

Silver-chlorexidine-liquid is effective to sterilize screw retaining abutment to implant space in already inserted fixture: An in vitro study

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INTRODUCTION

Contamination of screw retaining abutment to implant space (SRAIS) and microbial leakage between implant-abutment junction (IAJ) can cause

or maintain peri-implantitis.¹ Many implant systems have been created in order to prevent bacterial penetration/leakage and bone resorption at IAJ level, but, even in the most advanced implant

ABSTRACT:

Contamination of screw retaining abutment to implant space (SRAIS) creates an infected room at the implant abutment junction (IAJ) that can cause or maintain peri-implantitis. Aim of the present study is to determine whether the treatment of SRAIS with silver-chlorexidine liquid (named SBC-40) is able to sterilize SRAIS.

Materials and methods: A total of eight implants were used (Edierre Implant System, Edierre SpA, Genova, Italy). The SRAIS of four fixtures were firstly contaminated with 10 micro-litres of pure bacteria (i.e. genetically modified *Escherichia coli*). Subsequently SRAIS were treated with SBC-40 for 20 seconds and then replaced with Lysogeny Broth (LB). The remaining four implants were just filled with LB. Abutment were then screwed. All 8 fixtures were placed in tubes and immersed in LB plus *E. coli* to cover outer implant-abutment junction (IAJ). After 24 hours IAJ were opened and liquid collected from SRAIS by using paper tips. Same sampling was performed in LB out of implants. The tips were immersed in sterile LB and bacteria viability was determined by measuring their Optical Density (OD) at three time points.

Results: In untreated implants, bacteria grew (internally and externally) for the first 48 hours but subsequently they started to die. In treated implants, instead, bacteria grew just in the space surrounding fixtures suggesting that, even if bacteria were able to enter in SRAIS, they immediately died, thanks to the presence of SBC-40.

Conclusions: SBC-40 is potentially able to sterile the SRAIS in fixtures already inserted in patients.

Key words: Bone resorption, implant-abutment connection, microbiological leakage, peri-implantitis.

systems, there is no sealant connection.² Microbial leakage is an important factor for chronic inflammatory infiltration and marginal bone resorption.³ New designs of IAJ aim to improve precise tight mechanical connection and thus minimize the bacterial leakage. This process is technically very difficult as bacteria are around 1–10µm in length.⁴

The most successful two-pieces dental implant systems use screw-retained or cemented-retained abutments. This system provides two interfaces: one between implant and abutment and the other between abutments and prosthetic crown, with a significant accumulation of bacterial bio-film. When IAJ is under loading, the size of gaps increases due to micro-movements which also have a pump effect, sucking bacteria from the external environment or pumping them around implant.⁵

Some studies quantify microbiological penetration between micro-gaps at IAJ level, showing that there is no sealing IAJ till now.^{6, 7} Antibacterial agents has been studied to cleaning SRAIS , but none have been demonstrated to be completely effective.⁸ Thus a new silver-clorexidine liquid named SBC-40 is here investigated in an in vitro model to verify its effectiveness in sterilising SRAIS.

Material and Methods

Silver-clorexidine liquid named SBC-40

The liquid is an aqueous solution containing a thermally and photochemically stable anionic silver complex, where the silver ion is coordinated to the sulfur of the 2-mercapto-5-benzimidazole sulfonic ligand (Figure 1A). Pharmaceutical compositions based on photochemically stable silver complexes chlorexidine and cationic surfactants; (PCT/IB2013/054649) in mixture with didecyldimethylammonium chloride (Figure 1B) and with chlorhexidine digluconate (Figure 1C).

Preparation of the anionic complex ($\text{Ag}^+ \cdot [\text{L} \cdot \text{Na}^+]$) as Sodium salt.

The anionic complex ($\text{Ag}^+ \cdot [\text{L} \cdot \text{Na}^+]$) with $[\text{L} \cdot \text{Na}^+] = 2\text{-Mercapto-5-benzimidazolesulfonic acid sodium salt}$ was prepared by addition of the ligands $[\text{L} \cdot \text{Na}^+]$ to an aqueous solution containing a stechiometric amount of AgNO_3 . The product, formed instantly, was precipitated by addition of ethanol/diethyl ether and the solid dried at 50 °C for 12 hr in a ventilated oven.

