Impact of salivary contamination during placement of implants with simultaneous bony augmentation in iliac bone in sheep

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Abstract

Our aim was to investigate the possible impact of contamination with saliva on osseointegration during placement of implants with simultaneous bone augmentation. Six hemispheric shape bone defects (8 mm in diameter × 4 mm deep) were prepared in each iliac bone of six sheep. A dental implant (2.9 mm in diameter × 10 mm long) was placed in the centre of each defect, and then pairs of defects were filled with one of the following bone augmentation materials: autogenous bone, autogenous bone plus bovine bone, or resorbable biphasic ceramic bone substitute. One site in each augmentation group was impregnated with saliva (contaminated group), while the other was not (non-contaminated group). Bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO) within implant threads were measured after a healing period of five weeks, both in respect of the implant inserted in the augmented bone and in that inserted in the residual bone. Overall results showed that there was a significant difference between the contaminated and non-contaminated group for BIC in the augmented implant (p = 0.028), while there were no significant differences in the implant in residual bone (p = 0.722). For BAFO, there were no significant differences between the contaminated and non-contaminated groups among the different augmentation materials. The results showed that contamination with saliva during placement of an implant with simultaneous bone augmentation had a serious deleterious effect on osseointegration of the aspect of the implant within the augmented defect. Contamination with saliva during placement of an implant with simultaneous bone augmentation should therefore be avoided.

Introduction

According to the original protocol for implantation of a dental implant,[1] an optimal surgical technique should include aseptic procedures to minimise the risk of infection and failure of the implant.[2] In certain clinical conditions, however, contamination of the implant during operation may be inevitable as a result of contact with saliva, which is known

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to be a protein-rich fluid that contains more than 700 bacterial species.[3] Studies on the failures of orthopaedic implants and biomaterial-associated infections have also reported that early failures are strongly correlated with bacterial contamination.[4,5] However, according to a recent systematic review, there is insufficient scientific evidence about the possible impact of bacterial contamination of dental implants during the procedure.[6]

To achieve predictable results in terms of aesthetics and function, dental implants should be surrounded by a sufficient amount of bone.[7] When there is inadequate bone for proper placement of an implant bony augmentation is recommended either before, or at the same time as, placement of the implant. In a recent in vivo study, implants contaminated with saliva during implantation in previously augmented areas showed reduced bone-to-implant contact (BIC) compared with non-contaminated implants.[8] However, we know of no information on the possible effect of contamination with saliva on osseointegration during placement of an implant with simultaneous bony augmentation.

The aim of this study was therefore to investigate the possible impact on osseointegration of contamination with saliva during placement of an implant with simultaneous bony augmentation.

Material and methods

Study design and surgical techniques

The animal study took place at the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France). The in vivo experimental design was approved by the local ethics committee (reference number: B940462) and is reported according to ARRIVE guidelines.[9] Six adult Finnish Dorset cross-bred sheep (mean (SD) age 64 (2) months) were used. The procedures were done under general anaesthesia. Ketamine (4–6 mg/kg) and diazepam (0.5–1 mg/kg) were injected into a jugular vein and 2.5% isoflurane was used for inhalation anaesthesia. Every second hour during the operation intravenous injections of morphine (0.1–0.5 mg/kg) were given and the same drug was also injected subcutaneously for three days postoperatively.

Preoperatively the iliac region was shaved and disinfected with iodine solution. After an incision into the skin, the muscular plane was dissected, and the iliac bone exposed with a periosteal elevator. In each animal, six bony defects 8 mm in diameter × 4mm deep were created with a round burr (Fig. 1). A dental implant 2.9 mm in diameter × 10 mm long and a dual acid-etched surface (Unitite® Slim implants, SIN) was placed in the centre of each defect using drills rotating at 1500 rpm under abundant sterile saline irrigation. The remaining space in each of two defects was filled with one of the three following augmentation materials: first, autogenous bone harvested from the iliac crest with a bone collector (Safescraper® TWIST curve, C.G.M. S.p.A. Divisione Med-

icale Meta); secondly, autogenous bone mixed with an equal volume of deproteinised bovine bone (Direct Oss^{TM}, Implant Direct); or thirdly, a resorbable biphasic ceramic bone substitute consisting of 40% calcium phosphate (hydroxyapatite) and 60% calcium sulphate (Cerament^{TM}, Bonesupport). In half the sites – that is, one from each augmentation material - implants and augmentation material were impregnated for 15 seconds with fresh human saliva from one donor with periodontitis (contaminated group) before placement. The other half of the implants and augmentation material was handled under aseptic conditions (non-contaminated group).

