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A comprehensive study into the impact of a chitosan mouthwash upon oral microorganism's biofilm formation *in vitro*

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ABSTRACT

Modern dentistry emphasizes the importance of dental plaque control to improve oral health. To that end the development of oral care formulations has been geared toward the incorporation of antiplaque agents that may play a crucial role in oral health maintenance. In later years the research into antiplaque agents has led to the discovery of compounds with significant capability to affect biofilm formation. Among these compounds was chitosan, a polysaccharide which showed great ability to interfere with *Streptococcus mutans* biofilm formation. As such the aim of this work was to incorporate chitosan into a mouthwash matrix and assess its effect upon biofilm formation of oral microorganisms. This assessment was performed *via* study of the impact the mouthwash upon microbial adherence, biofilm formation and mature biofilms. Additionally, the action of the chitosan mouthwash was compared with two commercially available mouthwashes. The results here obtained show that only the chitosan containing mouthwash was capable of interfering with all microorganisms' adherence, biofilm formation and mature biofilms while at the same time showing vastly superior activity than both commercial mouthwashes assayed. As such a chitosan mouthwash shows great potential as a natural and efficient alternative to traditional mouthwashes.

1. Introduction

The primary factor in good oral health is the routine control of dental plaque–a natural biofilm formed on tooth surfaces and compacted at gingival margins (Gilbert, McBain, & Sreenivasan, 2007). Dental plaque is a structurally and functionally organized multispecies microbial biofilm and plays a major role in the etiology of oral diseases (Flemmig & Beikler, 2011; Marsh, 2010). While oral diseases can be controlled through a meticulous mechanical hygiene most individuals have difficulty in maintaining a good oral health consequently, many oral care products have antimicrobial and antiplaque agents in their composition (Marsh, 2010). In later years, the use of mouthwashes has become quite common in a variety of situations, ranging from halitosis to the treatment of minor infections. Their widespread usage as lead to several reports concerning secondary effects and microbial resistances have surfaced (Gagari & Kabani, 1995). As such the search for new, natural alternatives to the existing mouthwashes is of great importance. In recent years, natural polymers have received much attention because they can be an alternative to synthetic polymers in many technological processes, presenting very interesting features such as their biocompatibility and biodegradability, easy availability, and especially as a novel type of functional materials (Jayakumar, Nwe, Tokura, & Tamura, 2007).

Chitin is a linear polymer of N-acetyl glucosamine units linked by β-(1-4) bonds. It is the primary structural component of the shells of crustaceans, arthropods and fungal cell wall and is obtained mainly as a byproduct of the fishing industry. It is considered one of the most abundant polysaccharides in nature after cellulose and its production is estimated at 10^9–10^{11} tons per year (Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Kurita, 1998). Partial deacetylation of chitin leads to chitosan, a polysaccharide composed of units of glucosamine (2-amino-2-deoxy-D-glucose) and N-acetyl glucosamine (2-acetamido-2-deoxy-D-glucose) linked by β(1→4) bonds. Chitosan is the only natural polysaccharide that presents cationic character due to its amino groups which, at low pH, are protonated and can interact with negatively charged compounds such as proteins, anionic polysaccharides (*e.g.* alginates, carragenates, pectins) fatty acids, bile acids and phospholipids (Ramos, Rodriguez, Rodriguez, Heras, & Agulló, 2003). This behavior, along with its biocompatibility, biodegradability and lack of toxicity, has led to the usage of chitosan in diverse fields as technology, food, cosmetics, medicine, biotechnology, agriculture and the paper industry (Kim & Rajapakse, 2005; Kittur, Kumar, Gowda, & Tharanathan, 2003; Kurita, 1998).

In later years various authors (Bae, Jun, Lee, Paik, & Kim, 2006; Choi et al., 2001; Costa, Silva, Pina, Tavaria, & Pintado, 2012; Costa, Silva, Tavaria, & Pintado, 2013; Ji et al., 2009a; Tarsi, Muzzarelli,

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Table 1
Commercial mouthwashes tested full composition.

