

THE ASSESSMENT OF ANTIBACTERIAL EFFECT OF 0.1% OCTENIDINE SOLUTION ON *IN VITRO* BIOFILMS OF STREPTOCOCCUS SALIVARIUS USING BACTERIAL LIVE/DEAD VIABILITY STAINING AND CFU COUNTING

¹DERAR AL-SEBAIE

ABSTRACT

The purpose of this study was to assess the antibacterial effect of 0,1% octenidole solution on Streptococcus salivarius biofilms when using live / dead staining and standard CFU (colony forming units) counting for determination of the bacterial survival rate.

Streptococcus salivarius biofilms were grown in vitro for 42h on 12 mm titanium discs in a flow chamber system. Formed Biofilms were exposed to 0,1% octenidine (Schülke,..) solution for 30s, 60s, 120s or 300s. The bacterial kill rate was determined by plating on TSB agar and CFU counting as well as live / dead viability staining (BacLight Viability Kit, Invitrogen,) and confocal laser scanning microscopy (CLSM) analyses.

Using the plating method and CFU counting, complete killing of adherent could be observed after 30s treatment. Live / dead staining showed complete killing of bacteria even after 5 minutes immersion of biofilms in octenidine solution.

According to this study, significant differences of bacterial survival rates were observed with the two methods used. Therefore, it was concluded that special care should be taken when choosing a laboratory method for the evaluation of antibacterial effects.

Key Words: *Streptococcus salivarius, flow chambers, titanium discs ,octenaidol mouth wash 0.1% concentration, live / dead staining and culture plating over night culture, titanium disc.*

INTRODUCTION

Streptococcus salivarius is the most common bacterium normal flora of human oral cavity. It may enter the blood stream by accident during dental treatment or even when brushing the teeth. It is the first bacterium which colonizes the dental plaque, before being joined by numerous other species of various genera.⁶ Therefore it seems to be the pioneer in colonizing dental plaque. It creates favorable conditions for other species to colonize. It is also a bacterium which plays the role of moderator, permitting the implantation of bacteria which are harmful to the health of the oral cavity.

METHODOLOGY

Biofilms of *Streptococcus salivarius* DSM 20067 (DSMZ, Braunschweig, Germany) were grown on 12 mm polished titanium discs (Pic 3), surface roughness < 1µm, Titanium grade 4). Test specimens were placed in flow-chambers, each manufactured with a

12 mm circular socket (Pic 1, 2). Discs were overflowed for 42 hours at room temperature by a suspension of *Streptococcus salivarius* in TSB media (Oxoid,...). Bacterial cultivation took place in a glass bioreactor under constant stirring (250 rpm). To ensure a constant flow rate of 16 ml/min during the complete experiment, a multi-channel peristaltic pump (Ismatec IPC,..., Germany) was used to transport the medium from the external reservoir to the flow chambers.¹⁻³

Octenidine treatment of biofilms

Titanium discs with adherent biofilms were removed from the flow chambers. Non adherent cells were washed away by rinsing three times with ddH₂O. Test specimens were immersed in 0, 1% octenidine solution (Schulke Chemie,..., Germany) for 30s, 60s, 120s or 300s and subsequently rinsed three times with ddH₂O. Then, bacterial cells were stained or plated on solid media. Each experiment was performed in triplicate.^{4,9}

Live/Dead staining

After octenidine treatment, cells were stained with the BacLight viability staining kit (Invitrogen, Germany) according to the manufacturer's instructions. The

¹ Specialist Dental Prosthesis & Implantology, Prince Rashid Hospital, Royal Medical Services, Jordan Email: derar25@yahoo.com

