ANTIMICROBIAL ACTIVITY OF MELIMINE OR CATHELICIDIN BOUND TO CONTACT LENSES

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INTRODUCTION

Contact lens wear is a risk factor for the development of microbial keratitis (MK).[1] A variety of microorganisms have been implicated in MK (Figure 1), such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Other bacterially driven adverse events during contact lens wear include contact lens-induced acute red eye, contact lens-induced peripheral ulcer and infiltrative keratitis.

Previous studies have confirmed that surface attached antimicrobial peptides (AMP) such as melimine[2] or cathelicidin (LL-37)[3] show high biocidal activity. LL-37 is a naturally occurring AMP that has been found in tears. Melimine is a synthetic peptide and a chimera of the active regions of protamine (from salmon sperm) and melittin (from bee venom).

Figure 1: *Microbial Keratitis (MK)*

PURPOSE

Development of antimicrobial contact lenses may reduce the rate of contact lens related adverse events. The purpose of this study was to compare LL-37 and melimine when bound to contact lenses for their activity against *P. aeruginosa* and *S. aureus*.

METHODS

- Minimum inhibitory concentration (MIC) of LL-37 and melimine against *P. aeruginosa* ATCC 19660, *P. aeruginosa* 6294, *S. aureus* ATCC 29213 and *S. aureus* 31 were determined following a established procedure.[4]
- Peptides were attached individually onto the surface of contact lenses (Etafilcon A; ACUVUE® 2®) using a previously described method.[2]
- Varying concentrations of peptides were covalently bound to contact lenses.
- The amount of peptide associated with the lens was quantified using amino acid analysis (AAA).
- Antimicrobial activity of peptide coated contact lenses were evaluated against *P. aeruginosa* 6294 *and S. aureus* 31.
- Biocidal activity was evaluated by viable plate count of bacteria after exposure to the coated lenses.
- The adhesion data were $\log_{10}$ (x+1) transformed prior to data analysis where x is the number of adherent bacteria in CFU mm^{-2}. A linear mixed model ANOVA was used for statistical analysis and significance was set at 5% level.

RESULTS

- Table 1. represent the MIC of LL-37 and melimine against *P. aeruginosa* and *S. aureus* strains.

Table 1: Minimum inhibitory concentration (µg ml^{-1}) of LL-37 and melimine.

Bacteria	**LL-37** (µg ml^{-1})	**Melimine** (µg ml^{-1})
P. aeruginosa ATCC 19660	3.9	500
P. aeruginosa 6294	62	250
S. aureus ATCC 29213	3.9	250
S. aureus 31	250	125

- Figures 2 and 3 show quantification of LL-37 and melimine associated with contact lenses. In addition the figures show the antimicrobial activity of the respective peptides attached to the contact lenses.

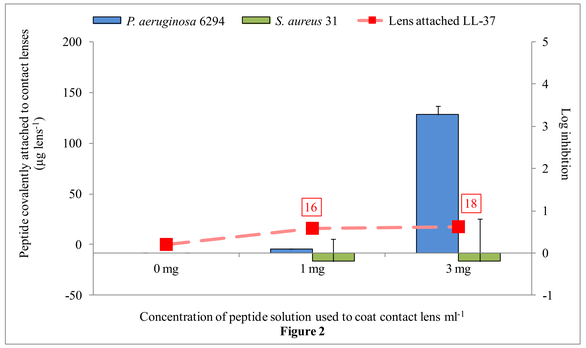

Figure 2

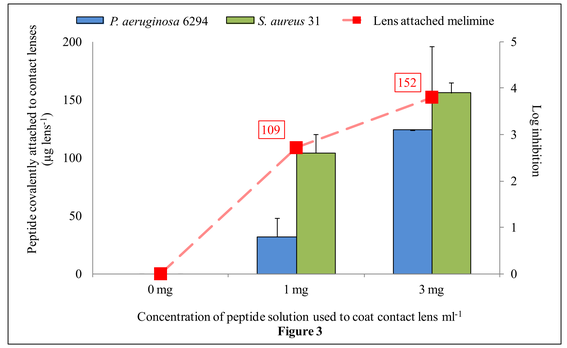

Figure 3

Figure 2 & 3: *Amount of covalently bound LL-37 or melimine attached to contact lenses and the respective antimicrobial activity of the lenses against P. aeruginosa and S. aureus.*

- The covalent attachment technique was able to bind much higher amounts of melimine onto contact lenses compared to LL-37.
- There was 109 ± 4 µg and 152 ± 44 µg melimine and 16 ± 0.7 µg and 18 ± 0.5 µg LL-37 associated with each contact lens after covalent reaction with 1 mg ml^{-1} and 3 mg ml^{-1} respectively.
- Due to the low level of LL-37 that was able to be attached to the lenses using the EDC reaction, bound LL-37 showed no antimicrobial activity against *S. aureus* 31, but did show 3.3 log reduction against *P. aeruginosa* 6294 when 18 µg per lens was attached.
- The higher levels of melimine on lenses resulted in greater anti-bacterial activity against both *P. aeruginosa* and *S. aureus.*
- LL-37 was more active against *P. aeruginosa* on a molecule/molecular basis than melimine, but was inactive against *S. aureus*.

DISCUSSION

- Both LL-37 and melimine showed high antimicrobial activity in solution against *P. aeruginosa* and *S. aureus*, the activity varies with different strains of bacteria.
- However, probably due to the lower level of cationic amine groups on LL-37 (11) compared to melimine (16), much less LL-37 was able to be bound to the contact lenses
- This lower level of LL-37 resulted in no activity against *S. aureus*, although there was good activity against *P. aeruginosa* even with only 18 µg/lens

CONCLUSION

Covalent surface attachment of AMPs offers excellent potential for development as an antimicrobial coating for contact lenses and thus for other biomaterials. Biocidal mechanisms of various AMPs vary with their innate structure and therefore AMP specific surface attachment techniques may be necessary for optimal outcomes.

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