The wound was then closed in two layers using a resorbable suture (Vicryl®, Ethicon) for the muscular layer and a non-resorbable suture (Ethilon®, Ethicon) for the skin.

After seven days of wound healing, the non-resorbable sutures were removed. For five days postoperatively, benzylpenicillin 114 mg, dihydrostreptomycin 164 mg, and procaine 13 mg (Peni Dhs Coophavet, M.C. Santé Animale,) were given. Meloxicam 0.4 mg/kg (Metacam®, Boehringer Ingelheim Vetmedica,), was given for the first three postoperative days.

Collection of samples, histological preparation, and histomorphometric analysis

The animals were killed five weeks postoperatively with an intravenous overdose of embutramide 4000 mg, mebezonium 538.4 mg, and tetracaine 87.8 mg (Tanax® T61, Intervet International), and the iliac bone blocks containing the implants were retrieved.

After fixation in 4% formalin for three days, the samples of bone were progressively dehydrated in a graded series of ethanol and embedded in light curing glycolmethacrylate (Technovit 7200 VLC) for non-decalcified ground sectioning. The embedded specimens were sectioned parallel to the long axis at the centre of the implants using a diamond saw cutting machine (EXAKT 300, EXAKT Advanced Technologies GmbH), and ground and polished until a final section thickness of 40 μm was achieved (EXAKT 400CS). The non-decalcified sections were stained with toluidine blue and pyronin G, and photographed using light microscopy with a digital imaging system (BZ-9000, KEYENCE).

We used quantitative histomorphometry for the following variables using software for image analysis (Image J v.1.43u, National Institute of Health) (Fig. 1).

First, bone-to-implant contact (BIC): on each side of the implant (excluding the bottom), the percentage of its outline that presented bone in direct contact with its surface was measured. The mean value of the two measurements was used for each implant. BIC was calculated for the augmented area and for the residual bone area. The calculated length for the residual area was the same as that for the augmented area in each side of implants.

Secondly, bone area fraction occupancy (BAFO): In each side of the implant in the augmented defect, the percentage of the area within the implant threads occupied by visibly
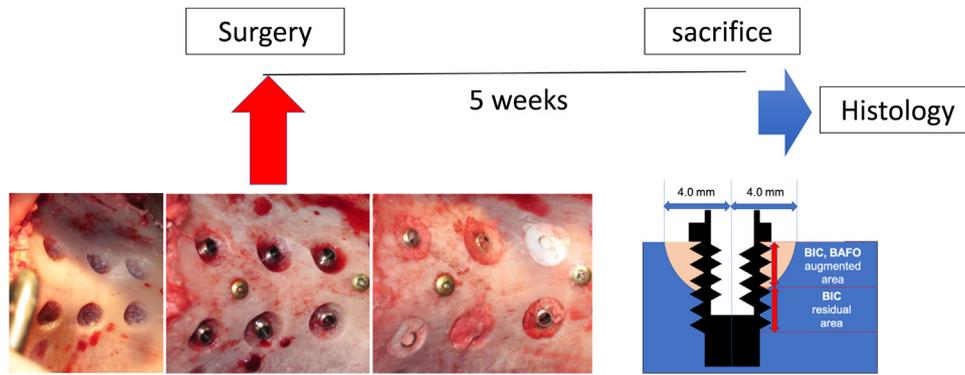
Fig. 1. Flow design of study time line, and scheme of the histomorphometric analysis for calculation of bone-to-implant contact (BIC), bone-area fraction occupancy (BAFO).
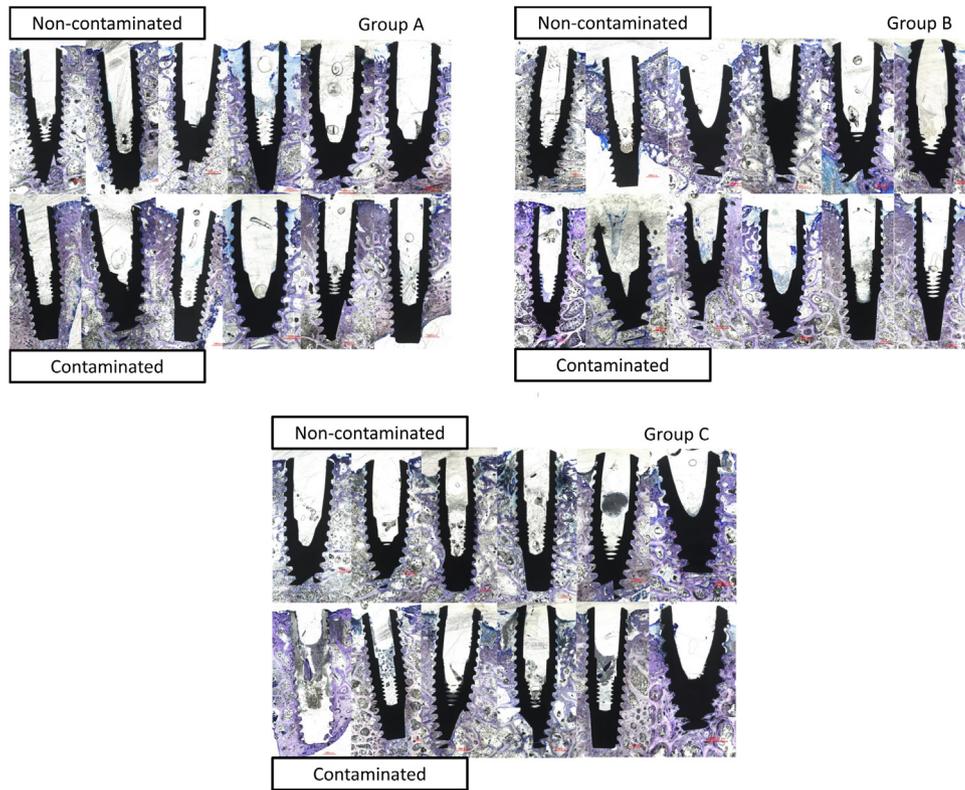