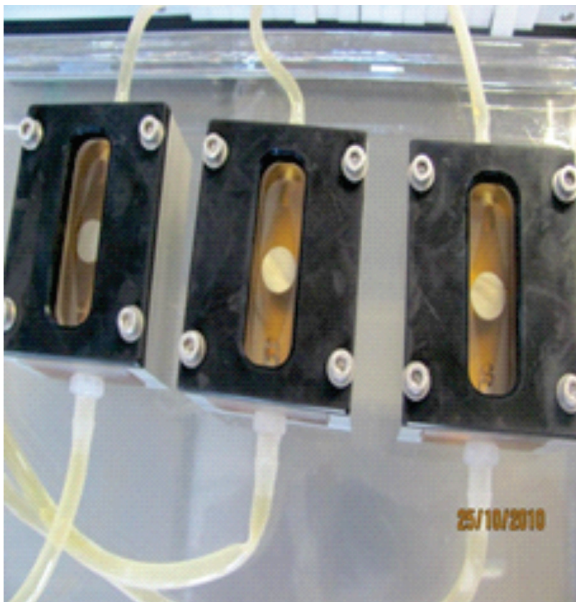
Received for Publication: April 1, 2014

Revision Received: April 23, 2014

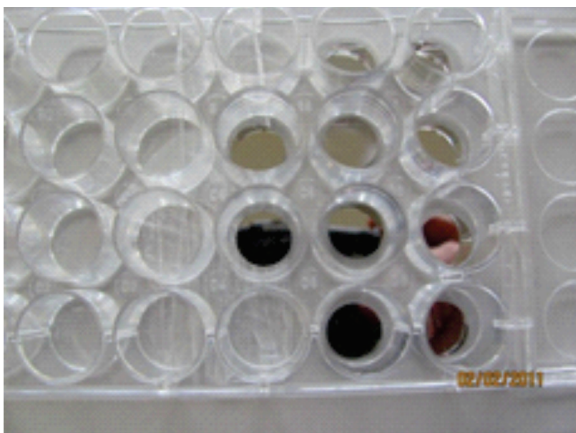
Revision Accepted: April 26, 2014



Pic 1: Complete system



Pic 2: Flow chambers with titanium discs



Pic 3: Titanium discs

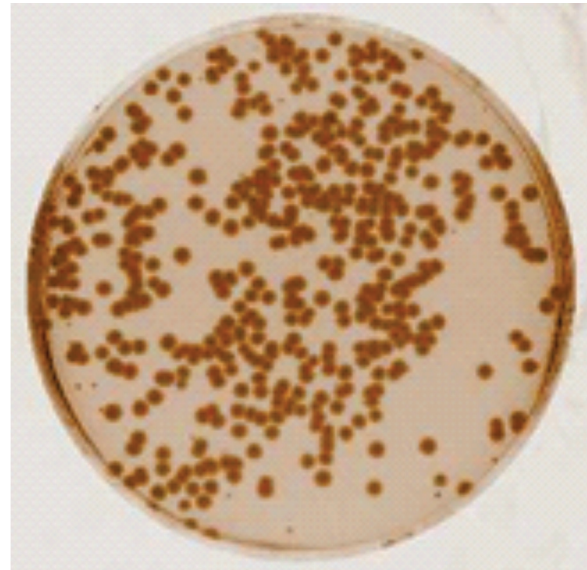


Fig 1: Negative control

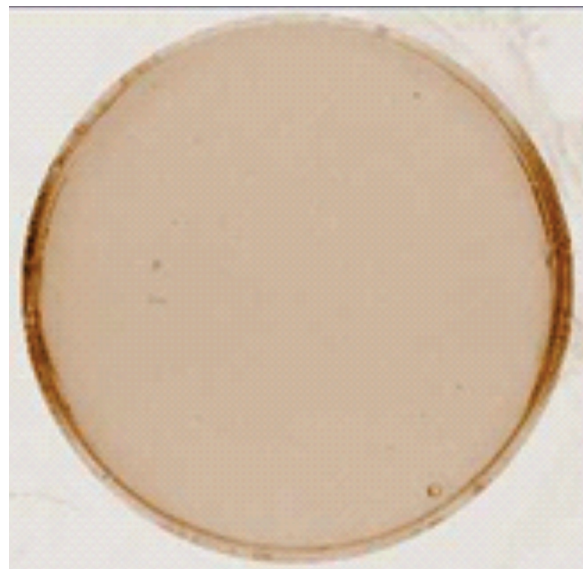


Fig 2: 30 Sec.

