OSSEOUS COAGULUM COLLECTED IN BONE TRAPS: POTENTIAL FOR BACTERIAL CONTAMINATION AND METHODS FOR DECONTAMINATION

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Because of its excellent osteogenic potential, autogenous bone is the preferred grafting material for dental procedures; however, bone collected in osseous coagulum traps is subject to contamination by oral bacteria. This study assessed bacterial contamination of osseous coagulum and tested treatments for reducing contamination. Fifty bone samples from patients undergoing implant osteotomy procedures were collected in osseous coagulum traps, divided into groups of 10, and rinsed with normal saline, 0.12% chlorhexidine, or 50 mg/mL tetracycline. Twenty control samples received no treatment. The bone samples were plated in triplicate on selective and differential media to assay aerobic and anaerobic bacteria and potential bacterial pathogens, including staphylococci, streptococci, enterics, and black-pigmented bacteria (BPB). Inoculations were performed with an Autoplate 4000, and plates were incubated at 37°C either aerobically or in a Coy anaerobic chamber. Bacteria were isolated from all samples. In control samples, the mean colony-forming units (cfu) per milliliter of suspended osseous coagulum was $6.5 \times 10^4 \pm 9.6 \times 10^4$ in aerobic cultures and $4.8 \times 10^4 \pm 6.9 \times 10^4$ in anaerobic cultures. Viridans streptococci were isolated from 46 samples, with a mean of $2.9 \times 10^4 \pm 4.1 \times 10^4$ cfu/mL. Enterics were in 16 samples with cfu ranging from 200 cfu/mL to 3.4×10^4 cfu/mL. Mannitol nonfermenting staphylococci were found in one sample at 106 cfu/mL. BPB were not isolated. A Mann-Whitney *U* test with significance set at $P = .05$ determined that the only statistically significant reductions in bacterial numbers occurred in tetracycline-treated samples of anaerobic bacteria (5-fold decrease, $P = .02$) and aerobic bacteria (6-fold decrease, $P = .01$). Tetracycline treatments effected a 7-fold decrease in streptococci, but the difference was not significant ($P = .07$). These data indicate significant bacterial contamination of bone collected in osseous coagulum traps and justify further research into methods for eliminating that contamination.

Key Words: oral flora, contamination, peri-implantitis, autogenous bone, osseous coagulum, osteotomy, chlorhexidine, tetracycline

INTRODUCTION

Bone grafting is used in many dental procedures, including sinus grafting, treatment of periodontal osseous defects, and alveolar ridge augmentation. Autogenous bone is considered the "gold standard" for bone grafting procedures because of its potential for regeneration. When used as a grafting material to treat bony defects caused by chronic periodontitis, regeneration of an attachment

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apparatus that includes cementum, bone, and a functionally oriented periodontal ligament has been demonstrated.[1]

Osseous coagulum traps have been used since the early 1990s to aid in the collection of autogenous graft particles.[2] During the course of periodontal or dental implant surgery, an osseous coagulum trap can be applied to the surgical suction tip and bone particles can be collected. This material can then be used, either alone or in combination with a bone substitute.[3,4] Retaining this bone permits the use of autogenous grafting material without the morbidity of creating a second surgical site. In addition, the osteogenic properties that promote maximum regeneration at the defect site are still present.[5,6] As a result, the use of osseous coagulum traps has become an established method of obtaining bone for grafting purposes.

However, the potential for microbial contamination of the collected bone is a matter for concern. Normal flora that are typically present in the oral cavity average 10^{11} bacteria/g of plaque and 10^{8} bacteria/mL of saliva, including many pathogens, periodontal and otherwise.[7,8] Clinicians attempt to maintain a sterile environment during periodontal or implant surgery; however, it is likely that osseous coagulum traps harbor unwanted bacteria. Previously, Cravatta (unpublished data, 2001) found significant microbial contamination in autogenous bone collected in osseous coagulum traps. The majority of the isolates were streptococci; rods and motile organisms were rarely isolated. This indicated that most of the contamination was from normal oral flora. Nevertheless, these microbes, if placed in a grafting site, could potentially cause infection and failure of the grafting procedure.