Fig. 2. Histological specimens stained with toluidine blue and pyronin G (original magnification × 20).

distinguishable bone was calculated. The mean value of the two measurements was used for each implant.

Statistical analysis

We made descriptive analyses of the BIC in the augmented and in the residual bone, and BAFO. Normal distribution and equal variance were not assumed with respect to the small numbers of samples (n = 6). The significance of differences was calculated by the Friedman two-way analysis of variance, and a probability of <0.05 was accepted as significant. We used the software IBM SPSS for Mac (version 25.0, IBM Corp).

Results

One sheep showed signs of infection in the skin of iliac region during the early stage of healing, but this did not necessitate the exclusion of the animal from the study.

Histological observation

No inflammatory reaction or total failure of osseointegration was found in any of the samples. In both groups A and B, newly-formed bone was found to be in direct contact with all the surfaces of the implant in the augmented area. However

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

| Group | Bone implant contact (%) | | | | | | Bone area fraction occupancy (%) | | |
| | Augmented area | | | Residual area | | | Augmented area | | |
	Not contaminated	Contaminated	p value	Not contaminated	Contaminated	p value	Not contaminated	Contaminated	p value
A	10.72 (5.60)	5.90 (6.07)	0.280	36.98 (13.05)	31.45 (14.74)	0.463	38.92 (24.85)	43.11 (23.51)	1.00
B	11.51 (4.64)	7.36 (8.19)	0.045*	37.49 (12.73)	32.47 (14.05)	0.345	46.66 (12.77)	25.81 (17.37)	0.06
C	4.44 (4.47)	2.51 (2.57)	0.758	28.01 (18.69)	25.97 (19.59)	0.463	22.40 (19.32)	12.11 (14.10)	0.53
Overall p value			0.028*			0.722			0.007*

A = autogenous bone; B = bovine bone; and C = resorbable biphasic ceramic bone substitute.
  * $p < 0.05$.

in Group C, it was in contact only in the lower part of the augmented area (Fig. 2).

Histomorphometric analysis

Bone-to-implant contact (%): the mean values in the augmented and residual areas of contaminated and non-contaminated subgroups are shown in Table 1. Statistical analysis showed a significant difference between contaminated and non-contaminated implants only in the augmented area ($p = 0.028$); there was no significant difference in the residual area ($p = 0.722$).

In particular, the BIC was significantly lower in the contaminated compared with the non-contaminated ones, in Group B ($p = 0.045$). A lower BIC was also found in the contaminated implants compared with the non-contaminated ones in the other groups, but the differences were not significant.

BAFO (%): the mean values are shown in Table 1. There was a significant difference between contaminated and non-contaminated implants overall ($p = 0.007$). However, we found no statistical differences between contaminated and non-contaminated sites within Groups A, B, and C.

