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In situ neutralisation of the antibacterial effect of 0.2% Chlorhexidine on salivary microbiota: Quantification of substantivity

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ABSTRACT

Objective: To quantify the substantivity of a single 0.2% Chlorhexidine mouthwash in saliva after its neutralisation with tooth-brushing and 1% acetic acid, in order to identify the effect of Chlorhexidine substantivity in regard to the re-growing period of the salivary bacteria.
Methods: Unstimulated saliva samples were collected from a group of 15 healthy individuals at baseline (BS), and then 30 s and 1, 3, 5 and 7 h after the following protocols were performed: a single sterile water mouthwash (M-WATER) (negative control), a single 0.2% Chlorhexidine mouthwash (M-0.2% CHX) (positive control) and a single 0.2% Chlorhexidine mouthwash followed by a complete and detailed tooth-brushing, and a single 1% acetic acid mouthwash (M-0.2% CHX + NP). The samples were analysed using an epifluorescence microscope in combination with LIVE/DEAD® BacLight™ fluorescence solution.
Results: After the M-0.2% CHX treatment, the bacterial vitality was significantly lower than BS until 7 h (87.6 ± 6.5% *vs.* 73.6 ± 8.8%; $p < 0.001$). However, after M-0.2% CHX + NP, the bacterial vitality remained significantly lower until 3 h with regard to BS (81.4 ± 3.8% *vs.* 68.1 ± 10.6%; $p = 0.001$), increasing at 5 and 7 h (no differences from BS).
Conclusion: The immediate antibacterial effect of a single 0.2% Chlorhexidine mouthwash is so potent that the bacterial population needs more than 3 h to return to baseline bacterial vitality levels. The substantivity of a 0.2% Chlorhexidine mouthwash is a property that significantly increases its antibacterial activity from the first hour and contributes to extend the duration of its effect by at least double.

1. Introduction

It was not until the beginning of the 1970s, when Löe first used Chlorhexidine (CHX) as a mouthwash, testing its efficacy for reducing dental plaque and gingivitis.[1] From then on, it has been widely used for the control of dental plaque, as well as for the treatment of gingivitis and periodontitis.[2]

CHX is a cationic antimicrobial agent which has a broad-spectrum of action on both gram-positive and gram-negative bacteria as well as on fungi and some viruses.[3] Its activity is particularly linked to this cationic nature, as this leads to a

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strong bonding with the anionic sites of the cell membrane and wall.[4] This bonding triggers events that affect the osmoregulatory and metabolic capability of the cell membrane and its contained enzymes.[5] At higher concentrations, CHX can cause a loss of structural integrity of the membrane, allowing catastrophic leakage of cellular materials.[6]

The action of CHX on the membrane structure permits the use of fluorescence staining techniques based on combinations of dyes, which can differentiate vital from non-vital cells due to membrane permeability and integrity. A common combination used in studies on the activity of antimicrobial agents both on dental plaque[7,8] and on salivary microbiota[7–12] has been found in the LIVE/DEAD® BacLight™ Viability Kit (Molecular Probes, Leiden, The Netherlands), which is composed of Syto 9 and propidium iodide. Their staining principle is simple, as both are based on membrane permeability; Syto 9 penetrates all vital and non-vital cells due to its ability to penetrate intact cells, while propidium iodide only penetrates those cells with higher cell membrane permeability (*i.e.* damaged, non-vital cells). This differentiation offers the possibility to compare the percentage of live/dead bacteria present in a sample before and after treatment with different CHX rinses.[13]

The cationic nature of CHX,[14] as well as other factors such as the formation of reservoirs on teeth and oral mucosa, concentration, time, temperature and pH,[15,16] have been shown to be essential because this leads to this antimicrobial agent having prolonged antimicrobial activity inside the oral cavity; this characteristic has been called substantivity. When this term is used to refer to a topical antimicrobial agent, it is defined as its ability to adhere to the substrate and persist at effective concentrations.

