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Microbiological analysis of autologous bone particles obtained by low-speed drilling and treated with different decontamination agents

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Abstract. The aim of this study was to compare the effectiveness of three agents – two antibiotics (amoxicillin and clindamycin) and an antiseptic (chlorhexidine) – to decontaminate bone grafts obtained by low-speed drilling. The study included 248 bone tissue samples harvested from 62 patients by low-speed drilling before dental implant placement. Each of four samples obtained from every patient was dropped, using a sterile instrument, into a sterile tube containing a 500-μl solution of 400 μg/mL amoxicillin, 150 μg/mL clindamycin, 0.12% chlorhexidine, or physiological saline for 1 min. The number of colony-forming units (CFU) was determined at 48 h of culture. The use of clindamycin, amoxicillin, or chlorhexidine as decontaminant for 1 min significantly reduced the CFU count when compared to physiological saline (control agent). In both anaerobic and CO_2-rich atmospheres, significant differences in CFU/mL were found between the control and chlorhexidine groups ($P < 0.001$), control and amoxicillin groups ($P < 0.001$), control and clindamycin groups ($P < 0.001$), chlorhexidine and amoxicillin groups ($P < 0.0001$), and chlorhexidine and clindamycin groups ($P < 0.0001$). In conclusion, clindamycin had the highest decontaminating effect on bone particles obtained by low-speed drilling, followed by chlorhexidine and amoxicillin. Clindamycin may therefore be a valid alternative option for the routine decontamination of intraoral bone grafts.

Key words: low-speed drilling; bone decontamination; amoxicillin; clindamycin; chlorhexidine.

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The predictability of implant procedures and the long-term maintenance of implant stability are directly related to the quality and quantity of bone tissue available for their placement, as widely demonstrated in the literature over the past few years[1]. However, this bone tissue can be lost through trauma, periodontal disease, or physiological maxillary bone resorption after tooth loss, or may be absent due to a congenital malformation, producing deficits in the amount and quality of bone in the implant area[2–4]. Grafts must be used to augment the width and height of bone around implants in many cases of bone loss, and various procedures have been applied for this purpose. The use of autologous bone is the gold standard for bone augmentation, because it contains bone morphogenetic proteins among other proteins, minerals, and vital bone cells, unlike allografts, xenografts, or other types of bone graft. Block or particulate autologous bone grafts are used alone or in combination, and numerous extraoral and intraoral donor sites have been proposed[5,6].

Guided bone regeneration techniques and bone grafts are used to fill adjacent bone defects and achieve optimal implant placement[7]. Only a relatively small bone volume is usually needed to correct small osseous defects such as fenestration or dehiscence, and a sufficient amount can generally be obtained utilizing a simultaneous technique (implant placement plus bone regeneration). Previous studies by our group demonstrated that particulate bone obtained by low-speed drilling had superior biological properties in comparison to that harvested by other methods[8]. It also proved less susceptible to bacterial contamination in comparison to bone fragments or shavings collected during ostectomy using a bone filter or back-action chisel[7].

Nevertheless, there is a high risk that bones gathered by low-speed drilling are contaminated by the numerous microorganisms that constitute the habitual flora of the oral cavity, and the transplantation of contaminated bone can cause infectious complications or even augmentation failure. Reported rates of infection after graft contamination range between 5% and 50%[9], and there is a need to destroy contaminating organisms while maintaining the viability of bone and osteoprogenitor cells[10]. Numerous agents have been proposed for the decontamination of harvested particles, mainly antibiotics and antiseptics, but none have proven completely effective[11–13].

The objective of this study was to compare the effectiveness of three agents – two antibiotics (amoxicillin and clindamycin) and one antiseptic (chlorhexidine) – to decontaminate bone grafts obtained by low-speed drilling.

