Bacterial Contamination of Oral Implant Associated Autogenous Bone Grafts

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I.Purpose:

The successful longevity of dental implants is supported by adequate quantity and quality of alveolar bone. Autogeneous bone graft is considered to be the best choice for reconstructive implant surgery. However, it has been reported that microbial infection has a detrimental effect upon the success rates of endosseous dental implants and on guided bone regeneration[1]. Hence, we investigated the bacterial contamination of autogeneous bone (CBD) that was harvested from the implant's site during the surgery. In addition, the excellent sterilization potential of the strongly acidic electrolyzed water (SAEW) had gotten a lot of attention recently and its use is widely increasing. (Table 1,2,3)[2] A decontaminating method of CBD using SAEW was examined at the same time.

Table 1 The features of strongly acidic electrolyzed water (SAEW)

Specialized electrolysis processing (0.05% NaCl solution)
High disinfection effect of high oxidoreduction potential (ORP)
(pH 2.7, ORP 1100mV)

Broad antibacterial spectrum No allergy No side effect No residue	Strong virucidal effect (including HIV, HBV) Maintains high safety level No resistant bacteria	Reasonably cheap (240£/$ only) Environment friendly

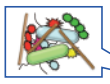


Table 2 The sterilization potential on fungal disinfection effect of a strongly acidic electrolyzed water (SAEW) [2]

Bacteria / Fungous		Infection value Untreated	SAEW-treated
Bacteria	*Staphylococcus aureus*	10^{7}	0
	Escherichia coli	10^{8}	0
	Bacillus subtilis	10^{8}	10^{2}
Fungous	*Candida spp.*	10^{8}	0

Each suspension of the bacteria (0.1ml) and SAEW (0.9ml) were inoculated for three minutes at room temperature. The suspension was diluted 10-fold with nutrient broth and the viable cells were calculated.

Table 3 Inactivation effect of SAEW on various viruses [2]

Virus	Infection value Untreated	SAEW-treated
Herpes simplex,Type II	$10^{5.8}$	0
Cytomegalovirus	$10^{5.2}$	0
Human immunodeficiency virus (HIV)	$10^{4.5}$	0
Polio virus Types 1,2,3	$10^{5.3}$	0

After 0.1ml of each virus solution and 0.9ml of SAEW(1st day after preparation) were reacted at room temperature for 3 minutes, 0.1ml of the 10-fold stepwise diluted solutions was individually inoculated into sensitive cells to obtain the infection value. (Shimizu, Tohoku University)

II.Materials and Methods:

Seventeen patients (6 men, 11 women; mean age 52.6 years; range, 18 to 69) who received implant surgery in a private dental clinic in Saitama prefecture were selected for this study. The study protocol was explained to each patients and signed informed consent was obtained.Oral cavity was made asepsis as much as possible before the surgical procedures and bone samples were harvested.
Collected bone samples were divided into five groups as follows;

G-I: Swab collection from alveolar bone surface on flap opening,
G-II: CBD collection by a disposable Osseous Collector {OTA, California, USA (Figure 1-1, 1-2) } from a donor site; a stringent aspiration protocol,
G-III: CBD by using a suction tip with bone trap {Osseous Collection Trap; SALVIN®, Charlotte, NC, USA, (Figure 2, 3)}from the donor site; a non-stringent aspiration protocol,
G-IV: CBD immersed in alcohol solution before grafting (as positive control),
G-V: CBD processed with a strongly acidic electrolyzed water (SAEW) with pH level of 2.7 {PANACEE®, JAPAN INTEK, Hiroshima,(Figure 4)}.

The features of a strongly acidic electrolyzed water (SAEW) are shown in Table 1. All these samples from the above five groups were immediately transported for bacterial culture using Trypticase Soy Broth (Japan BD, Tokyo) as a transporting medium. Samples were cultured in broth at 35ºC for 24 h and then plated separately on appropriate media; Trypticase Soy Blood Agar (Japan BD, Tokyo,35ºC for 48 h), Modified Drigalski Agar (Japan BD, Tokyo, 35ºC for 48 h), Chocolate Agar (Japan BD, Tokyo, 35ºC for 24 h) and Brucella HK Agar (Japan BD, Tokyo, 35ºC for 48 h).Incubation was carried out at microbiological facility of BML Co.(Tokyo, Japan) .
The number of colonies was caluculated by using a colony counter {Anaeropack system, MITSUBISHI GAS CHEMICAL Co.,(Figure 5)}.

Figure 1-1 A disposable CBD collector

Figure 1-2
Snout of CBD collector

Figure 2 Suction filter apparatus

Figure 3
Net for bone trap

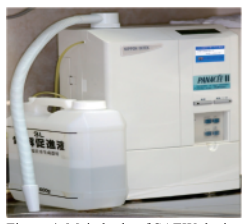
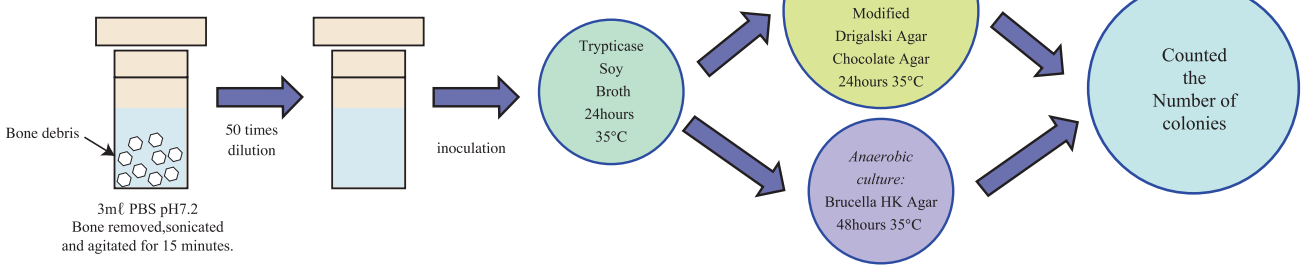


