

Insights into the effect of chitosan upon biofilm formation of several oral pathogenic microorganisms

Costa, E.M.^{a*}; Silva, S.^a; Tavarina, F.K.^a and Pintado, M.M.^a

^a Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal

* Corresponding author: emcosta@porto.ucp.pt



Introduction

The establishment of biofilms is a fundamental step in the survival of oral microorganisms (1). Among the biofilm forming microorganisms *Streptococcus mutans* and *Porphyromonas gingivalis* have received considerable attention as the main pathogens responsible for dental caries and adult periodontitis (2).

Generally, mechanical treatment of oral biofilm (dental plaque) formation is insufficient to prevent oral diseases, and an on-going quest exists for new antimicrobials to be used in oral healthcare (3). Chitosan is a high molecular weight (HMW), linear, polycationic heteropolysaccharide derived from chitin. The high percentage of amino groups (6,89 %), which provide chelating capability, in conjunction with antimicrobial, antitumoral and immunoadjuvant activity, acceleration of wound healing, biodegradability and biocompatibility make chitosan a high sought biomaterial. Moreover chitosan possesses bioadhesive capabilities thus making it capable of residing in the oral cavity (4). The aim of this work was to assess the potential of chitosan as a viable antibiofilm agent through adhesion, biofilm formation and quorum sensing inhibition assays.

Methods

MIC determination

Sub-MIC

Adhesion

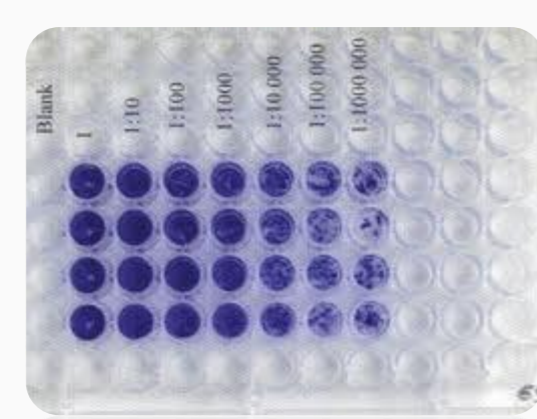
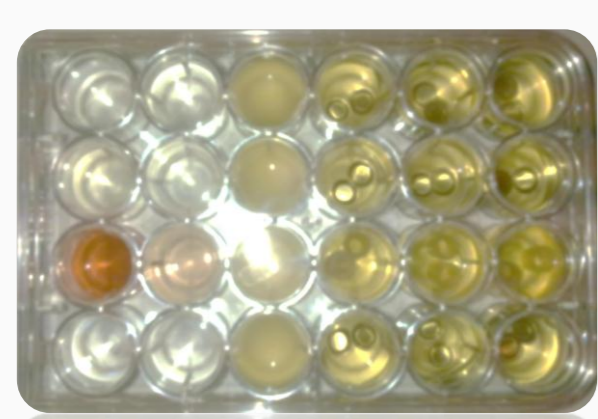
Biofilm Formation

Quorum sensing

Surface coating

96 wells microplate assay

C. violaceum reporter system



Results

MIC's

	HMW*	LMW*
<i>P. buccae</i>	2	1
<i>L. acidophilus</i>	3	1
<i>C. albicans</i>	1	3
<i>C. violaceum</i>	3	3