Stability tests did not show any change in colour or composition after 12 months to ambient light exposure. In comparable conditions, solutions of Silver salts, such as nitrate, acetate, perchlorate or hexafluorophosphate, showed formation of a black Ag° precipitate after few minutes.

Preparation of SBC-40

The product is composed by an aqueous solution containing the anionic silver complex, where silver ions are present at 0.00022%, didecyldimethylammonium chloride at 0.57% and of chlorhexidine digluconate at 0.11%. The product can be prepared by direct dissolution of the different components in sterile water (Pharmaceutical compositions based on photochemically stable silver complexes chlorexidine and cationic surfactants. PCT/IB2013/054649).

Implants treatment

To verify the antibacterial power of SBC-40 in implant already inserted in patients, an in vitro model was developed. A genetically modified *Escherichia coli* containing synthetic DNA target sequences in their plasmid was used. Plasmids contain two sequences specific for two bacterial species (i.e. *Porphyromonas gingivalis* and *Tannerella forsythia*) as well as two genes for antibiotic resistance (Kanamycin and Ampicillin).

Bacteria were cultured in Lysogeny Broth (LB) with both Kanamycin and Ampicillin (at a final concentration of 50ug/ml), in order to avoid the growth of non-specific bacteria. Cultures were then let at 37°C for 12-18 hours in a shaking incubator.

A total of eight implants were used (Edierre Implant System, Edierre SpA, Genova, Italy). A drawn picture of the IAJ is given in Figure 2. The SRAIS of four fixtures were contaminated with 10 micro-litres of pure bacteria and subsequently washed for 20 seconds with SBC-40. Then SBC-40 was replaced with pure LB and abutment screwed. The remaining four implants were not treated with SBC-40 and just filled with LB. Subsequently, 20 micro-litres of *E. coli* culture were used to “contaminate” fresh LB contained in a micro-centrifuge tube where implants were placed. The volume of the solution was enough to submerge the IAJ. Tubes were then incubated at 37°C for 24 hours in a heater, in order to allow bacterial growth.

To check for the presence of any bacterial contamination, a negative control containing only LB with antibiotics both inside and outside implant was included in the study.

Twenty-four hours later, each implant was opened and samples were collected by dipping a paper probe both outside and inside implants (Figure 3). A total of 4 paper probes for implant were collected. Two out of these four probes were used to measure the bacteria optical density outside the implant, and the remaining were employed for the internal measurement.

Optical density (OD) measurement

Paper probes were put, in a pair, in a micro-centrifuge tube with 200ul of LB with selective antibiotics, and let at 37°C in a heater for optical density measurement at four time point: 24, 48, 72 and 96 hours. Bacterial OD600 was measured by using Nano-Drop 2000c spectrophotometer (Thermo scientific, Wilmington, USA). For each sample 1uL of LB with or without bacteria was measured. Sterile LB broth with antibiotic, instead, was used to blank the spectrophotometer.

Statistical analysis

To evaluate if the different bacterial growth among SBC-40 treated and untreated implants was statistically significant, Student's t-test was applied on average OD both for external and internal measurement, at each time point.

RESULTS

Figure 4 shows the bacterial growth by measuring optical density at 600nm (OD600). Average OD values between treated and untreated implants were compared, between internal and external medium at each time point. In untreated implants, bacteria grew (internally and externally) for the first 48 hours but subsequently they started to die, probably as a consequence of nutrient consumption. In SBC-40 treated implants, bacteria grew just out of implants suggesting that, even if bacteria were able penetrating the screw-retaining-implant-space, they immediately died thanks to the presence of SBC-40.

Comparing treated and untreated-implants, differences in bacterial growth were statistically significant at 24, 48 and 72 hours. After 96 hours, in fact, bacteria died both in treated and in untreated implants and thus no difference was detectable

(Table 1). Externally, no differences were revealed (Table 2).