Figure 4 Main body of SAEW device

Figure 5
Methods of processing the samples and bacterial culture



III.Results:

Aerobic cocci dominated the isolates in almost all groups from patients (G-I to G-III; ranged from about 300,000 to 30,000,000 CFU/ml , Figures 6,7-1,7-2). *Streptococcus salivarius* found to be predominant, identified almost in every sample and *Streptococcus mitis* was second predominant. In all G-III samples *α-Streptococcus spp., γ-Streptococcus spp., Neisseria spp., Pseudomonas aeruginosa* were identified almost regularly. However, *α-Streptococcus spp*. and *γ-Streptococcus spp*. were less and irregular in G-II samples. Interestingly, the contaminated CBD that showed high contamination in G-III (about 30,000,000 CFU/ml) displayed a sharp reduction in the number of revived bacteria (G-V; about 4,000 CFU/ml) after processing with SAEW. SEM observation also demonstrated the reduction of bacterial cells in SAEW treated CBD (Figures 7-3,7-4).

Figure 6 Bacterial cell counts of CBD during implant surgery (mean±SD,n=17)

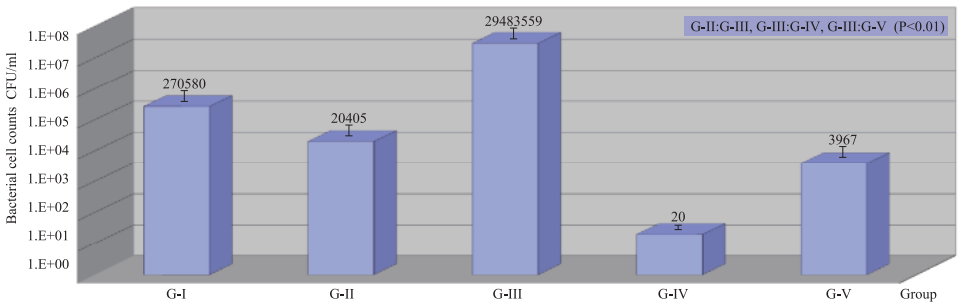
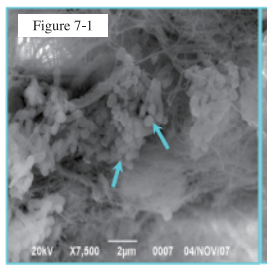


Figure 7-1, 7-2: SEM pictures of CBD. Blue arrows indicate colonies of bacteria (cocci), white arrow indicates a colony of rod shaped bacteria.



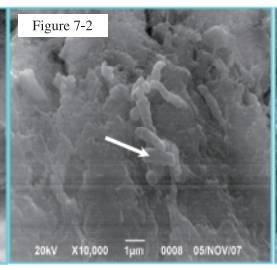
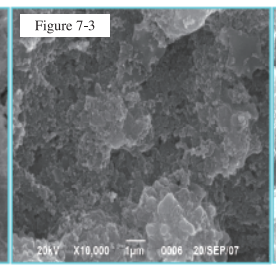
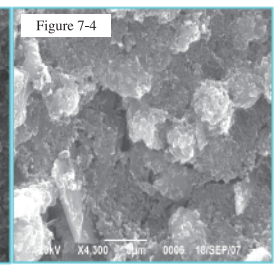


Figure 7-3, 7-4 SEM pictures of SAEW treated disinfected CBD. Colonies of bacteria (cocci) and rod shaped bacteria could not observe.





IV.Discussion:

The CBD during implant surgery was contaminated by aerobic bacteria regardless of the harvesting method. The indirect method with a suction filter apparatus (G-III) showed significantly more bacterial contamination than the direct method (G-II). The number of total bacterial cell counts of G-III corresponded to the report that viable micro-organisms may reach up to 10^{9} colony forming units (CFU) per ml of saliva [3]. Therefore, it was suggested that more effective additional sanitizing methods other than conventional stringent bone collection protocol is needed to reduce microbial contamination of CBD.
As for the method of aseptic processing of CBD, the number of bacteria in processed group (G-IV, G-V) was statistically smaller than non-processed group (G-III). Though sterilization effect of SAEW was somewhat inferior to alcohol, SAEW didn't show any adverse effect to mucous membrane or any other tissues that was observed in case of alcohol solution. The safety of SAEW was also proven by animal experiment with rats [4]. Moreover, the price of SAEW is cost effective compared with other disinfectants. While the use of SAEW disinfected bacteria and viruses in ten minutes [2], it didn't produce resistant bacteria [5]. The elimination method of bacteria by SAEW should be further examined.

V.Conclusion:

A stringent bone collection protocol showed less bacterial contamination than a non-stringent bone collection protocol. The antiseptic process with SAEW was effective to reduce bacterial contamination level.

References:

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