Other researchers have recognized this problem and attempted to reduce the numbers of contaminating bacteria in osseous coagulum. Tetracycline, which has been effective in eliminating periodontal pathogens, has been mixed with bone grafting materials prior to placement into osseous defects.[9–11] Chlorhexidine, an effective topical antimicrobial rinse, has been shown to significantly reduce bacterial contamination when used as both a presurgical and postsurgical rinse.[12–14]

The goal of this study was to investigate methods of decreasing the number of bacteria contaminating osseous coagulum. These methods included limiting the duration and field of use of the osseous coagulum traps during the surgical procedure and treating the osseous coagulum samples with rinses of normal saline, chlorhexidine, or tetracycline.

MATERIALS AND METHODS

Patient selection

Fifty samples were collected from 50 healthy patients scheduled for dental implant placement at the Southern Illinois University (SIU) School of Dental Medicine Implant Clinic. The patients who participated in the study met the following criteria: (1) they were at least 18 years of age, (2) they required dental implant therapy, and (3) they consented to have samples taken for analysis. Patients excluded from the study included: (1) pregnant patients, (2) nursing mothers, (3) immunocompromised patients, (4) those who had received antibiotic therapy within 3 months prior to surgery, (5) those with a history of systemic disease, and (6) patients with untreated periodontal disease. The SIU Edwardsville Institutional Review Board approved the human subjects protocol. Informed consent was obtained prior to surgical therapy. Patients remained anonymous, and no labels were placed on the autogenous bone samples to identify patients throughout the course of the study.

Surgical protocol

All procedures were performed under sterile conditions. Prior to surgery, all patients rinsed with 0.12% chlorhexidine for 30 seconds. Plaque was removed from the surfaces adjacent to the surgical site. Patients were anesthetized with 2% lidocaine with 1:100 000 epinephrine and/or 0.5% bupivacaine with 1:200 000 epinephrine. Incisions were made according to the location of the surgery and the number of implants to be placed. Full-thickness mucoperiosteal flaps were reflected. Osseous coagulum traps (Implant Innovations, Palm Beach Gardens, Fla) were placed onto the surgical suction tip to collect autogenous bone that was removed during preparation of the implant site. Two suction tips were used during osteotomy preparation. One surgical suction tip was attached to the osseous coagulum trap and was used only during osteotomy preparation at the osteotomy site. The second suction tip was used throughout the remaining implant placement procedure to collect saliva, blood, and debris. The osseous coagulum trap collected autogenous bone that is normally discarded. Initially, 20 samples were collected and used as controls for this study. An additional 10 samples were rinsed by administering 20 mL of sterile saline directly into the surgical suction tip and through the osseous coagulum trap following bone collection. Ten samples were rinsed in the same manner with 20 mL of 0.12% chlorhexidine solution. The final 10 samples were rinsed with 20 mL of 50 mg/mL tetracycline solution.

TABLE 1

Selective and differential media used to assess the presence of certain bacterial species in osseous coagulum samples

Medium	Bacteria Taxa or Species	Incubation Conditions/ Confirmatory Tests
Eosin methylene blue	Enterics	Aerobic, 37°C, 2 to 3 d/ purple colony, green sheen
Schaedler's vitamin K blood agar	Total aerobic and anaerobic	Aerobic: 37°C, 2 to 3 d/ colony, hemolysis Anaerobic: 37°C, 5 to 7 d/ black colony, hemolysis
Mannitol salt agar	Staphylococci	Aerobic, 37°C, 2 to 3 d/ yellow or white colony
Mitis-salivarius	Streptococci	Aerobic, 37°C, 2 to 3 d/ blue or black colony

The tetracycline solution was prepared by suspending 1 g of tetracycline powder into 20 mL of sterile saline prior to the surgical procedure. The traps were removed prior to flap closure and placed in sterile bags. The bags were sealed and placed into a cooler at 4°C for transportation to the microbiology laboratory.

Microbial sampling

The trap was removed from the sterile bag and disassembled under a laminar flow hood (Labconco, Kansas City, Mo). The osseous coagulum was removed with a sterile spatula and placed into a tared weighing boat, and the weight of the sample was recorded. The sample was then placed into 1 mL of prereduced anaerobically sterilized (PRAS) liquid dental transport medium (Anaerobe Systems, Morgan Hill, Calif) and sonicated for 30 seconds. For each of the 50 samples, two 10-μL aliquots were removed for phase-contrast microscopy in a Petroff-Hausser chamber (Hausser Scientific Partnership, Horsham, Penn). The number of bacteria per milliliter of osseous coagulum in PRAS was determined using the following formula: the total number of bacteria was counted in each of 10 small squares of the Petroff-Hauser chamber, and then the average number of bacteria per square was multiplied by 20 000 and by the sample dilution (see Table 2). This number was used to calculate the appropriate dilutions of the samples for plating on agar media. The remaining sample was placed in a vortex mixer for 30 seconds to disperse the microorganisms in the sample, and serial 10-fold dilutions in PRAS were made prior to plating on selective and nonselective microbiologic culture media.