DISCUSSION

Silver ion is an effective antimicrobial agent and shows a rather broad spectrum of bactericidal activity consistent with different mechanisms of action, which depends on the binding site. For instance, when binding occurs at the bacterial cell wall, ruptures can occur.⁹ When bound to proteins involved in respiration and nutrition of the organism, silver can block these processes with the consequent elimination of the bacteria.¹⁰ When binding DNA, silver can affect the replication and division of the organism.¹¹

Since bacterial passage through IAJ has been demonstrated in several in vitro studies (12-14) and SRAIS is a reservoir of bacteria which potentially cause peri-implantitis, the effect of SBC-40 in sterilizing SRAIS was proven in the present in vitro model.

Colonization of the IAJ is associated to many factors: tight connection between the implant and prosthetic components, torque used to connect abutment to fixture, and overloading after prosthetic rehabilitation. A perfect sealing between implant and abutment was never found (to our best knowledge) and micro-gaps are colonized by periodontal bacteria being risk factor for peri-implantitis.

Even if the design of IAJ can limit the bacteria penetration into the internal part of a dental implant, microbiological studies confirmed the passage of bacteria around IAJ at level of peri-implant tissues.^{12, 13} In an in vitro study, Dias et al demonstrated microbial penetration of IAJ micro-gap of fixtures with an external hex design.¹² Cosyn et al studied microbial leakage in different implant-abutment connections, showing microbial contamination in implant with an internal connection.¹³ Kotouzis et al evaluated bacterial penetration along the IAJ micro-gap and established bacterial colonization in an in vitro experiment using loading forces.¹⁴ Other studies investigated micro-organism penetration through IAJ in order to find an efficient bacterial seal system.¹⁵⁻¹⁸ With the two-piece implant system, the abutment is retained to fixture with mechanical methods. This results in gaps and cavities between the IAJ becoming a bacteriological reservoir. This gap favours an

Table 1: Inner average OD values of SBC40 treated and untreated implants, and their comparison

Time point	OD600 Untreated Implants	OD600 Treated Implants	P-value ^(a)
24h	0,050	0,007	0,0264
48h	0,064	0,004	0,0248
72h	0,018	0,003	0,0299
96h	0,003	0,001	0,1901

^(a)data were considered statistically significant for P-value <0.05

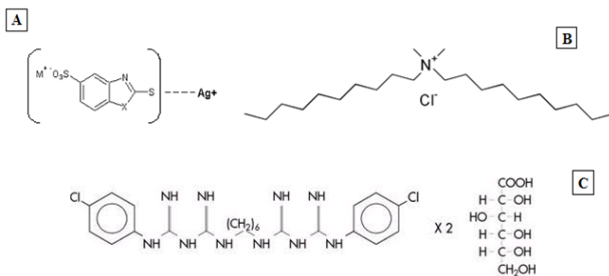


Figure 1: Silver-chlorexidine liquid named SBC-40. Silver ion coordinated to the sulfur of the 2-mercapto-5-benzimidazole sulfonic ligand (A) in a mixture with Didecylmethylammonium chloride (B) and Chlorhexidine digluconate (C).

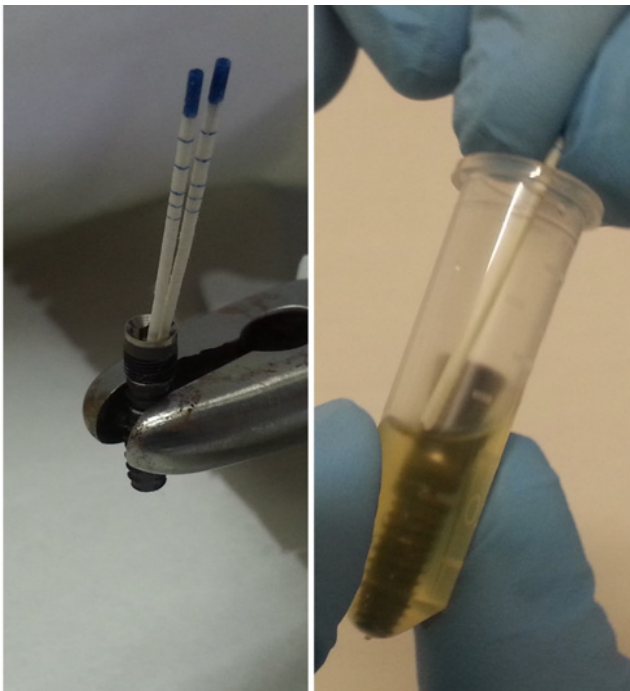


Figure 3: Samples collection- Samples were collected by dipping a paper probe both inside (on the left) and outside (on the right) the implants.