Materials and methods

Patient selection

A total of 248 bone samples were gathered using low-speed drilling, as described by Anitua et al.[14] (50 rpm without irrigation), from 62 patients undergoing the placement of multiple implants for complete rehabilitation in the Oral Surgery and Implantology Master's Clinic of the University of Granada (Spain). All participants provided written informed consent to participate in the study, in accordance with the guidelines of the Declaration of Helsinki, and the study was approved by the Ethics Committee of the University of Granada (reference number 721). Participants were selected from among volunteers meeting study eligibility criteria. Study exclusion criteria were history or presence of systemic disease, immunocompromised state, pregnancy, clinical or radiographic evidence of active oral disease, receipt of any medication that might interfere with the surgical procedure or postoperative wound healing, or receipt of antibiotic therapy in the month before the study. In addition, the patients received no systemic antibiotics before or at the time of surgery.

Surgical protocol

The same experienced surgeon (FJHB) performed all procedures. Before the surgery, patients were fully covered with sterile drapes to avoid bacterial contamination, and 10% povidone iodine (Corsodyl; SmithKline Beecham, Brentford, UK) was applied to the perioral skin and lips as antisepsis. Immediately before the surgery, the patients rinsed with 10 ml 0.12% chlorhexidine mouth rinse for 2 min (Perio-Aid; Dentaid SL, Barcelona, Spain). Surgical sites were isolated by placing sterile gauze swabs on the upper buccal vestibule (sulcus) to prevent saliva flow from the Stenson duct and on the lingual aspect of the surgical site, extending sublingually. All surgical procedures were performed under local anaesthesia using 4% articaine with 1:100,000 epinephrine (Ultracain; Normon SA, Madrid, Spain).

All samples were taken from the implant site using low-speed drilling (20–80 rpm) with no irrigation. Each of four samples obtained from every patient was dropped, using a sterile instrument, into a sterile tube (Eppendorf Ibérica SLU, Madrid, Spain) containing a 500-μl solution of 400 μg/mL amoxicillin, 150 μg/mL clindamycin, 0.12% chlorhexidine, or physiological saline (control) for a period of 1 min. Immediately afterwards, the solutions in the tubes were replaced with 1000 μl of brain–heart infusion agar medium (BHI; Becton Dickinson Co., Cockeysville, MD, USA). All tubes were weighed with an electronic balance before and after the introduction of bone samples to ensure that a similar amount of bone (approximately 1 g) was always obtained. Samples reached the laboratory within 5–10 min for immediate processing.

Sample processing

Once received in the laboratory, the tubes containing the bone samples were brought to a volume of 1 ml with BHI medium for subsequent serial dilution. They were then placed in a vortex to mix the samples for 30 s.

Besides the initial suspension, two serial dilutions (1:10) were performed in BHI medium for seeding on Columbia human blood agar plates (Becton Dickinson Co., Cockeysville, MD, USA). Next, 20 μl of the initial suspension and each dilution were plated on blood agar plates that had previously been divided into three equal parts. Eight plates were seeded for each patient, i.e., two for control and two for each treatment, in order to incubate one in a CO_2-rich atmosphere and the other in an anaerobic atmosphere at 37 °C for 48 h.

Colony counts

After 48 h of incubation, the number of colony-forming units (CFU) in each blood agar plate in a CO_2-rich or anaerobic atmosphere was determined visually. The CFU count in each agar medium was multiplied by the dilution factor of the sample to obtain the total number of CFU/mL with the following formula:

$$\mathrm{CFU/ml} = \frac{\text{CFU count}}{\text{Dilution factor}(10^{-x})\times \text{Volume of sample(ml)}}$$

Statistical analysis

IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA) was used for the data analysis. A descriptive analysis

was conducted to calculate the mean value of the variables, 95% confidence interval (CI), standard error, standard deviation, and median value, estimated by 1000 bootstrap resampling with 95% CI. The Anderson–Darling test was applied to evaluate the normality of the variable distributions. The Kruskal–Wallis test was used to compare the agents. The equality of variance among the different samples was evaluated with the Levene test. The Mann–Whitney test was used for multiple comparisons among agents. The level of significance (α) was 0.05 in all tests.

Table 1. Colony-forming units per millilitre in each group (anaerobic and CO_2-rich conditions) after 48 hours of culture; mean (SD) and median values.