Cultivation of bacteria

The samples were diluted in PRAS based on bacterial counts obtained with phase contrast microscopy prior to inoculation on selective and nonselective media (Table 1). The inoculations were done with a spiral plating system (Autoplate 4000, Spiral Biotech Inc, Bethesda, Md). The media, the bacterial species detectible with the media, and the incubation conditions are provided in Table 1. All samples were plated on all media in triplicate. Total colony-forming units (cfu) were determined on both anaerobic and aerobic Schaedler's blood agar plates (Gibson Laboratories, Lexington, Ky). For the selective and differential media, the total cfu of the organism for which the medium was selective and/or differential were determined.

TABLE 2

Average number of bacteria/mL of osseous coagulum*

Sample Number	Bacteria/mL ± SD	Sample Number	Bacteria/mL ± SD
1	$4.82 \times 10^5 \pm 1.81 \times 10^5$	26	$4.50 \times 10^5 \pm 1.68 \times 10^5$
2	$3.48 \times 10^5 \pm 0.94 \times 10^5$	27	$4.18 \times 10^5 \pm 1.66 \times 10^5$
3	$4.78 \times 10^5 \pm 0.90 \times 10^5$	28	$3.40 \times 10^5 \pm 1.65 \times 10^5$
4	$8.58 \times 10^5 \pm 2.36 \times 10^5$	29	$4.08 \times 10^5 \pm 1.64 \times 10^5$
5	$7.82 \times 10^5 \pm 2.17 \times 10^5$	30	$3.92 \times 10^5 \pm 1.62 \times 10^5$
6	$7.60 \times 10^5 \pm 2.08 \times 10^5$	31	$4.14 \times 10^5 \pm 1.61 \times 10^5$
7	$7.12 \times 10^5 \pm 2.02 \times 10^5$	32	$3.96 \times 10^5 \pm 1.60 \times 10^5$
8	$4.26 \times 10^5 \pm 2.08 \times 10^5$	33	$3.68 \times 10^5 \pm 1.59 \times 10^5$
9	$4.58 \times 10^5 \pm 2.05 \times 10^5$	34	$3.80 \times 10^5 \pm 1.58 \times 10^5$
10	$4.94 \times 10^5 \pm 1.97 \times 10^5$	35	$3.82 \times 10^5 \pm 1.57 \times 10^5$
11	$3.78 \times 10^5 \pm 1.99 \times 10^5$	36	$4.18 \times 10^5 \pm 1.55 \times 10^5$
12	$6.22 \times 10^5 \pm 1.92 \times 10^5$	37	$4.28 \times 10^5 \pm 1.54 \times 10^5$
13	$3.56 \times 10^5 \pm 1.97 \times 10^5$	38	$4.48 \times 10^5 \pm 1.53 \times 10^5$
14	$4.18 \times 10^5 \pm 1.95 \times 10^5$	39	$4.26 \times 10^5 \pm 1.51 \times 10^5$
15	$4.22 \times 10^5 \pm 1.93 \times 10^5$	40	$4.50 \times 10^5 \pm 1.50 \times 10^5$
16	$4.80 \times 10^5 \pm 1.89 \times 10^5$	41	$4.28 \times 10^5 \pm 1.49 \times 10^5$
17	$4.36 \times 10^5 \pm 1.86 \times 10^5$	42	$4.30 \times 10^5 \pm 1.48 \times 10^5$
18	$4.32 \times 10^5 \pm 1.83 \times 10^5$	43	$3.98 \times 10^5 \pm 1.48 \times 10^5$
19	$4.66 \times 10^5 \pm 1.80 \times 10^5$	44	$3.96 \times 10^5 \pm 1.47 \times 10^5$
20	$4.70 \times 10^5 \pm 1.77 \times 10^5$	45	$3.96 \times 10^5 \pm 1.46 \times 10^5$
21	$4.94 \times 10^5 \pm 1.74 \times 10^5$	46	$3.80 \times 10^5 \pm 1.45 \times 10^5$
22	$3.82 \times 10^5 \pm 1.73 \times 10^5$	47	$4.02 \times 10^5 \pm 1.43 \times 10^5$
23	$3.70 \times 10^5 \pm 1.72 \times 10^5$	48	$3.94 \times 10^5 \pm 1.43 \times 10^5$
24	$3.74 \times 10^5 \pm 1.71 \times 10^5$	49	$3.94 \times 10^5 \pm 1.42 \times 10^5$
25	$4.38 \times 10^5 \pm 1.69 \times 10^5$	50	$4.42 \times 10^5 \pm 1.41 \times 10^5$