Mouthwash	Composition
C1	Water, alcohol, sorbitol, benzoic acid, eucalyptol, menthol, thymol, methyl salicylate, sweetener, sodium saccharin, sodium benzoate, CI42053, CI47005
C2	Water, alcohol, propylene glycol, sodium benzoate, lactic acid, poloxamero 338, sodium citrate, CI42051, allantoin, sweetener, chlorhexidine digluconate, hydroxyethil cellulose, limonene, PEG-12 dimethicone, sodium bicarbonate, sodium saccharin, sodium borate

Guzman, & Pruzzo, 1997) reported on chitosan's antimicrobial and antibiofilm activity upon several oral disease related microorganisms thus validating the utilization of chitosan as an alternative to traditional antimicrobials used in oral health care products. However, to the best of our knowledge, all the works published in this field contemplated only chitosan solutions and not a market ready mouthwash (with stabilizers, flavoring, coloring, *etc.*).

In view of the above data, this research effort was aimed at evaluating the effect of a chitosan mouthwash in adherence and biofilms of several known oral commensals and potential pathogens.

2. Material and methods

2.1. Source of chitosan and mouthwash formulations

The commercial mouthwashes (Table 1) tested had either essential oils as active principle (**C1**) or chlorhexidine (**C2**). Chitosan mouthwash formulation (**Ch**) was prepared using High Molecular Weight (DD > 75%; MW 624 kDa) and Low Molecular Weigh chitosan (75% < DD < 85%; MW 107 kDa) (Sigma–Aldrich (St. Louis, USA)) with the final concentration of either chitosan being 0.4% (v/v). The chitosan based mouthwash, prepared for a final pH of 5, contained 0.5% (w/v) salt (NaCl), 1% (w/v) stabilizer (arabic gum), 5% (w/v) sweetener (mannitol). Food grade flavoring and coloring were gently provided by Frulact S.A. and added at 0.1% (v/v) each.

2.2. Microorganisms

Five oral microorganisms (4 bacteria and 1 yeast) were used as model microorganisms. Four of the five microorganisms used in this study were obtained from the culture collection of the Göteburg University (CCUG) (Sweden) – *Streptococcus mutans* (CCUG 45091) origin not discriminated; *Lactobacillus acidophilus* (CCUG 33386) and *Enterococcus faecium* (CCUG 34441) obtained from human abscess; *Candida albicans* (CCUG 49242) human clinical sample. *Prevotella intermedia* is a clinical isolate and was isolated from a patient with periodontitis. *S. mutans*, *E. faecium* and *P. intermedia* were grown in Wilkins-Chalgren Anaerobe Broth (WCB) (Oxoid, Hampshire, England) and were plated in Wilkins-Chalgren Anaerobe Agar (WCA) (Oxoid, Hampshire, England), *L. acidophilus* was grown in de Man, Rogosa and Sharpe Broth (Biokar Diagnostics, Beauvais, France) and plated in de Man, Rogosa and Sharpe Agar (Biokar Diagnostics, Beauvais, France) and *C. albicans* was grown in Yeast Malt Broth (Difco, New Jersey, USA) and plated in Yeast Malt Agar (Difco, New Jersey, USA).

2.3. Determination of MIC

Determination of Minimal Inhibitory Concentration (MIC) was performed using the broth microdilution assay as described by Costa et al. (2012). Briefly, an inoculum of 0.5 MacFarland (*ca.* 1.5×10^8 CFU/mL) of each microorganism was prepared from overnight cultures and inoculated in the proper medium with mouthwashes concentrations ranging from 12.5 to 75% (v/v). Two controls were simultaneously assessed: one with 12.5% mouthwash but without inoculum and another where mouthwash was replaced by sterile water and added inoculum. The MIC was determined by observing the lowest concentration of mouthwash which inhibited visible bacterial growth. All assays were done in duplicate.

2.4. Adherence

The effect of the studied mouthwashes upon on bacterial adhesion was tested in accordance with protocol described by Costa et al. (2013). Briefly, 1 cm aluminum disks were dipped for 60 s in wells containing either chitosan mouthwash or one of the two commercial mouthwashes. Following that disks were rinsed with sterile water and submerged in a well containing inoculum for 60 s, after which disks were placed in wells containing the appropriated medium and incubated for 24 h at 37 °C. Two controls were simultaneously assessed. In the first disks were dipped in sterile water, inoculated and incubated and in the second disks were dipped in the test solutions, rinsed and then incubated without inoculum. After 24 h disks were retrieved and viable counts were assessed by the Miles, Misra, and Irwin (1938) technique. Results were given as inhibition percentages using the following formula:

$$\text{Adhesion inhibition percentage} = 100 - \left(\frac{\log \text{CFU sample}}{\log \text{CFU control}}\right) \times 100$$

All assays were done in duplicate.