Methods developed for the study of antimicrobial activity and its duration include *in vitro* and *in vivo* models. The first, can give information on the mechanism standpoint such as minimum inhibitory concentration or bacterial kill-time curves, but give very limited clinical perspective on a so dynamic ecosystem like the oral cavity. On the other hand, *in vivo* models vary from methods based on plaque re-growth assessments on short periods of time[17] or others using saliva[8–10,12,18,19] and/or dental plaque after a single application.[19–21]

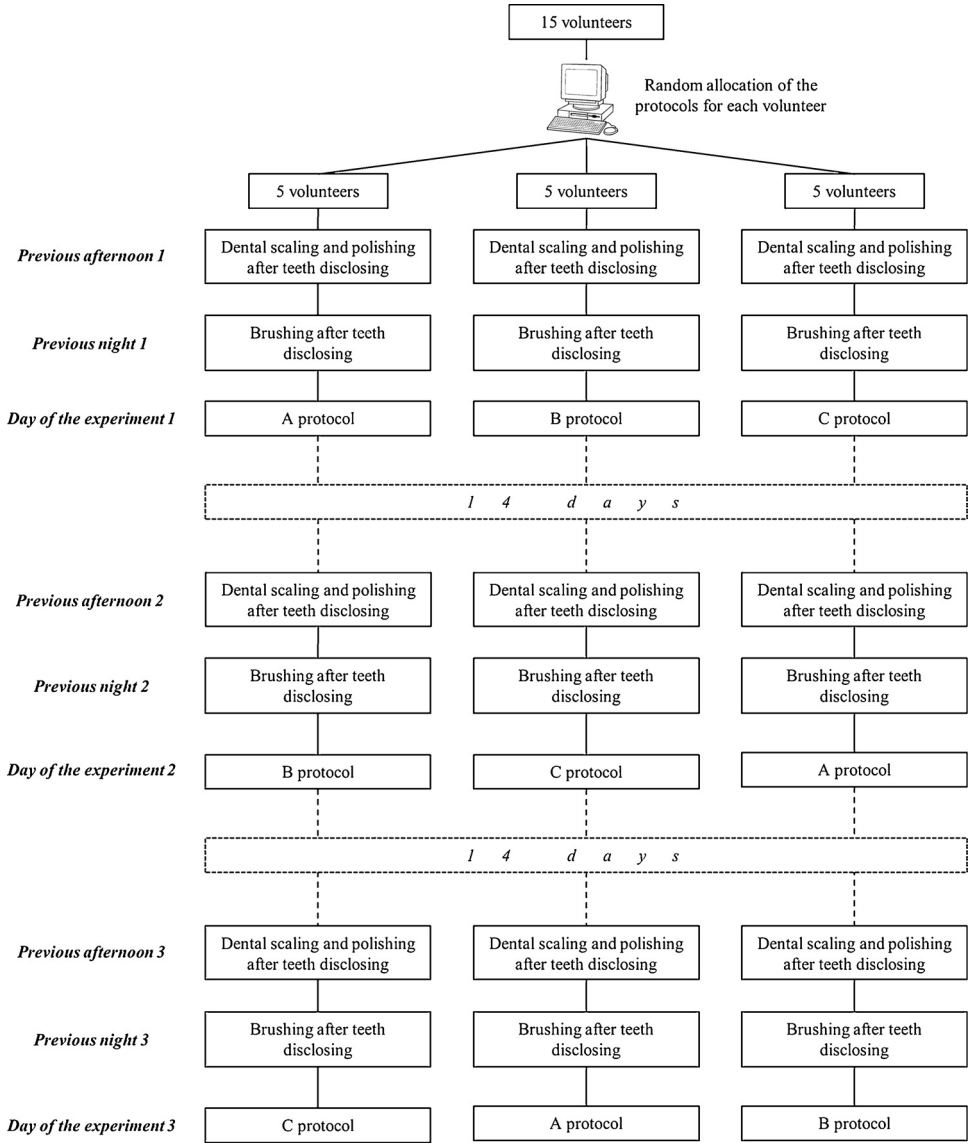

Fig. 1 – Scheme of the protocol followed by all volunteers from the randomization to the application of the different tests.