*To determine the average number of bacteria/mL of osseous coagulum suspended in prereduced anaerobically sterilized liquid dental transport medium, the total number of bacteria was counted in each of 10 small squares of a Petroff-Hauser chamber. The average number of bacteria per square was multiplied by 20 000 and by the sample dilution.

Statistical analysis

Statistical analysis was performed with the aid of statistical software (SPSS, Chicago, Ill). Regression analysis was used to assess possible relationships between bone weight and aerobes, anaerobes, enterics, streptococci, or staphylococci. This analysis was used to determine whether there was a linear relationship between bone weight and microbial numbers of the various groups. A Mann-Whitney *U* test with significance set at $P = .05$ was performed to

assess the effects of the treatments on each group of bacteria. For every triplicate sample, replicates with extremely low or high colony counts were eliminated prior to Mann-Whitney testing. This lowered the coefficient of variation and thus increased the statistical power of the study.

RESULTS

Osseous coagulum was collected from 50 subjects accepted into the study. The average bone weight of each sample was 87.67 ± 13.32 mg and ranged from 38.45 to 124.25 mg.

Phase-contrast microscopy

Phase-contrast microscopy revealed bacteria in all 50 bone samples. Following preparation of each sample for microscopic analysis, the number of bacterial cells in 10 small squares in a Petroff-Hauser chamber was counted, and the mean number of bacteria was calculated (Table 2). For the 50 samples evaluated, the average number of bacteria observed in each sample ranged from $3.40 \times 10^5 \pm 1.05 \times 10^5$ to $8.58 \times 10^5 \pm 2.32 \times 10^5$ per mL of osseous coagulum suspended in PRAS liquid dental transport media. This average was used to estimate the dilution factor that would allow plating of a statistically significant yet countable number of viable bacteria on selective and nonselective media.

Bone weight vs microbial contamination

Bivariate correlation analysis was used to assess possible linear relationships between bone weight and the numbers of bacteria isolated in the categories examined (ie, anaerobes, aerobes, enterics, staphylococci, and streptococci). The Pearson correlation (r) reported a weak to very weak relationship between bone weight and bacterial numbers in most samples. A correlation was found between bone weight and enterics in the control group ($P = .00$) and between bone weight and aerobic bacteria in the saline group ($P = .03$). This indicated an increase in bacterial numbers in these groups, as larger quantities of bone were harvested. However, the correlation for all samples as a cumulative was not significant at $P = .05$, and there was no statistically significant relationship between bone weight and bacteria numbers in the remaining groups.

Detection of viable bacteria

Viable bacteria were present in all 50 bone samples (Table 3). In the controls, anaerobic counts ranged from 200 to 3.9×10^5 cfu/mL, while aerobic counts ranged from 300 to 2.5×10^5 cfu/mL. Streptococci were detected on the selective mitis-salivarius (M/S) medium in 46 of the 50 samples, with a range of 40 to 1.3×10^4 cfu/mL. Staphylococci were detected in only 1 sample at (106 cfu/mL). These staphylococci did not ferment mannitol on mannitol salt agar (MSA). Growth on eosin methylene blue detected enterics in 16 of the 50 samples in numbers ranging from 200 to 3.8×10^4 cfu/mL. No black-pigmented bacteria (BPB) indicative of *Porphyromonas* and *Prevotella* sp were observed.