Group	Number	Mean (SD)	Median
Anaerobic			
Physiological saline	62	85.87 ± 379.25	5.00
Chlorhexidine	62	12.52 ± 68.09	0.00
Amoxicillin	62	60.03 ± 380.98	1.00
Clindamycin	62	5.16 ± 9.12	1.00
CO_2			
Physiological saline	62	71.03 ± 290.25	6.00
Chlorhexidine	62	13.31 ± 77.49	0.00
Amoxicillin	62	58.38 ± 381.17	1.00
Clindamycin	62	7.73 ± 1.86	1.00
Total			
Anaerobic	248	40.90 ± 271.39	1.00
CO_2	248	37.60 ± 15.43	1.00

SD, standard deviation.

Results

All samples showed bacterial growth after incubation in either the anaerobic or CO_2-rich atmosphere. As the CFU/mL values were not homogeneously distributed within each group, median values were considered.

The mean anaerobic CFU/mL in the samples was 85.87 CFU/mL (median 5) for the control group, 12.52 CFU/mL (median 0) for the chlorhexidine group, 60.03 CFU/mL (median 1) for the amoxicillin group, and 5.16 CFU/mL (median 1) for the clindamycin group. The corresponding mean values under CO_2-rich conditions were 71.03 CFU/mL (median 6) for the control group, 13.31 CFU/mL (median 0) for the chlorhexidine group, 58.38 CFU/mL (median 1) for the amoxicillin group, and 7.73 CFU/mL (median 1) for the clindamycin group (Table 1).

As shown in Table 2, the number of CFU/mL in both anaerobic and CO_2-rich atmospheres differed significantly between the control and chlorhexidine groups ($P < 0.001$), control and amoxicillin groups ($P < 0.001$), control and clindamycin groups ($P < 0.001$), chlorhexidine and amoxicillin groups ($P < 0.0001$), and chlorhexidine and clindamycin groups ($P < 0.0001$). No significant difference was observed between the amoxicillin and clindamycin groups in anaerobic ($P = 0.7$) or CO_2-rich ($P = 0.79$) atmosphere.

Table 2. *P*-values (Mann–Whitney test) for between-group comparisons.

	Anaerobic	CO_2	Total
Physiological saline vs chlorhexidine	<0.001	<0.001	<0.001
Physiological saline vs amoxicillin	<0.001	<0.001	<0.001
Physiological saline vs clindamycin	<0.001	<0.001	<0.001
Chlorhexidine vs amoxicillin	<0.001	<0.001	<0.001
Chlorhexidine vs clindamycin	<0.001	<0.001	<0.001
Amoxicillin vs clindamycin	0.7	0.79	0.56

Discussion

Numerous techniques are available to gather intraoral bone particles in areas adjacent to defects requiring regeneration, thereby avoiding the need for a second surgical field; however, bacterial contamination of these particles can cause infectious complications around dental implants[14–16]. Although various methods have been proposed to avoid this contamination during particle gathering, none have proven completely effective[5,11].

Bone particles collected with a bone filter, even when utilizing an astringent protocol, have very high contamination levels in comparison to particles gathered with low-speed drilling and back-action[7]. In addition, the growth and differentiation capacity of osteoblasts are higher in bone particles obtained by low-speed drilling than in those from intraoral bone collected with other techniques[8]. Nevertheless, even tissue samples obtained by low-speed drilling can show bacterial colonization[7], and it is therefore necessary to further reduce the bacterial load of autografts around implants.

In comparison to physiological saline (control agent), all of the decontaminating agents used in this study significantly reduced the bacterial load of bone particles obtained by low-speed drilling. The highest decontaminating effect was achieved with clindamycin, followed by chlorhexidine and amoxicillin. Tezulas et al.[13] reported that chlorhexidine and clindamycin applied at concentrations similar to those in the present study, but for 3 min, were effective at decontaminating bone particles obtained in implant bed preparation, although they recommended further comparison of the two agents in future studies. Unlike the present study, they found no differences between bone graft decontamination techniques, which may be attributable to the use of preoperative oral antibiotics by all of their patients, an exclusion criterion in our study. A review of the literature by Tezulas and Dilek[11] concluded that the antiseptic chlorhexidine and antibiotics such as tetracycline, rifamycin, and clindamycin can be used for bone particle decontamination. However, they believed that further research was required to identify the most effective disinfectant for bone particles obtained by ostectomy.