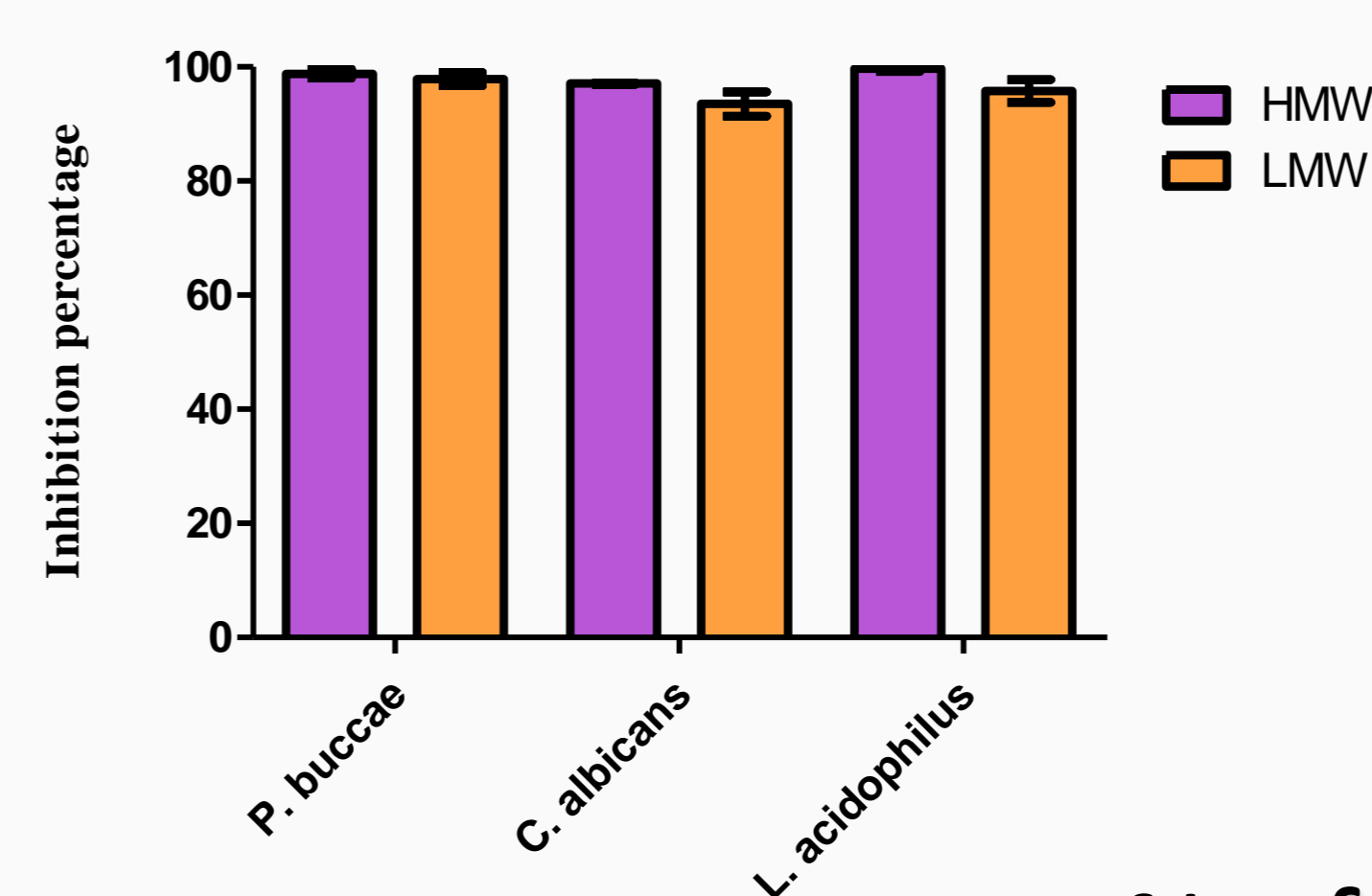
*Values in mg/mL

Sub-MIC's

	HMW*		LMW*	
	1/2	1/4	1/2	1/4
<i>P. buccae</i>	1	0,5	0,5	0,25
<i>L. acidophilus</i>	1,5	0,75	0,5	0,25
<i>C. albicans</i>	0,5	0,25	1,5	0,75
<i>C. violaceum</i>	1,5	0,75	1,5	0,75

