Levels of Bacterial Contamination in Fresh Extraction Sites After a Saline Rinse

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Purpose: To determine the level of bacterial contamination in immediate implantation or augmentation sites vs pristine bone, before and after saline rinses. **Materials and Methods:** Bacterial samples were taken from fresh extraction sites (17 patients) and pristine bone (15 patients) before performing implant dentistry surgical procedures. Levels of bacterial contamination were estimated before and after saline rinses. Samples from the socket were placed on an agar plate for total bacterial account and on selective plates for Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis counts. **Results:** The level of bacterial contamination before saline rinse was 1.2×10^4 units of bacterial colony (CFU)/mL in fresh extraction sites (study group) and 5×10^2 CFU/mL in pristine bone sites (control group). After a saline rinse, the bacterial level was lowered significantly to 5.2×10^3 CFU/mL in the study group and to zero in the control group ($P < .05$). Levels of bacterial contamination were higher in the mandibular sockets (7.5×10^3 CFU/ml) than in the maxillary sockets (5.6×10^3 CFU/mL), and the difference was statistically significant ($P = .034$). All implanted/augmented fresh or pristine sites survived in the follow-up period. **Conclusion:** Fresh extraction sockets with clinical signs of infection show bacterial presence. Pristine bone shows a lower bacterial level. Saline rinse in addition to a decontamination protocol may reduce the level of bacterial contamination significantly both in fresh extraction sites and pristine bone. INT J ORAL MAXILLOFAC IMPLANTS 2015;30:1362–1368. doi: 10.11607/jomi.3980

Key words: bacterial contamination, immediate implantation and augmentation sites, pristine bone sites

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Immediate dental implantation/augmentation in fresh extraction sockets is a common procedure that has a high survival rate. This procedure has several advantages, including a shorter healing period, fewer surgical interventions, and prevention of bone loss during the remodeling period of the extraction socket.[1–4]

One of the disadvantages of this procedure is the risk of failure of the dental implant if the socket is infected.

Several researchers have found high survival rates (93%–95%) of immediate implantations during a 3- to 5-year follow-up period, and they have recommended a protocol for maintaining antiseptic conditions for immediate dental implantation.[5,6] The high survival rate obtained in several studies supports the hypothesis that implants may be successfully osseointegrated when placed immediately after extraction of teeth presenting with endodontic and periodontal lesions, provided that appropriate clinical procedures are performed before the implant surgery; these procedures include meticulous cleaning, socket curettage/debridement, and use of a chlorhexidine 0.12% rinse.[7] In a study of 1,081 immediate implantation sites that were followed up for at least 5 years, the overall survival rate was 95%.[8]

Aggregatibacter actinomycetemcomitans and *Porphyromonas gingivalis* are two periopathogens seen in periodontal diseases. These bacteria also play an important role during the healing period and the implant's functional period.[9–12] In many cases, the bone near the extracted teeth is somewhat absorbed as a result of periapical lesions and/or periodontal disease. Dental implantation in infected sites is contraindicated.[13] Several articles deal with dental implantation in infected sites, and show predictable osseointegration of the implants under adequate preoperative and postoperative treatment.[14,15] The patient's defense system also has an important role in the management of the infection by means of mechanical and chemical processes. Evian et al[16] found a higher tendency toward implant failure in periapical infected areas. It is also known that periodontal or endodontal infections are risk factors for implant failure.[17–22] Residual microbial infection in a clinically healed socket can lead to lack of osseointegration in alveolar bone.[23,24]

Conversely, a few research studies have shown that the survival rates of immediate dental implants after extraction of cracked teeth, perforations, and endodontic and periodontal lesions are similar to the survival of implants placed in healthy sockets.[24–30]

The aim of the present study was to determine the level of bacterial contamination in fresh extraction sockets and pristine sites before and after saline rinse and to correlate the data with the clinical outcomes, that is, the implant survival rate.

MATERIALS AND METHODS

The levels of bacterial contamination in extraction sockets that were planned for immediate implantation or augmentation were estimated before and after saline rinses, with particular attention to the bacterial infection levels in upper vs lower extraction sockets. These contamination levels were compared to the levels of contamination noted in pristine sites of implantation.

The patients were followed up for at least 3 years after surgery, for the clinical outcome of the procedure. The survival rate of immediate implantation and augmentation vs late implantation was compared.