2.5. Biofilm formation assay

Quantification of biofilm production was carried out by adapting the microtiter biofilm formation protocol described by Stepanovic, Vukovic, Dakic, Savic, and Svabic-Vlahovic (2000). Briefly, in a flat bottom 96 microplate, wells were filled with 200 μL of test solutions at sub-MIC concentrations with inoculum being added at 2% (v/v). Following this the microplate was incubated at 37 °C for 48 h. To visualize biofilms, the contents of each well were discarded and the well washed 3 times with sterile deionized water in order to remove non-adherent cells. The remaining attached bacteria were fixed with 200 μL of ethanol (Panreac, Barcelona, Spain) for 15 min. Ethanol was then discarded and the wells air dried. After that, 200 μL of crystal violet solution (Merck, Germany) were added to the wells for 5 min. Excess stain was removed by rinsing the plate under tap water and the air dried.

Adherence was quantified by measuring the Optical Density (OD) at 660 nm using a microplate reader (FlUOstar, OPTIMA, BGM Labtech).

Results for this test were given as percentage of biofilm formation inhibition applying the following formula:

$$\text{Biofilm formation inhibition percentage} = 100 - \left(\frac{\text{OD}_{\text{assay}}}{\text{OD}_{\text{control}}}\right) \times 100$$

All assays were done in triplicate.

2.6. Mature biofilms assay

The assessment of the mouthwashes effect upon mature biofilms was performed through the adaptation of the biofilm formation protocol as described by Stepanovic et al. (2000). Briefly, in flat bottom 96 wells microplate wells were filled with 200 μL of medium, inoculated at 1% (v/v) and incubated 48 h at 37 °C. After 48 h the medium was carefully aspirated and the wells were rinsed with phosphate buffer. Following that 200 μL of medium, with the

Table 2
MIC's and MBC's obtained for the different mouthwashes tested against the studied microorganisms. Values in percentage (v/v) of mouthwash.

	Ch		C1		C2	
	MIC	MBC	MIC	MBC	MIC	MBC
S. mutans	12.5%	12.5%	12.5%	12.5%	50.0%	50.0%
L. acidophilus	25.0%	37.5%	25.0%	37.5%	n.d.[a]	n.d.[a]
E. faecium	25.0%	50.0%	37.5%	n.d.[a]	n.d.[a]	n.d.[a]
C. albicans	37.5%	37.5%	12.5%	25.0%	75.0%	n.d.[a]
P. intermedia	25.0%	50.0%	12.5%	37.5%	62.5%	n.d.[a]

[a] Not detected.

mouthwashes at sub-MIC concentration, was added and incubated at 37 °C for 24 h.

To visualize biofilms, the contents of each well were discarded and the well washed 3 times with sterile deionized water in order to remove non-adherent cells. The remaining attached bacteria were fixed with 200 μL of ethanol (Panreac, Barcelona, Spain) for 15 min. Ethanol was then discarded and the wells air dried. After that, 200 μL of crystal violet solution (Merck, Germany) were added to the wells for 5 min. Excess stain was removed by rinsing the plate under tap water followed by being air dried.

Adherence was quantified by measuring the OD at 660 nm using a microplate reader.

All experiments were done in triplicate for each microorganism. OD values from wells with only WCB were used as negative controls. A positive control with sterile deionized water for each bacterium was used.

Results for this test were given as reduction of present biofilm applying the following formula:

$$\text{Mature biofilm inhibition percentage} = 100 - \left(\frac{\text{OD}_{\text{assay}}}{\text{OD}_{\text{control}}}\right) \times 100$$

2.7. Statistical analysis

The statistical differences between the methods were evaluated using PASW Statistics v. 18.0.0 (New York, USA). The normality of the results distribution was evaluated through Shapiro–Wilk's test. The differences were evaluated using one way ANOVA test associated with Scheffe's test (for normal distributions) or the non-parametric Mann–Whitney Test for the rest. The differences were considered significant at a 0.05 level.