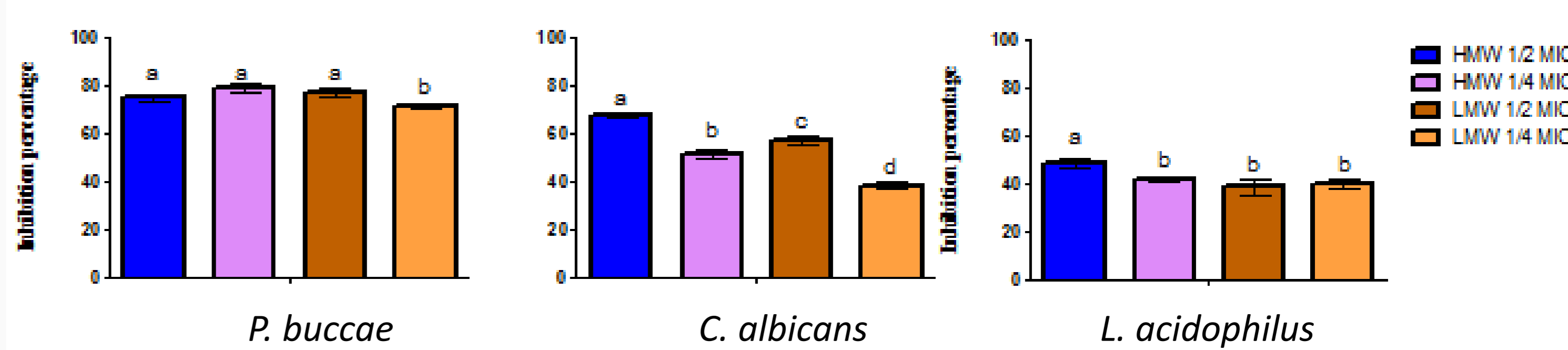
*Values in mg/mL

Adhesion

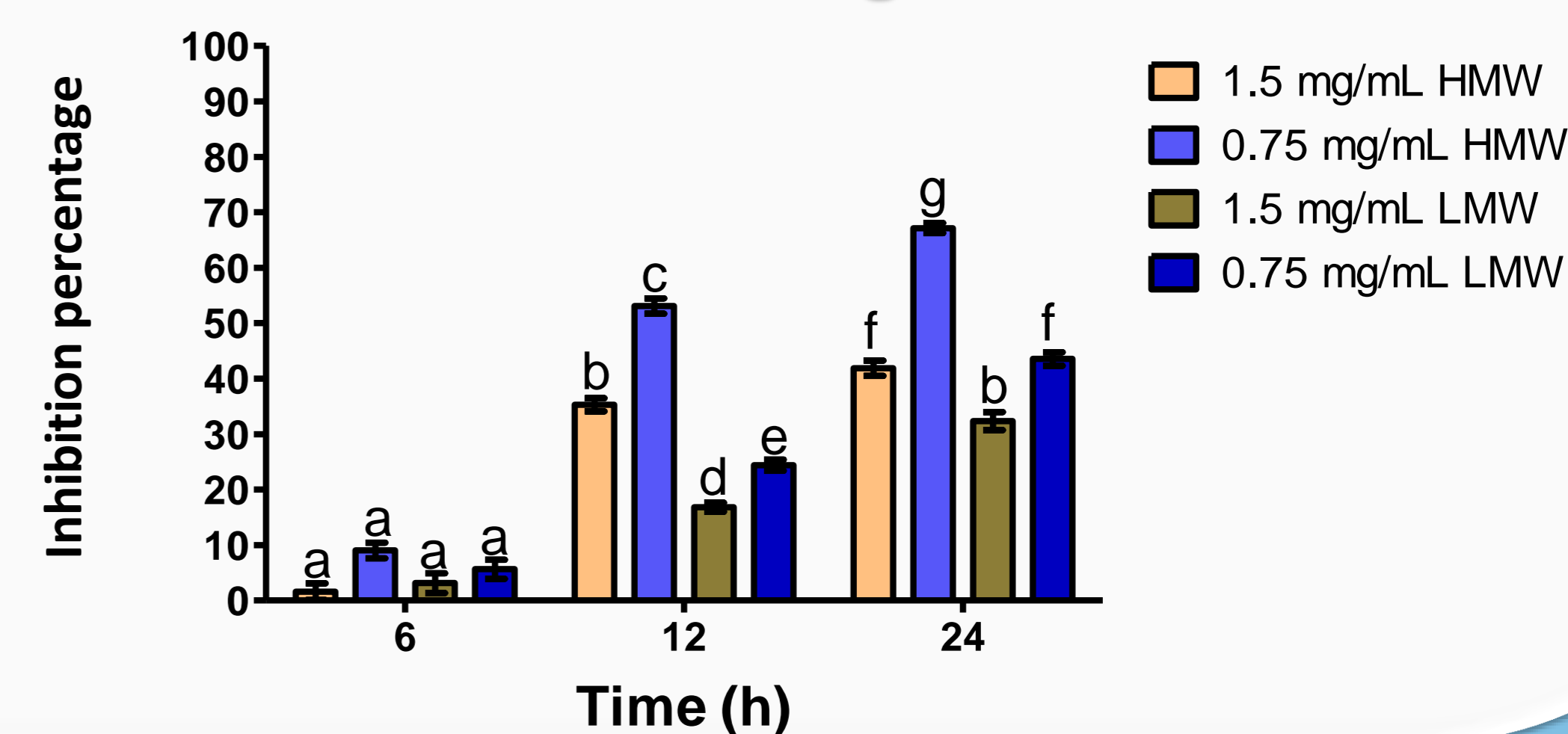


~ 100% adhesion inhibition
Biofilm formation reduced at all sub-MICs (minimum 40% at 1/4)
Quorum sensing inhibited

Biofilm formation



Quorum sensing



Conclusions

- ✓ Chitosan presented low MICs
- ✓ Chitosan inhibited microbial adhesion
- ✓ Biofilm formation was inhibited at sub-MICs
- ✓ Chitosan disrupted Quorum sensing after 6 h, increasing throughout the time
- ✓ Chitosan proved to be an efficient antibiofilm agent

References

1. Willey et al. Microbiology: McGraw-Hill Higher Education; 2008.
2. Takahashi N. International Congress Series. 2005 9//;1284(0):103-12.
3. Busscher et al. European journal of oral sciences. 2008 Oct;116(5):493-5..
4. Kockisch et al.,. European Journal of Pharmaceutics and Biopharmaceutics, 2005. 59(1): p. 207-216.

Acknowledgements

Agency of Innovation (Agência de Inovação, ADI, Portugal) and Quadro de Referência Estratégico Nacional (QREN, Portugal) which through the project "QUITORAL – Desenvolvimento de novas formulações de quitosanos com aplicação em medicina oral" (QREN-ADI 3474) and the National Funds from FCT – Fundação para a Ciência e a Tecnologia through project PEst-OE/EQB/LA0016/2011 provided funding for the realization of this work.