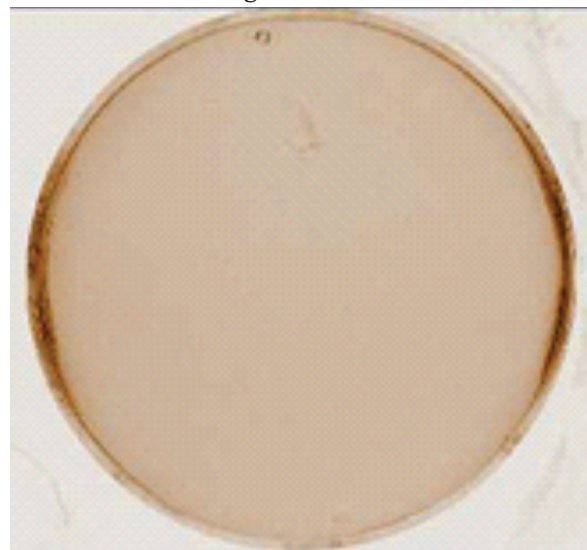


Fig 3: 60 Sec

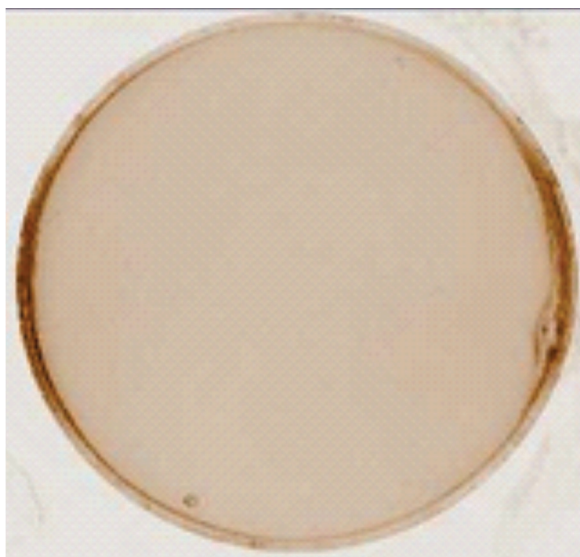


Fig 4: 120 sec.

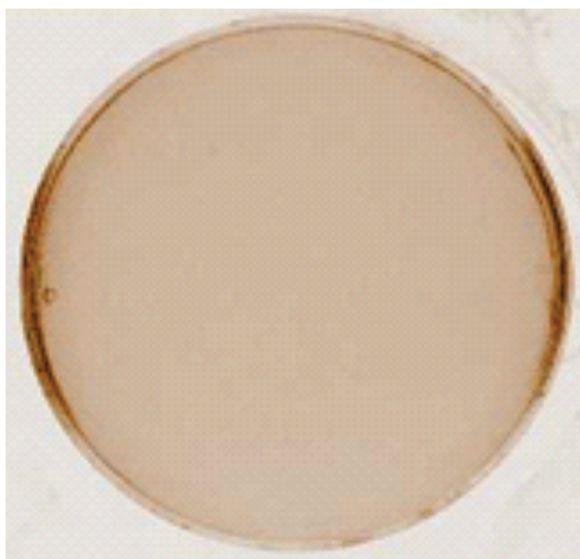


Fig 5: 300 sec.

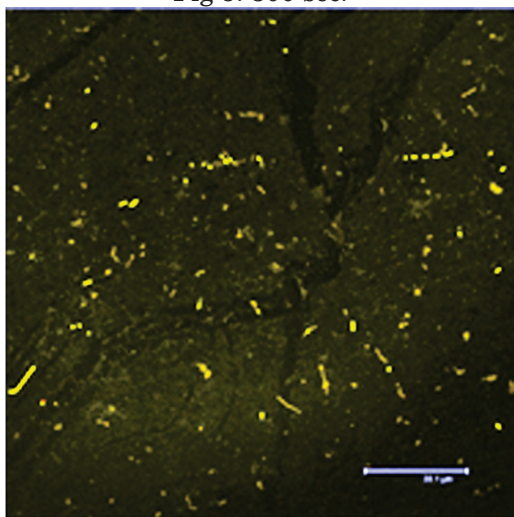


Fig 6: Negative control

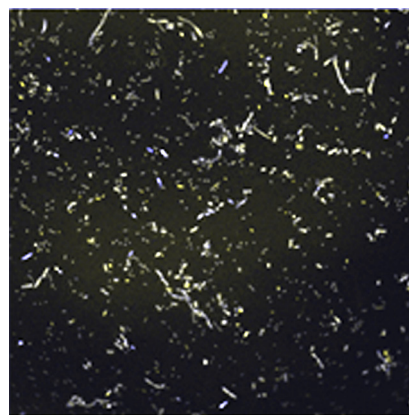


Fig 7: 30 sec.

