovine bone; and group C = resorbable biphasic ceramic bone substitute.
* No contamination compared with contamination.

Table 2
Mean values of bone and material area (%) and bony area (%) in regions of interest of all experimental groups.

Group	Bone and material area (%)			Bony area (%)		
	No contamination	Contamination	p value	No contamination	Contamination	p value
A	46.57	46.00	–	46.57	46.00	0.917
B	49.40	55.19	–	46.43	50.19	0.600
C	49.11	41.46	–	21.52	20.60	0.753
Overall p value*			0.847			0.855

Group A = autogenous bone; group B = bovine bone; and group C = resorbably biphasic ceramic bone substitute.
* No contamination compared with contamination.

Bony area (%)
The mean values are shown in Table 2, and again there were no significant differences among the groups.

Discussion

We have tried to find out (using a calvarial defect model in sheep) whether bacterial contamination during insertion of an implant influences its osseointegration in areas augmented with different type of grafts. This model seems to be suitable for this purpose, as it allows submerged installation of implants in defects of a clinical size in cortical bone with poor vascularisation, and without other interfering factors (such as loading).

The results of histomorphometric analysis suggested that contamination by saliva during insertion of an implant may affect bone implant contact in augmented areas, but this was significant only in group C. The overall test, including groups A, B, and C, showed that there was a significant difference between the contaminated and uncontaminated subgroups. Interestingly, there were no differences in bone implant contact between them and in the area where the implant was fixed into the non-augmented bone. In the rabbit study by Yuan et al[18] four types of implant surface with and without contamination were tested. Bacterial contamination (*Prevotella intermedia*) had an adverse effect on the bone implant contact of the surface of every implant including turned, titanium-anodic-oxidised, sandblasted, acid-etched, and sprayed with hydroxyapatite plasma. A more profound significant reduction was noted for rougher surfaces after contamination when compared with uncontaminated groups. Oosterbos et al[19] showed that contamination with *Staphylococcus aureus* had an adverse influence on bone implant contact on rough surface implants including grit-blasted surfaces and surfaces coated with hydroxyapatite. In the group of rougher surfaces with hydroxyapatite-coated implants, the infection was more severe. In the current study, we used implants with turned surfaces. The use of these could be why there was no significant difference in bone implant contact in the mother bone between the contaminated and uncontaminated subgroups, which confirms the results of Ivanoff et al who placed machined-surface implants into the tibias of rabbits with or without contamination in the surrounding connective tissue beforehand.[20]

Even though turned surface implants were used, bone implant contact in the augmented areas nevertheless showed lower values in the contaminated group, suggesting that the bone tissue in augmented areas has poor resistance to contamination of the surface of the implant compared to that in non-augmented bony areas. Bone implant contact in the augmented areas of group C was less than in groups A and B among both contaminated and uncontaminated subgroups, which could be explained by the lower osteogenic ability of the ceramic bone substitute, as also shown by the lower percentage of bony area within ROI in this group.

There were no significant differences between the contaminated and uncontaminated subgroups in bone area fraction occupancy, which was also found by Bonsignore et al[21] who found that surface contamination inhibits bone implant contact without affecting peri-implant bone. There were also no

significant differences between contaminated and uncontaminated subgroups in the bone and material, and bony, areas.

We suggest, therefore, that both surface contamination and surface roughness primarily affect bone formation only on the implant surface and have less effect on more distant bone formation.

Conclusion

Contamination with saliva during insertion of implants may have an adverse effect on osseointegration in augmented areas, and clinicians should avoid it when implants are being placed in augmented areas. We propose that the effect of the contamination might be limited to bony formation on the surface of the implant, but it has less effect on the formation of bone in areas more distant from the surface of the implant. Further studies are needed to validate these results.

Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

This animal study was approved by the ethics committee of the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France) (reference number: B940462). No permission required: animal study.

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