3. Results

Microbial survival in the oral cavity is only possible for microorganisms capable of adhering and forming biofilms. As such is of crucial importance the study of the effect of the novel chitosan mouthwash on these properties.

3.1. MIC determination

Chitosan by itself is a polymer with a well-known antimicrobial activity with several authors reporting its wide range of activity against fungi and aerobic and anaerobic bacteria (Costa et al., 2012; Fernandes, Eaton, Gomes, Pintado, & Xavier Malcata, 2009; Fernandes et al., 2008; Raafat & Sahl, 2009). However, at present, there are no reports regarding the *in vitro* antimicrobial activity of a chitosan containing mouthwash.

As can be seen from Table 2, the chitosan containing mouthwash presented a wide range of antimicrobial activity, with bacteriostatic and bactericidal effect being present for all studied microorganisms. MIC values obtained were in the same range as those obtained for the **C1** mouthwash with the only differences being observed for *C. albicans*. On the other hand, the chitosan mouthwash presented consistently lower MIC values than the **C2** mouthwash. At the present time there are no previous reports regarding the effect of the incorporation of chitosan in a mouthwash matrix upon its *in vitro* antimicrobial activity. Nevertheless, some comparisons can be made about the chitosan concentration present in each MIC and the chitosan MICs found in previous works. Several studies (Bae et al., 2006; Choi et al., 2001; Costa et al., 2012; İkinci et al., 2002; Ji et al., 2009b; Tarsi et al., 1997) have shown that, for the studied bacteria, chitosan's MIC's varied between 0.5 and 5 mg/mL and, despite the fact that these studies incorporated modified chitosans with several MWs, comparisons can still be drawn. When considering the individual activity of the chitosan's used in this study previous work by Costa et al. (2012) reported MICs values that ranged between 1 and 5 mg/mL for both MW chitosans, values that are equal or slightly superior to those obtained for the chitosan mouthwash in this study (*S. mutans* – 0.5 mg/mL; *P. intermedia* – 1 mg/mL). In prior studies LMW chitosan showed MIC values that were superior to those obtained in this study – Tarsi et al. (1997) who reported a MIC of 2 mg/mL (1.5 kDa; no DD given) and Bae et al. (2006) a MIC of 1.5 mg/mL (3–5 kDa; DD 70%), both for *S. mutans*. On the other hand Tayel et al. (2010) reported a MIC of 1.25 mg/mL (32 kDa; DD 86%) for *C. albicans*, a value slightly inferior to the one obtained in this work (1.5 mg/mL). A similar behavior was found for HMW chitosan, with Ji et al. (2009b) (1080 kDa; DD 86%) reporting MIC values of 2.5 mg/mL for *S. mutans* and *P. intermedia*, values well above those reported in this study.

Overall the chitosan mouthwash presented MICs that fitted into the 0.5–5 mg/mL concentration range described for the chitosan molecule, with the highest concentration needed being found for *C. albicans* (1.5 mg/mL) and the lowest for *S. mutans* (0.5 mg/mL). These values seem to indicate that chitosan's was successfully incorporated and stabilized into the mouthwash matrix as it did not register any loss in antimicrobial activity. Furthermore, when comparing the MIC's obtained for the chitosan mouthwash with the ones obtained for the tested commercial mouthwashes one can see that the chlorhexidine based mouthwash (**C2**) presented significantly higher MICs while the essential oils based mouthwash (**C1**) presented similar values than the ones obtained for the chitosan mouthwash.