Patients

This prospective cohort study was conducted between 2009 and 2011 at the Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Tel Aviv University. The research was approved by the ethical committee of the university. All participants signed an informed consent form before participating in the study; all planned to undergo dental implantation as a means of oral rehabilitation.

Inclusion criteria were:

- Age more than 20 years old
- Inflamed tooth that was scheduled for extraction
- Oral rehabilitation of the extracted tooth was planned with implantation, with or without bone augmentation

Exclusion criteria were:

- Patients who refused to participate in the study
- Systemic condition that would not allow dental implantation
- Missing data

Patient Groups and Data

Patients were divided into two groups based on the treatment:

- Immediate implantation or immediate augmentation in fresh extraction sockets
- Late implantation (more than 3 months after extraction)

Detailed information regarding each patient was recorded: epidemiologic data, reason for extraction, radiologic and clinical evaluation of the tooth and surrounding bone, clinical symptoms (edema, redness of site, pus release, local sensitivity, bleeding on probing, spontaneous pain, bad odor, bad taste, and recurrent abscess). Intraoperative and postoperative information regarding the extraction and implantation, implantation details, healing period, and implantation and augmentation survival rate was recorded.

The implantation areas were checked for bacterial contamination before and after saline rinses as will be detailed later.

Experimental Protocol

Samples from each site were collected using sterile paper points (SpitzenSz 80, RoekoPapier) which were absorbed in the site for 5 seconds before and after a 30-second rinse with 10-mL sterile saline. The immediate implantation sites were debrided thoroughly before the saline rinse. Each site's three paper points were transferred into a separate Eppendorf tube containing 1 mL of Hytransport medium (Hy Lab, Rehovot). An effort was made to prevent the paper points from coming in contact with saliva or oral cavity organs.

The tubes were vortexed for 30 seconds and five 10-fold serial dilutions were prepared. Samples of 100 μL were plated in triplicate on blood agar plates (Hy Lab, Columbia blood agar, Rehovot) for total bacterial counts. Plates were incubated under anaerobic

conditions for 48 hours at 37°C. The appropriate dilutions were placed on trypticase soy serum bacitracin vancomycin (TSBV), a selective agar medium for isolating and identifying *A actinomycetemcomitans,* and on a selective agar medium (vancomycin and kanamycin blood agar) for isolating and identifying *P gingivalis.* *A actinomycetemcomitans* plates were incubated under capnophilic conditions for 72 hours at 37°C, and *P gingivalis* plates were incubated under anaerobic conditions for 1 week at 37°C.

After incubation, bacterial colonies were detected and enumerated according to colony morphology, before and after the saline rinses.

The clinical situation of the operated area was followed up for at least 3 years and for a maximum of 5 years.

Statistical Analysis

Comparison between levels of contamination in immediate implantation/augmentation sites vs late implantation sites, and the effect of level of contamination on implant and augmentation success, was performed using the χ^2, Fisher exact, and Mann-Whitney tests.

Differences between bacterial levels before and after the saline rinse were analyzed using the Wilcoxon rank test. Differences between the groups regarding age and implant measures were analyzed by means of one-way analysis of variance.

RESULTS

Thirty-two patients participated in the study in an equal proportion of men and women, with a mean age of 49 years (range, 20–83 years). Most patients were categorized as ASA 1 (75%) with a few ASA 2 (according to American Society of Anesthesiology [ASA] system).[31]

Bacterial samples were collected and compared between the immediate implantation/augmentation and late implantation groups, and in each group before and after saline rinse, with the added protocol of infection removal, as appropriate: antibiotic therapy, chlorhexidine mouthwash, or curettage of the socket.

Samples were distributed into two groups according to the sites and treatments: group 1 included bacterial samples from fresh extraction sites planned for immediate implantation or augmentation before saline rinse (17 sites and patients); and group 2, bacterial samples from pristine bone in the late implantation sites (15 sites and patients).

Clinical Signs of Infection

In group 1, there was evidence of clinical infection before the extraction and the immediate implantation/augmentation: edema (30%), redness of area (30%), pus (17%), and local sensitivity (23%). Other symptoms found in the groups were bleeding, spontaneous pain, bad odor, bad taste, and recurrent abscess. The reasons for tooth extraction were mainly cracked teeth and also lost teeth because of periodontitis or dental caries.