Table 2. Outer average OD values of SBC40 treated and untreated implants, and their comparison.

Time point	OD600 Untreated Implants	OD600 Treated Implants	P-value ^(a)
24h	0,110	0,079	0,2293
48h	0,127	0,116	0,3268
72h	0,045	0,040	0,5765
96h	0,009	0,007	0,5496

^(a)data were considered statistically significant for P-value <0.05

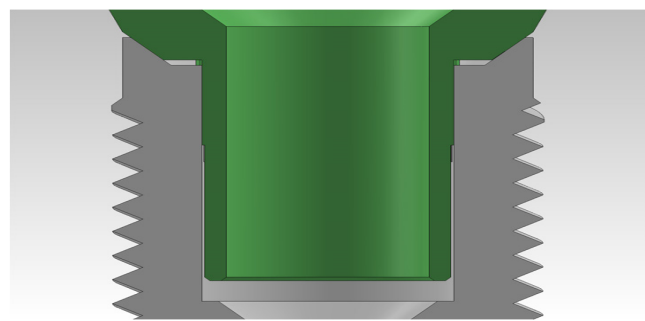


Figure 2: Implant-abutment junction. (Drawn picture of the IAC.)

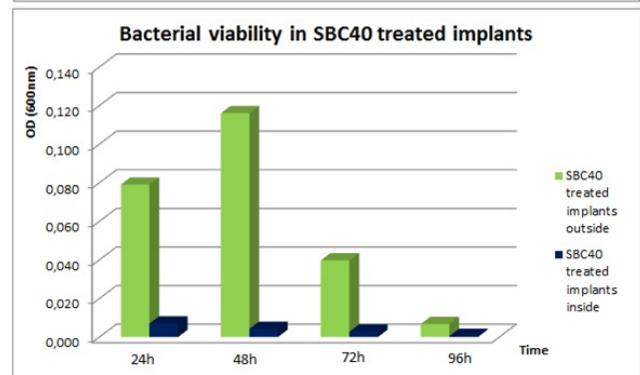
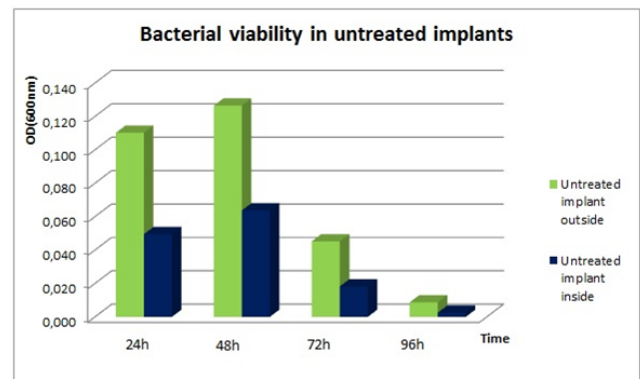


Figure 4: Bacterial viability- Optical density (OD) measurement inside and outside untreated and treated implants, at four different time points.

inflammatory process in peri-implant tissues. Microbial colonization of the IAJ has consequences as bone resorption as clearly demonstrated by Lazzara in his work on "Platform Switching".¹⁹

CONCLUSION

Since there is no IAJ which is sealant, today, a solution able to clean and to sterilize the screw retaining abutment to implant space can be very useful in the dental daily practice. The present report demonstrated that SBC-40 is an effective antibacterial solution in an in vitro model which can be potentially used in already inserted two-piece implants. SBC-40 could be considered a new frontier in the era of peri-implantitis prevention.

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