3.2. Adherence

Several authors have reported on the problem raised by bacterial adhesion to surfaces, with the oral cavity being given as one of the most problematic sites for adherence and consequential biofilm management (Hannig & Hannig, 2009; Nobbs, Jenkinson, & Jakubovics, 2011). As can be seen from Fig. 1 the chitosan mouthwash was capable of inhibiting adherence of all studied microorganisms, both commensal (*L. acidophilus* and *E. faecium*) and pathogenic. Moreover all inhibition percentages obtained were above 80%, with *S. mutans*, *C. albicans* and *P. intermedia* inhibition percentages being equal or higher than 90%. Considering the importance of these microorganisms in dental caries, oral candidiasis and periodontitis the capability to inhibit its establishment in the oral cavity is of upmost importance. Additionally, comparing these results with those obtained for the commercial mouthwashes, one can see that **Ch** presented a significantly ($p < 0.05$) higher anti-adherence capability with **C1** and **C2** presenting at best an inhibition percentage of approximately 40%. Furthermore both **C1** and **C2** displayed a smaller range of action than **Ch**, with **C1** incapable of inhibiting *C. albicans* and **C2** being incapable of inhibiting *P. intermedia*. These results are supported by those of Tarsi et al. (1997) who reported that LMW chitosan molecules (MW 1.5 kDa) were capable of inhibiting *S. mutans* adhesion to hydroxyapatite, Busscher, Engels, Dijkstra, and van der Mei (2008) also showed

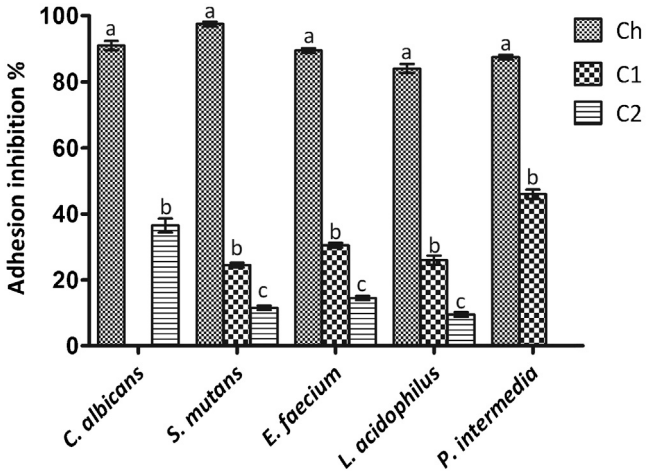

Fig. 1. Inhibitory effect of chitosan upon oral microorganisms adhesion. Values obtained given in percentage of adhesion inhibition. Different letters represent statistically significant differences found ($p < 0.05$). **Ch** – chitosan mouthwash; **C1** – essential oil based commercial mouthwash; **C2** – chlorhexidine based commercial mouthwash.

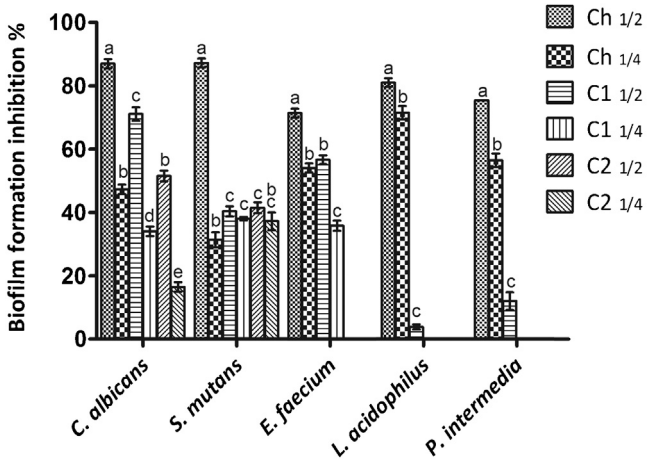

Fig. 2. Effect of sub-MIC concentrations (½ and ¼ of the MICs) of the tested mouthwashes upon oral microorganism's biofilm formation. Values obtained given in percentage of biofilm formation inhibition. Different letters represent statistically significant differences found ($p < 0.05$). **Ch** – chitosan mouthwash; **C1** – essential oil based commercial mouthwash; **C2** – chlorhexidine based commercial mouthwash.

that LMW chitosan (MW 182 kDa; DD 82%) reduced the adhesion of several streptococci strains and Costa et al. (2013) who showed that both HMW (MW 624 kDa; DD > 75%) and LMW (MW 107 kDa; DD 85%) chitosans reduced *S. mutans* adherence by percentage of almost 100%. Additionally, Sarasam, Krishnaswamy, and Madihally (2006) showed that chitosan films were capable of reducing *S. mutans* adhesion to negligible levels and Pasquantonio et al. (2008) stated that after 4 h *S. mutans* adhesion was completely inhibited by chitosan. Furthermore Sano, Shibasaki, Matsukubo, and Takaesu (2001) reported that chitosan reduced adhesion of oral bacteria *in vitro* to human anterior teeth surface, Barembaum et al. (2003) showed that HMW chitosan was capable of inhibiting *C. albicans* adhesion and Liu et al. (2010) revealed that coating of biliary stents with chitosan completely reduced enterococcal adhesion.