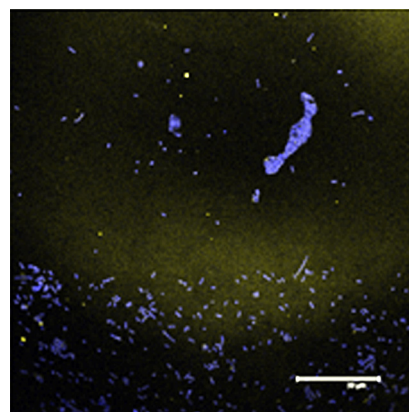


Fig 8: 60 sec.

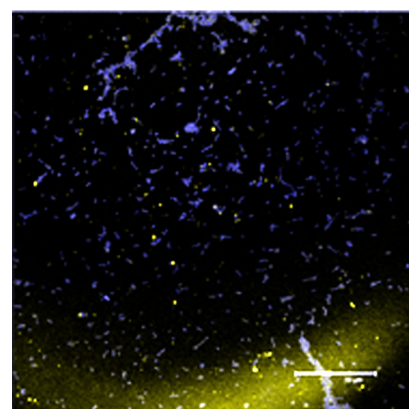


Fig 9: 120sec.

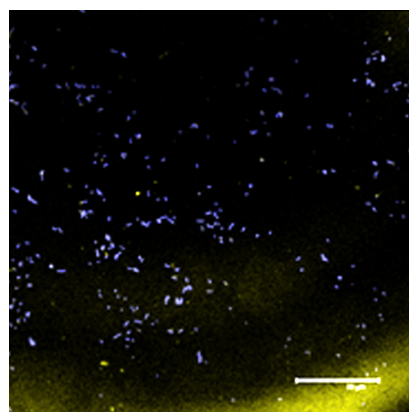


Fig 10: 300 sec.

biofilms were fixed with 2,5% glutaraldehyde solution, (Carl Roth, ..., Germany). and the ratio of live: dead bacterial cells was estimated by confocal laser scanning microscopy (Zeiss,).⁹⁻¹¹

Plating method

Adherent cells on the titanium surfaces were removed by ultrasonification in a Bandelin XYZ ultrasonic bath (20 minutes at 37°C power: 100%) in a total volume of 5 ml PBS buffer (Dublecco,). A dilution series was prepared and (what dilutions) and equal volumes of XXX µl were plated on TSB agar. After incubation at 37°C for 24h the number of CFU was determined (Fig 1, 2, 3, 4, 5).^{5,6}

RESULTS

After exposure of *Streptococcus salivarius* biofilms to octenidine for at least 30s a complete killing of bacteria were detected when using the plating method to evaluate the antibacterial effect. In contrast, CLSM microscopy analysis showed complete killing even after 5 minutes exposure to octenidine. Exposure to octenidine for 30s-120s in combination with staining resulted in an overall survival rate between 20-50%. (Fig 6, 7, 8, 9, 10).¹³⁻¹⁴

DISCUSSION

The experiments showed that a significant difference between the antibacterial effect of octenidine analysed by staining and the one analysed by conventional plating.⁴ Plating is a relatively fast and easy to perform laboratory method. Dead or heavily damaged cells can not divide anymore. This results in complete absence of bacterial growth on solid or in liquid growth media. Under the given experimental conditions live/dead staining was inferior to the plating method and proved to be an unreliable technique for the analyses of the antibacterial effect of octenidine on *Streptococcus salivarius* biofilms. Probably short exposure times of *Streptococcus salivarius* cells to octenidine do not lead to structural damages big enough to allow the fluorophor propidium iodide to enter the cells.^{5,6} We could show that the assessment of an antibacterial effect could be influenced to a great extent by the applied method. Therefore we conclude that special care should be taken when choosing a method for the evaluation of antibacterial effects.^{15,16}