Levels of Contamination: Immediate vs Late Implantation/Augmentation Sites

Significant differences were found between the two groups with regard to the level of bacterial contamination (total counts and the two periopathogen counts). Bacterial contamination was 1.2×10^4 CFU/mL in the immediate implantation/augmentation sites, and the level was higher than in the late implantation sites (5×10^2 CFU/mL) for the total bacterial counts ($P < .001$, Mann-Whitney test; Table 1 and Fig 1).

Levels of Contamination: Before vs After Saline Rinse

In group 1 immediate implantation/augmentation sites, bacterial samples were compared before and after saline rinse (17 sites). In group 2, bacterial samples from pristine bone were compared before and after saline rinse (15 sites). Significant differences were found before and after the saline rinse in both groups (Mann-Whitney test; Table 2 and Fig 1). In group 1, the total bacterial counts were 1.2×10^4 CFU/mL and 5.2×10^3 CFU/mL before and after saline rinse, respectively. In group 2, the counts were 5×10^2 CFU/mL before rinse and zero after rinse. No clinical signs of infection were found in group 2 before and after the rinse.

The microbiological examinations revealed bacterial contamination in 100% of the cases in group 1 and in 20% of cases in group 2 (Columbia agar plates showing total counts). Bacterial samples after saline rinse revealed bacterial contamination in 5% of cases in group 1 and no bacterial contamination in group 2. The reduction of total bacterial contamination levels was 57%; the reduction of *A actinomycetemcomitans* and *P gingivalis* levels were 64% and 54%, respectively, in the immediate implantation group. The late implantation group exhibited 100% reduction of bacterial contamination (Fig 2). No case in either group showed evidence of late infection of the graft/implant. All the augmentations and dental implantations survived during the follow-up period (3–5 years).

Levels of Contamination: Maxilla vs Mandible

Statistically significant differences were found between the groups in the level of bacterial contamination in the maxilla and the mandible in the total counts; levels of bacterial contamination with *A actinomycetemcomitans* were higher in mandibular sockets ($P < .05$; Table 3). This finding had no effect on the long-term outcome.

Table 1 Bacterial Counts in the Groups

Bacteria*	Group no.[†]	No. of patients	Mean bacterial counts (CFU/mL)[†]	P
Total	1	17	1.2×10^4	< .000
	2	15	5×10^2	< .000
Aggregatibacter actinomycetemcomitans	1	17	7.1×10^3	< .000
	2	15	0	< .000
Porphyromonas gingivalis	1	17	5.2×10^3	< .001
	2	15	0	< .001

*Columbia agar was used for total bacterial counts; trypticase soy serum bacitracin vancomycin agar for *Aggregatibacter actinomycetemcomitans*; and a selective blood agar for *Porphyromonas gingivalis*.
[†]Group 1 = immediate implantation sites before saline rinse; Group 2 = late implantation before rinse.
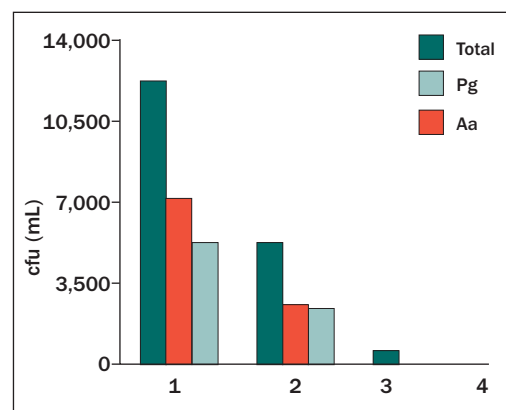

Fig 1 Mean level of bacterial contamination in the three groups. 1 = immediate implantation sites before saline rinse; 2 = immediate implantation sites after saline rinse; 3 = pristine sites before saline rinse; 4 = pristine sites after saline rinse. Columbia agar was used for total bacterial counts; TSBV agar for *A actinomycetemcomitans*; and a selective blood agar for *P gingivalis* ($P < .005$).

Correlations

Significant positive correlations were found between prevalence of the periopathogens *A actinomycetemcomitans* and *P gingivalis* and *(1)* bleeding on probing ($P = .021$ and $P = .038$, respectively), and *(2)* the presence of oral malodor ($P = .034$ and $P = .048$, respectively). No correlation was found between these periopathogens and other symptoms, such as spontaneous pain, recurrent abscess, and bad taste (Mann-Whitney test).