3.3. Biofilm formation

Biofilms in the oral cavity are characterized as being a protective environment, where microorganisms are protected from host and antimicrobials, which allows for the colonization of more fastidious bacteria (Marsh, 2010; Terefework et al., 2008). As such is of the upmost importance the development of valid biofilm control strategies. Analyzing the results presented in Fig. 2 we can see that **Ch** presented the widest range of action of the three tested mouthwashes. In fact **Ch** was the only one capable of inhibiting all studied microorganisms at both ½ and ¼ of the MIC. Actually, **C1** and **C2** had both a significant lower ($p < 0.05$) antibiofilm activity when in comparison with **Ch**, and both commercial mouthwashes inhibition percentages were, at best, *ca.* 20% inferior. More concretely, **C2** only presented activity against *C. albicans* and *S. mutans* (albeit reduced when comparing with **Ch**) and **C1** presenting at ½ of the MIC inhibition percentages similar to those of **Ch** at ¼ of the MIC, while only displaying activity against three of the five studied microorganisms at ¼ of the MIC. These results are supported by previous studies which showed that chitosan reduced biofilm viable cell numbers of *C. albicans* (Carlson, Taffs, Davison, & Stewart, 2008), metabolic activity by 70% (after 30 min) and 87.5% (after 24 h) (Cobrado et al., 2012; Martinez et al., 2010) and biofilm viability by >95% (after 24 h) (Martinez et al., 2010). Decker, Von Ohle, Weiger, Wiech, and Brecx (2005) reported that chitosan was capable of reducing the vitality of attached streptococci by approximately 90%, Busscher et al. (2008) stated that chitosan treated biofilms presented a decrease in viability by a factor of 3× when in comparison with a control. Furthermore, Chavez de Paz, Resin, Howard, Sutherland, and Wejse (2011) reported that chitosan nanoparticles at neutral pH were still capable of damaging up to 95% of cells present in a *S. mutans* biofilm. Additionally, early studies by Sano, Shibasaki, Matsukubo, and Takaesu (2003) and Bae et al. (2006) reported that chitosan was capable of reducing dental plaque formation. Moreover one has to consider that the assay was executed at sub-MIC concentrations and that while Cerca, Martins, Pier, Oliveira, and Azeredo (2005) stated that sub-MIC concentrations of antibiotics were capable of inhibiting bacterial biofilm formation more recently Dong et al. (2012) showed that the same sub-MIC concentrations of antibiotics, among which chlorhexidine, may be responsible for the formation of an extensive extrapolymeric matrix in *S. mutans* biofilms and an up regulation of the genes related to *S. mutans* biofilm formation. This may help explain why the lack of antibiofilm activity found for the chlorhexidine based mouthwash used in this assay (**C2**).

3.4. Mature biofilms assay

One of the main deficits of traditional antimicrobials is their incapability to act upon and destroy mature biofilms. As can be seen from Fig. 3, of the tested commercial mouthwashes only **C2** was capable of acting upon mature biofilms. On the other hand **Ch** showed activity against all of the studied bacteria at both tested sub-MIC concentrations. Comparing the results obtained between mouthwashes one can see that, similarly to what happened in the biofilm formation assay, the best inhibition percentages obtained for **C2** at ½ of the MIC were equal, at best, to the values obtained for **Ch** at ¼ of the MIC. Considering that the active principle of **C2** is chlorhexidine, one of the most used antimicrobial in oral health, these results show the vastly superior activity and efficiency of chitosan, as an active principle of oral care products, in biofilm removal when in comparison with the currently used antimicrobials. Most of these antimicrobials are either inefficient or when efficient require high concentrations, for example *C. albicans* mature biofilms are notoriously difficult to destroy (Bink, Pellens, Cammue, & Thevissen, 2011) with high concentrations of antimicrobials (*e.g.* 2081 mg/mL of miconazole; and 2 mg/mL of caspofungin) being necessary to produce substantial reductions of *C. albicans* biofilm. Furthermore the concentrations surpass, sometimes, the MIC values of these antimicrobials for the planktonic phase several times as can be seen in the values reported by Wei, Campagna, and Bobek (2006) for the MIC and for the minimum