TABLE 3

Changes in bacteria/mL of osseous coagulum in response to treatment with saline and antimicrobials*

Treatment/bacteria	Mean cfu ($\times 10^4$)	Range	SD	N
Control (no treatment)				
Anaerobes	6.5	0.02–39.2	9.6	20
Aerobes	4.8	0.03–25.1	6.9	20
EMB	0.40	0–3.4	1.0	20
M/S	2.9	0–13.1	4.1	20
Saline treatment				
Anaerobes	3.0	0.29–7.8	2.5	10
Aerobes	1.8	0.05–3.1	1.3	10
EMB	2.0	0–1.6	0.5	10
M/S	0.7	0.02–1.6	0.6	10
Chlorhexidine (0.12%) treatment				
Anaerobes	3.2	0.3–12.4	3.9	10
Aerobes	2.5	0.02–11.0	3.3	10
EMB	0.14	0–0.9	0.31	10
M/S	1.6	0.008–6.0	1.9	10
Tetracycline (50 mg/mL) treatment				
Anaerobes	1.2†	0.04–7.1	2.2	10
Aerobes	0.8†	0.04–3.9	1.2	10
EMB	0.002	0–0.02	0.01	10
M/S	0.4	0.03–1.7	0.5	10

*cfu indicates colony-forming units; N, number of agar plates counted; EMB, eosin methylene blue, which is selective for enterics; M/S, mitis-salivarius, which is selective for streptococci.

†Indicates statistically significant ($P < .05$) differences from the control.

Treatment effectiveness

The effectiveness of saline, chlorhexidine, and tetracycline treatments in reducing the numbers of bacteria in the control samples is summarized in Table 3. The statistical significance of the changes in response to the treatments is summarized in Table 4. A nonparametric Mann-Whitney *U* test was used to determine that tetracycline was the only treatment that effected a statistically significant reduction in numbers of bacteria. Furthermore, only the 5-fold decrease in anaerobic bacteria ($P = .02$) and the 6-fold decrease in aerobic bacteria ($P = .01$) were statistically significant. Although the streptococci growing on M/S

TABLE 4

Statistical significance of treatments

	Treatment			Control			Mann-Whitney	
Organism	N	Median	Mean	N	Median	Mean*	z	P†
Anaerobes								
Saline	10	2.5	3.0	20	3.1	6.5	−0.33	.746
Chlorhexidine	10	1.7	3.2	20	3.1	6.5	−0.75	.475
Tetracycline	10	0.4	1.2	20	3.1	6.5	−2.29	.022‡
Aerobes								
Saline	10	2.1	1.8	20	2.3	4.8	−1.01	.328
Chlorhexidine	10	1.4	2.5	20	2.3	4.8	−0.99	.328
Tetracycline	10	0.2	0.8	20	2.3	4.8	−2.46	.013‡
Streptococci								
Saline	10	4.3	0.7	20	1.4	2.9	−1.32	.198
Chlorhexidine	10	0.9	1.6	20	1.4	2.9	−0.26	.812
Tetracycline	10	0.2	0.4	20	1.4	2.9	−1.90	.067
Enterics								
Saline	10	0	2.0	20	0	0.4	−0.61	.619
Chlorhexidine	10	0	0.1	20	0	0.4	−0.25	.846
Tetracycline	10	0	0.8	20	0	0.4	−1.85	.143

*Mean indicates mean colony-forming units $\times 10^4$.
†*P* is 1-tailed; significance of 2-tailed was the same as the 1-tailed for each data set.
‡Indicates treatment significantly different from control.

showed a 7-fold decrease in growth in the presence of tetracycline, the difference was not significant ($P = .07$).

DISCUSSION

Autogenous bone in the form of osseous coagulum may be used to graft osseous defects that are associated with natural teeth or sites for dental implant placement. The desire of every clinician using bone-grafting materials is to create an environment conducive to bone regeneration.[15] However, the potential for regeneration decreases when bacteria contaminate the grafting material. Previously in this laboratory, Cravatta (unpublished data, 2001) demonstrated significant contamination of osseous coagulum collected in bone traps. The goal of this study was to test procedures for minimizing bacterial contamination of bone grafting material collected in bone traps.