A correlation was also found between the reasons for tooth extraction and the level of bacterial contamination. Bacterial contamination levels were high in sites of extracted periodontal teeth (total = 6,220 CFU/mL; *A actinomycetemcomitans* = 17,000 CFU/mL; *P gingivalis* =11,100 CFU/mL), and in sites of extracted cracked teeth (total = 6,400 CFU/mL; *A actinomycetemcomitans* = 3,400 CFU/mL; *P gingivalis* = 3,300 CFU/mL). The lowest levels of bacterial contamination were found in sites of extracted carious teeth (total = 5,025 CFU/mL; *A actinomycetemcomitans* = 1,750 CFU/mL; *P gingivalis* = 1,138 CFU/mL).

All implanted/augmented sites were followed up for 3 to 5 years, and survival rates were 100%.

DISCUSSION

Immediate implantation and immediate augmentation are widespread procedures with many advantages and high degrees of success.[32–36] One of the causes for dental implantation and augmentation failures is bacterial infection.[37] The main purpose of this study was to evaluate levels of bacterial contamination in clinically infected fresh extraction sites before implantation or augmentation, before and after saline rinse to correlate them with clinical signs, and to investigate their effect on implant survival rate.

It was recently reported that reactive granular tissue from the socket can be used during augmentation and did not interfere with the success of the surgical procedure.[38] The implant survival was not affected in this chronically infected area, using the suitable protocol of meticulous curettage of the socket, saline rinse of the socket, and administration of an appropriate antibiotic protocol and chlorhexidine mouthwash (decontamination protocol). The present study demonstrates the existence of bacterial contamination in implanted augmented sites and that bacterial contamination level is higher in sites of immediate implantation and augmentation, as opposed to late implantation sites. However, the appropriate handling of the infected sites (curettage, rinses, and antibiotic therapy) enables reduction of levels of contamination. All sites of immediate implantation and augmentation showed microbiological evidence of active contamination during the procedure, and the level of contamination was reduced after the saline rinse. This fact may explain the high implant survival rate in the present study and in the study by Koga et al.[38] Late implantation sites without signs of clinical infection showed lower levels of contamination. During osteotomy for implant placement, saline irrigation is used as a coolant. The present authors found that the level of contamination was reduced to zero after the saline rinse. The amount of saline used for cooling is different from that used for decontamination, but based on the high survival rate of dental

Table 2 Bacterial Counts (CFU/mL) in the Groups Before and After Saline Rinse

Bacteria*	Group no.†	No of patients	Mean bacterial counts (CFU/mL)	P
Total	1 before	17	1.2×10^4	< .000
	1 after	17	5.2×10^3	< .000
	2 before	15	5×10^2	< .000
	2 after	15	0	< .000
Aggregatibacter actinomycetemcomitans	1 before	17	7.1×10^3	< .000
	1 after	17	2.55×10^3	< .000
	2 before	15	0	< .000
	2 after	15	0	< .000
Porphyromonas gingivalis	1 before	17	5.2×10^3	< .001
	1 after	17	2.4×10^3	< .001
	2 before	15	0	< .001
	2 after	15	0	< .001

*Columbia agar was used for total bacterial counts; trypticase soy serum bacitracin vancomycin agar for *Aggregatibacter actinomycetemcomitans;* and a selective blood agar for *Porphyromonas gingivalis.*
†Group 1 before= immediate implantation sites before saline rinse; Group 1 after = immediate implantation sites after saline rinse; Group 2 before = late implantation sites before saline rinse; Group 2 after = late implantation sites after saline rinse.
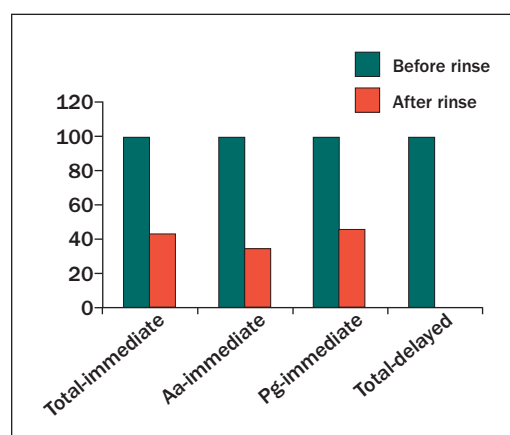

Fig 2 Percentage of bacterial contamination in the groups before and after saline rinse. Total immediate = total counts in immediate implantation sites; *Aa* immediate = *A actinomycetemcomitans* counts in immediate implantation sites; *Pg* immediate = *P gingivalis* counts in immediate implantation sites; total delayed = total counts in late implantation sites.