Infections caused by contaminated bone grafts pose a challenge in dental implantology. Pommer et al.[17] reported that treatment with 0.1% chlorhexidine was feasible when applied for 15 s, but that bone cells died after 30 s of contact time. It is difficult to follow this protocol in routine clinical practice, and there is a need for agents that are more bone-friendly. Antibiotics may be good candidates. In their review, clindamycin was also found to demonstrate decontaminating effectiveness, although slightly lower than that of chlorhexidine.

Etcheson et al.[18] compared the decontaminating effect of 0.12% chlorhexidine and 50 mg/mL tetracycline applied for 1 min on bone particles obtained by low-speed drilling. Tetracycline produced a markedly greater CFU reduction in comparison to chlorhexidine but did not achieve total decontamination, in agreement with the present findings.

In the present study, treatment with 0.12% chlorhexidine for 1 min achieved the highest possible decontaminating effect

without altering osteoblast cell viability, in agreement with previous studies[17–21]. Reported limitations of decontamination media include the cytotoxic effect of chlorhexidine on osteoblasts cultured in vitro at certain concentrations for 5–10 min[19]. Likewise, Lee et al.[20] found that high doses of chlorhexidine inhibit osteoblastic cell growth, proliferation, and collagen synthesis, although other authors described osteoblast viability after applying 0.12% chlorhexidine for >30 s[17] or 1 min[21].

Antibiotics have classically been used for the effective decontamination of bone allografts, third molar extraction sockets, and bone fractures, without impairing osteogenesis[22], although high concentrations have been found to inhibit osteoblast metabolic activity and proliferation[23]. Thus, it has been observed that clindamycin has a cytotoxic effect at high doses (500 μg/mL)[24], but that osteoblasts remain viable at low doses[23], which can even stimulate their metabolism[24]. In accordance with the aforementioned findings, we applied a low clindamycin dose of 150 μg/mL to benefit from its decontaminating potency without altering osteoblast metabolism.

Rathbone et al.[25] observed that amoxicillin at doses >500 μg/mL moderately diminished the osteoblastic cell count versus controls and reduced the alkaline phosphatase activity by 26–49%, and that the count was reduced by >75% at doses of ≥2000 μg/mL. Based on these previous studies, a dose of 400 μg/mL was used in the present study to maintain cell viability and metabolic activity while providing adequate decontamination.

A limitation of this study is that only a single dose of amoxicillin (400 μg/mL) and clindamycin (150 μg/mL) was tested, based on previous studies in the literature, in order to apply the maximum concentration compatible with the viability of bone tissue cells, i.e., a non-toxic dose. Another limitation is that the bacterial aspect was examined but not the viability of the bone cells. Further research is needed to elucidate the effect of the different decontaminating agents used in this study on the physiology of bone tissue cells to establish the maximum antimicrobial concentration that can be applied without compromising bone cell viability.

A 1-min application of each of the decontamination agents considered in this review significantly reduced the CFU count in comparison to the application of physiological saline. The most effective decontamination of bone particles obtained by low-speed drilling was achieved with clindamycin, followed by chlorhexidine and amoxicillin. The routine application of this antibiotic for the decontamination of intraoral bone grafts is simple, effective, and economical. As well as being more efficacious than chlorhexidine, as demonstrated in the present study, clindamycin has been reported to pose a lesser threat to the viability of osteoblasts. In addition, the fact that it is administered in a local rather than systemic manner reduces the possibility of generating bacterial resistance.

In conclusion, the utilization of clindamycin, amoxicillin, or chlorhexidine at appropriate concentrations and durations is effective at reducing the bacterial load of intraoral autologous bone grafts. However, further research is needed on the viability of the osteoblastic cells in bone particles that are in contact with these decontamination agents.

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Competing interests

The authors declare that they have no conflict of interest.

Ethical approval

All procedures conducted in this study involving human participants were in performed in accordance with the ethical standards of the Ethics Committee of the University of Granada and with the 1964 Declaration of Helsinki.

Patient consent

Informed consent was obtained from all individual participants in the study.

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