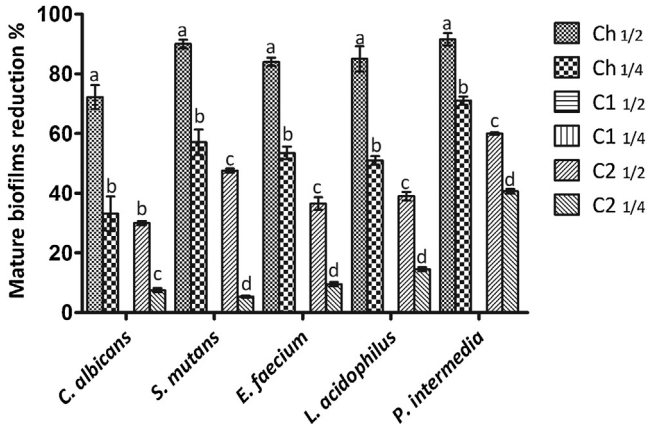

Fig. 3. Effect of sub-MIC concentrations of mouthwash upon oral microorganisms mature biofilms. Results given in mature biofilm reduction percentages. Different letters represent statistically significant differences found ($p < 0.05$). **Ch** – Chitosan mouthwash; **C1** – essential oil based commercial mouthwash; **C2** – chlorhexidine based commercial mouthwash.

biofilm reduction concentration (MBRC) of chlorhexidine regarding *S. mutans*. In this study the author reported a MIC of 1.3 mg/mL and a minimum biofilm inhibition concentration (MBIC) superior to 10 mg/mL contrary to what we see in the present work were all biofilm reduction was obtained at sub-MIC concentrations. Additionally, most of these antibiotics have a limited action spectrum, cannot be taken/used over a prolonged period of time or have side effects (*e.g.* chlorhexidine stains the teeth yellow after prolonged usage (Frank, Gent, & Hettinger, 2001)). Moreover, our results come in line with those of Orgaz, Lobete, Puga, and San Jose (2011) who showed that chitosan by itself was capable of reducing already formed biofilms of known food pathogen *Listeria monocytogenes* and with those of Costa et al. (2013) who showed that chitosan was capable of causing a 94% reduction in *S. mutans* mature biofilms.

4. Conclusions

The incorporation of chitosan in products, particularly oral care products, has not been extensively explored. While there are several antimicrobials/antibiotics that may possess higher activity or require lower concentrations than chitosan to be active against oral pathogens and their biofilms, neither possesses the biocompatibility nor the other inherent properties of chitosan. Furthermore due to chitosan's nature there are, at the moment, no reports of known antimicrobial resistances to chitosan. With these characteristics chitosan appears to be a perfect match for usage in oral care products however, most mouthwashes used nowadays are carefully crafted formulations with numerous compounds working together to obtain antimicrobial activity, among other properties. While the inclusion of chitosan in these formulations would be possible, chitosan's high reactivity, that makes it a high sought polymer, would be a double edged sword as it chemical interactions would create havoc and destabilize most formulations. Thus the best route for chitosan incorporation into oral care seems to be the development of novel products drawn specifically to harbor chitosan and potentiate its properties. As such, the results here obtained lay the groundwork for the application of chitosan in the oral cavity as this *in vitro* results show that a chitosan based mouthwash was capable of inhibiting microbial adhesion and biofilm formation while at the same time being capable of promoting the dissolution of already formed biofilms. Additionally, in comparison with two commercially available mouthwashes the chitosan containing mouthwash presented significantly superior activity in all assays.

Overall, these results demonstrate the potential of developing specific chitosan-based mouthwash formulations as they possess equal or better biological activity than the commercial products currently available.

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