Table 3 Mean Bacterial Counts Before Rinse in Maxilla vs Mandible

Bacteria*	Jaw	No of patients	Mean bacterial counts (CFU/mL)	P
Total	Maxilla	12	5.6×10^3	.034
	Mandible	20	7.5×10^3	
Aggregatibacter actinomycetemcomitans	Maxilla	12	2.7×10^3	< .05 (.034)
	Mandible	20	4.5×10^3	
Porphyromonas gingivalis	Maxilla	12	2.4×10^3	.136
	Mandible	20	3×10^3	

*Columbia agar was used for total bacterial counts; trypticase soy serum bacitracin vancomycin agar for *Aggregatibacter actinomycetemcomitans;* and a selective blood agar for *Porphyromonas gingivalis.*

implants, the difference may be negligible, and has no effect on the clinical outcome.

Levels of Contamination and Sites

Significantly higher contamination levels were found in the mandible (in total counts and *A actinomycetemcomitans* levels, and higher but not statistically significant *P gingivalis* levels) compared with the maxilla. Koga et al[38] also reported lower counts of bacterial contamination in the maxilla than those in the mandible (P < .01) in bone chips collected from dental implants sites.

One possible explanation is elevated saliva secretion and stasis near the mandibular sites, which in turn, supplies a richer environment for the bacteria. Another possible explanation is difference in vascular supply to the jaw bones and gums. In the maxilla, the higher vascular supply enables the body's defense mechanism to reach the infected area and fight against it quickly.

Different levels of contamination were found in the fresh extracted sockets based on the reason for extraction. The highest level of contamination was found in periodontal or cracked teeth extraction sockets and less in sites of carious teeth extraction. The anaerobic

conditions of periodontal sockets and cracked teeth enable *A actinomycetemcomitans* and *P gingivalis* to thrive and reach higher numbers of bacteria compared with carious teeth extraction sockets with no anaerobic conditions.

Levels of Contamination and Clinical Signs

A significant positive correlation was found between *A actinomycetemcomitans* and *P gingivalis* levels and oral malodor and bleeding index. Oral malodor was an expected finding. Because the metabolism of these two periopathogens is proteolytic, malodorous compounds such as volatile sulphur compounds are byproducts of such a metabolism. It is also reasonable to expect a higher bleeding tendency in the presence of bacterial contamination. This correlation may help the clinician treating hopeless teeth by paying more attention to sites with bleeding or malodor.

According to traditional conventions, infection in implantation sites is a contraindication for the procedure because of the risk for implant failure. In the present study, all immediate augmentation sites were preceded by cracked infected teeth and periodontally/endondotally lost teeth. The decontamination protocol before implantation or augmentation enabled immediate augmentation/implantation without compromising the result. The reduction in bacterial contamination provides the laboratory verification of the clinical results.

However, because of the small sample size in the present study, a cautious speculation may be reached regarding the high survival rate of dental implantation and socket augmentation in infected sockets.

A previous investigation showed bacterial presence in 32% of bone samples in healing sockets of 47 patients.[22] In that research, bacterial presence was concluded by detecting bacterial DNA in the bone, without clinical evidence of infection. The present study showed microbial evidence for infection in late implantation sites without clinical implications. Dental implantation and augmentation saline rinses are used routinely, and according to the study findings, lower the level of contamination, thereby leading to a low rate of postoperative infection or failure of procedure in fresh infected sockets and pristine bone.

CONCLUSIONS

Within the limitations of this study, it can be concluded that fresh extraction sockets with clinical signs of infection show bacterial presence. Saline rinse for decontamination reduces the bacterial level. The level of contamination is higher in periodontal or cracked teeth and in the mandible. Pristine bone shows lower bacterial level. Saline rinse used during the procedure in addition to the decontamination protocol showed a reduction in the level of bacterial contamination.

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