Discussion

We have investigated whether salivary contamination during bony augmentation with simultaneous placement of implants influences osseointegration. The in vivo model used in this study resembled the low-density bone of human jaw, and it allowed submerged healing without interfering factors.[10]

Although the level of evidence on this topic is scarce,[6] previous in vivo studies have indicated that contaminated implants might have a deleterious effect on the osseointegration of implants. Yuan et al[11] placed implants with four different surfaces into rabbit tibia. Bacterial contamination with *Prevotella intermedia* caused less BIC in all types of implants, including turned, titanium anodic-oxidised, sandblasted/acid-etched, and hydroxide-apatite plasma sprayed. However, bacterial contamination generated a greater effect when a rougher surface was used. Another study by Oosterbos et al,[12] using rabbit tibia, showed that the

contamination with *Staphylococcus aureus* had a detrimental effect on the healing process of implants with rough surfaces, which caused severe peri-implant infection and bony remodelling. The physical and chemical properties of the implant surface may play a part in the bacterial adhesion (being more retentive for rough surfaces) such as in the case of the present study.[13] It is established that adhesion of proteins and microorganisms to the surface of an implant with the formation of a contaminated biofilm is the preferable environment for biomaterial-associated infection,[14] and the exposure of an implant to a protein-rich fluid, such as saliva, creates a film at the surface, which could be easily colonised by bacteria.[3]

The incidence of such conditions, which lead to failure of the implant, may be prevented by the establishment of a balanced condition between inflammatory and immune response.[15] This equilibrium may be modulated by other factors, such as design and material of the implant, the state of the host bed, or surgical treatment.[16] The introduction of additional materials such as bone substitutes (which act as foreign bodies) could further worsen the prognosis of the integration of the biomaterials.[15] Augmentation procedures using heterologous materials at the same time as placement of an implant are known to increase the risk of early and late failure.[17,18]

The present study showed that the augmented area had a lower BIC than the residual bone, and the BIC in the augmented area was also significantly reduced by contamination, while it did not affect the BIC in the residual areas. Those findings are in accordance with our previous work,[8] in which we investigated the effect of contamination when turned implants were installed in augmented defects in a delayed fashion.

The residual and augmented areas might have reacted differently to contamination in response to the different immunological reaction.[19] Contamination of the implant in the residual bone may have been effectively addressed by a macrophage-mediated response recalled from the pristine vascular system. In contrast, the augmented area, which presents a poorly organised immunological response as a result of the immature vascularised structure, might have had a reduced resistance to contamination.

However, the present findings showed that there were no significant differences in BAFO between the uncontaminated

and contaminated groups, a result that was similar to those of Bonsignore et al.[20]

Another interest finding of the study was that the augmentation Groups A and B did significantly better than the augmentation Group C, both when contaminated and when not contaminated. This is not surprising, considering that Groups A and B contained both autogenous bone, which in the case of Group B was mixed with deproteinised bovine bone. Autogenous bone is still considered the gold standard for bony augmentation,[21] because it possess osteogenic, osteoinductive and osteoconductive properties, thanks to the pool of viable cells and growth factors that are transferred together with the graft from the donor to the recipient site. However, the procedure has the drawback of donor site morbidity, with the creation of a second trauma in the host. It is therefore of great importance to investigate the possibilities with new augmentation materials, as we have in the current study.

To conclude, our results have showed that salivary contamination during placement of implants with simultaneous bony augmentation materials inhibited BIC without affecting the peri-implant bone formation further away from the implant surfaces. It is therefore proposed that contamination of the implant surface mainly affects bone formation in direct contact with the implant, and not to the same extent at a distance from the implant.

One limitation of this study was that this was done extraorally. One has to be aware that bacterial infection of an implant is quite uncommon in the oral cavity. Previous research workers have reported how the immune system might have developed a sort of tolerance to the constant presence of normal bacterial flora.[22] Another limitation of the study was that the immunological and inflammatory responses were not assessed. Further studies are needed to investigate whether additional external factors, such as different surgical and augmentation techniques, might be detrimental to bone-to-implant formation.

Ethics statement/confirmation of patients' permission

The animal study took place at the École Nationale Vétérinaire d'Alfort (France). The in vivo experimental design was approved by the local ethics committee (B940462). No patients were involved.

Conflict of interest

We have no conflicts of interest.