A potential cause of the microbial contamination in the osseous coagulum collected by Cravatta was his use of the same surgical suction tip for collection of bone, saliva, and blood throughout the entire surgical procedure. In this study, the protocol was modified to include 2 surgical suction tips: one to aspirate saliva and blood and another to collect osseous coagulum from the osteotomy site. To avoid predictable contamination from adjacent teeth and periodontal pockets,[2] patients undergoing periodontal treatment were not used for procuring grafting material. Instead, the patients accepted into the study required dental implant placement, and the surgical sites were considered relatively uncontaminated, since a surgical flap procedure was needed to access the osteotomy site.[16] Since several studies have shown that preprocedural rinsing with chlorhexidine diminishes the number of bacteria,[13,17,18] the adjacent tooth surfaces were thoroughly débrided of plaque and a 0.12% chlorhexidine rinse was used prior to surgery.

The methods used in this study resulted in less bacterial contamination as compared to the results from Cravatta; however, significant contamination of the osseous coagulum collected with the bone traps still occurred. Streptococci, the predominant species in the normal oral flora, were found in all 50 samples, although the numbers did vary from subject to subject. BPB, indicative of the periodontal pathogens *Porphyromonas* and *Prevotella,* were not observed. Somewhat surprisingly, enteric bacteria were found in 16 of the 50 samples, and staphylococci were found in 1 sample. The staphylococci did not ferment mannitol on MSA; therefore, the species present was not the primary pathogen *Staphylococcus aureus*.

Reduction of microbial contamination following antimicrobial rinses with saline, 0.12% chlorhexidine, and 50 mg/mL tetracycline was tested. The only statistically significant reduction in microbial numbers was seen in aerobes and anaerobes treated with tetracycline. Streptococci, as measured on MSA, were

greatly reduced in the presence of tetracycline; however, this was not statistically significant ($P = .067$). It is possible that with a larger sample size this reduction would achieve significance.

Other studies have observed high numbers of both normal and pathogenic microbes when obtaining intraoral bone. Like our observations, the majority of these bacteria were streptococci.[19] Detection of a high bacterial load raises concerns that they could cause a negative effect on the regenerative potential of the osseous coagulum and cause infection or failure of the grafting procedure. Certain streptococci commonly found in saliva and the oral mucosa (ie, *S oralis* and *S mitis*) are early colonizers that coaggregate with other microorganisms to form plaque.[20] Although streptococci alone are not likely to cause failure of a grafting procedure, they are known to coaggregate with pathogenic bacteria, which could lead to failure. It has also been noted that nonpathogenic normal flora can become pathogenic when displaced to a wound site.[21]

The pathogenic bacteria assayed were the enterics, staphylococci, and BPB. The enterics were chosen because, although rare, they can be detected in the oral cavity,[22] and many enterics, for example, *Serratia marcescens*, can cause life-threatening wound infections.[23,24] Staphylococci are etiologic in purulent infections in the skin and mucosa, and they can lead to failure of grafted sites.[25,26] BPB are present in most abscesses and infections in the oral cavity. These genera are commonly found in patients with periodontal disease.[27] Aspiration of periodontal pockets could allow BPB to enter the osseous coagulum trap.[19] It appears that our exclusion of patients with periodontal disease may have successfully prevented contamination by BPB.

Very few studies have investigated antimicrobial treatment of contaminated osseous grafting material. Young et al[12] and Kuttenberger et al[28] achieved significant decontamination by modifying the surgical protocol to include collection of saliva and bone with different suction tips and preprocedural mouth rinsing with 0.12% chlorhexidine. However, Kurkcu et al[29] found that whereas chlorhexidine rinsing reduced the total numbers of microorganisms, it was not effective in reducing aerobic streptococci and anaerobes. Our study protocol was designed before the Young et al[12] study was published, and we did not test our modified surgical protocol or preprocedure mouth rinsing except to compare results with the previous study performed by Cravatta. We do not know of another study that has exposed the osseous coagulum to antimicrobial rinses while in the bone trap. However, our results are most like those of Kurkcu et al[29] in that chlorhexidine treatments did not cause significant reductions in microbial numbers. We found that tetracycline was the only effective treatment. Also, similar to Kurkcu et al,[29] we found that some streptococci were resistant to tetracycline.

CONCLUSION

The data presented in this study indicate significant bacterial contamination of osseous coagulum. We have shown that bone traps used with stringent surgical protocols that include segregated suction tips and preprocedural chlorhexidine rinsing, coupled with in situ rinsing with tetracycline, will effect significant yet incomplete reductions in bacterial contamination of osseous coagulum. Further work needs to be done to determine the optimum antimicrobials as well as concentrations and contact times that will decontaminate osseous coagulum while preserving the osteogenic potential of the material.

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