REFERENCES

- 1 Koburger T, Hubner NO, Braun M, Sibert J, Kramer A. Standardized comparison of antiseptic efficacy of triclosan, PVP-iodine, Octenadine dihydrochloride, polyhexadine and chlorhexidine digluconate. *J Antimicrob Chemother.* 2010 Aug; 65(8) 1712-9. Epub 2010 Jun 15
- 2 Rohrer N, Widmer AF, Waltimo T, Kulik EM, Weiger R, Filipuzzi-Jenny E, Walter C. Antimicrobial efficacy of 3 oral antiseptics containing Octenidine, polyhexamethylene biguanide, or Citroxx; can chlorhexidine be replaced? *Infect Control Hosp Epidemiol.* 2010 Jul; 31(7): 733-9.
- 3 Reinier Mutters, Helene Bykow, Majid Kulhat, Microbiocidal efficacy of anyiseptic Octenidine, or amine-/tin-fluoride-based oral rinses on periodontal pathogens, *GMS Interdisciplinary Hospital Hygiene* 2007, Vol 2(2).
- 4 Krishna BV, Gibb Ap. Use of Octenidine dihydrochloride in methicillin-resistant *Staphylococcus aureus* decolonisation regimens: a literature review. *J Hosp Infect.* 2010 Mar; 74(3) 199-203. Epub 2010 Jan 8.
- 5 Trili Re, Turan Y, Akal N, Karahan ZC. In vitro antimicrobial activity of several concentrations of NaOCl and Octenisept in elimination of endodontic pathogens, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009 Nov; 108(5): e 117-20.
- 6 Sennhenn-Krichner S, Wolff N, Klaue S, Mergaryan H, Borg-von Zepelin M. Decontamination efficacy of antiseptic agents on in vivo grown biofilms on rough titanium surfaces. *Quintessence int.* 2009 Nov-Dec; 40(10): e80-8.
- 7 Kocak MM, Ozcan S, Kocak S, Topuz O, Erten H. Comparison of the efficacy of three different mouthrinse solutions in decreasing the level of *Streptococcus mutans* in saliva, *Eur J Dent.* 2009 Jan; (1): 57-61.
- 8 Dogan AA, Adiloglu AK, Onal S, Cetin ES, Polat E, Uskun E, Koksall F. Short term relative anti bacterial effect of octenidine dihydrochloride on the oral microflora in orthodontically treated patients. *Int J Infect Dis* 2008 Nov; 12(6):e 19-25. Epub 2008 Jun 6.
- 9 Tandjung L, Waltimo T, Hauser I, Heide P, Decker EM, weiger R. Octenidine in root canal and dentine disinfectant ex vivo, *Int Endod J.* 2007 Nov; 40(11) : 845-51. E Pub 2007 Aug 30.
- 10 Shapiro S, Gierstn E, Guggenheim B. An in vitro oral biofilm model for comparing the efficacy of antimicrobial mouthrinses. *Caries Res.* 2002 Mar-Apr; 36(2): 93-100.
- 11 Braunwarth H. Octenidinedihydrochloride a new antiseptic agent. *Krankenpfl J.* 2000 Mar-Apr; 38(3): 63.
- 12 Karmer A, Hoppe H, Krull B, Pitten FA, Rosenau S. Anti septic efficacy and acceptance of octenisept computed with common antiseptic mouthwashes. *Zentralbl Hyg Umweltmed.* 1998 Feb; (5-6): 443-56.
- 13 Setz J, Heeg P. Disinfection of pumice. *J Prosthet Dent.* 1996 Oct; 76(4) 448-50.
- 14 Smith RN, Andersen RN, Kolenbrander PE. Inhibition of intergeneric coaggregation among oral bacteria by cetylpyridinium chloride, chlorhexidine digluconate and octenidine dihydrochloride. *J Periodontal Res.* 1991 Sep; 26(5): 422-8.
- 15 Beiswanger BB, Mallatt ME, Mau MS, Jackson RD, Hennon DK. The clinical effects of a mouthrinse containing 0.1% octenidine. *J Dent Res.* 1990 Feb; 69(2): 454-7.
- 16 Burton JP, Chilcott CN, Moore CJ, Speiser G and Tagg JR. A preliminary study of the effect of probiotic *Streptococcus salivarius* K12 on oral malodour parameters, *Journal compilation 2006 The Society for Applied Microbiology, Journal of Applied Microbiology* 100 (2006) 754-764.