Acknowledgement

References

1. Albrektsson T, Branemark PI, Hansson HA, et al. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand* 1981;**52**:155–70.
2. Buser D, von Arx T, ten Bruggenkate C, et al. Basic surgical principles with ITI implants. *Clin Oral Implants Res* 2000;**11**(Suppl 1):59–68.
3. Lima EM, Koo H, Vacca Smith AM, et al. Adsorption of salivary and serum proteins, and bacterial adherence on titanium and zirconia ceramic surfaces. *Clin Oral Implants Res* 2008;**19**:780–5.
4. Knobben BA, Engelsma Y, Neut D, et al. Intraoperative contamination influences wound discharge and periprosthetic infection. *Clin Orthop Relat Res* 2006;**452**:236–41.
5. Knobben BA, van Horn JR, van der Mei HC, et al. Evaluation of measures to decrease intra-operative bacterial contamination in orthopaedic implant surgery. *J Hosp Infect* 2006;**62**:174–80.
6. Johansson K, Jimbo R, Ostlund P, et al. Effects of bacterial contamination on dental implants during surgery: a systematic review. *Implant Dent* 2017;**26**:778–89.
7. Buser D, Martin W, Belser UC. Optimizing esthetics for implant restorations in the anterior maxilla: anatomic and surgical considerations. *Int J Oral Maxillofac Implants* 2004;**19**(Suppl):43–61.
8. Jinno Y, Jimbo R, Hjalmarsson J, et al. Impact of surface contamination of implants with saliva during placement in augmented bone defects in sheep calvaria. *Br J Oral Maxillofac Surg* 2019;**57**:41–6.
9. Berglundh T, Stavropoulos A. Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. *J Clin Periodontol* 2012;**39**(Suppl 12):1–5.
10. Jimbo R, Tovar N, Yoo DY, et al. The effect of different surgical drilling procedures on full laser-etched microgrooves surface-treated implants: an experimental study in sheep. *Clin Oral Implants Res* 2014;**25**:1072–7.
11. Yuan K, Chan YJ, Kung KC, et al. Comparison of osseointegration on various implant surfaces after bacterial contamination and cleaning: a rabbit study. *Int J Oral Maxillofac Implants* 2014;**29**:32–40.
12. Oosterbos CJ, HCh Vogely, Nijhof MW, et al. Osseointegration of hydroxyapatite-coated and noncoated Ti6Al4V implants in the presence of local infection: a comparative histomorphometrical study in rabbits. *J Biomed Mater Res* 2002;**60**:339–47.
13. Burgers R, Hahnel S, Reichert TE, et al. Adhesion of *Candida albicans* to various dental implant surfaces and the influence of salivary pellicle proteins. *Acta Biomater* 2010;**6**:2307–13.
14. Costerton W, Veeh R, Shirtliff M, et al. The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest* 2003;**112**:1466–77. Erratum: J Clin Invest 2007; 117:278.
15. Albrektsson T, Jemt T, Molne J, et al. On inflammation-immunological balance theory-A critical apprehension of disease concepts around implants: Mucositis and marginal bone loss may represent normal conditions and not necessarily a state of disease. *Clin Implant Dent Relat Res* 2019;**21**:183–9.
16. Albrektsson T, Canullo L, Cochran D, et al. Peri-Implantitis: a complication of a foreign body or a man-made disease: facts and fiction. *Clin Implant Dent Relat Res* 2016;**18**:840–9.
17. Jemt T, Karouni M, Abitbol J, et al. A retrospective study on 1592 consecutively performed operations in one private referral clinic. Part II: Peri-implantitis and implant failures. *Clin Implant Dent Relat Res* 2017;**19**:413–22.
18. Antoun H, Karouni M, Abitbol J, et al. A retrospective study on 1592 consecutively performed operations in one private referral clinic. Part I: Early inflammation and early implant failures. *Clin Implant Dent Relat Res* 2017;**19**:404–12.
19. Adeyemo WL, Reuther T, Bloch W, et al. Influence of host periosteum and recipient bed perforation on the healing of onlay mandibular bone graft: an experimental pilot study in the sheep. *Oral Maxillofac Surg* 2008;**12**:19–28.
20. Bonsignore LA, Colbrunn RW, Tatro JM, et al. Surface contaminants inhibit osseointegration in a novel murine model. *Bone* 2011;**49**:923–30.

21. Sakkas A, Wilde F, Heufelder M, et al. Autogenous bone grafts in oral implantology-is it still a gold standard? A consecutive review of 279 patients with 456 clinical procedures. *Int J Implant Dent* 2017;**3**: 23.

22. Yue C, Zhao B, Ren Y, et al. The implant infection paradox: why do some succeed when others fail? Opinion and discussion paper. *Eur Cell Mater* 2015;**29**:303–13.