INTRODUCTION
There remains a need to reduce microbial contamination of contact lenses, and thus the rate of adverse events during wear. We have earlier shown that the antimicrobial peptide melamine retains activity against various ocular pathogens.1 Although melamine-coated lenses retained antimicrobial activity following human wear, they were associated with occasional corneal staining.2

PURPOSE
To determine activity of a melamine-derived antimicrobial peptide Mel4 against drug resistant and clinical isolates of bacteria. In addition, the in vitro activity and clinical performance of Mel4 as an antimicrobial contact coating was evaluated in a human clinical trial.

METHODS
- Antimicrobial activity of Mel4 (K-N-K-R-K-R-R-R-R-R-G-G-R-R-G; >90% purity) was determined against bacteria (Table 1) by evaluating minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a modified broth microdilution assay.
- Etafilcon A lenses were coated by covalently binding the Mel4 peptide to the surface via EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) coupling.1
- Antimicrobial activity of Mel4-coated lenses against the P. aeruginosa and S. aureus strains were evaluated by viable plate count.
- A prospective, randomised, double-masked, clinical trial of one-week daily contralateral contact lens wear with 17 human subjects was conducted to assess the Mel4 coated lens safety and performance.
- For overnight lens storage Biotrue multipurpose solution and lens cases were used. Ethics approval was received from UNSW human research ethic committee.
- Clinical signs were monitored on Days 1, 2 and 7 of lens wear and 1 week and 3 weeks following study lens wear discontinuation.

Table 1: Bacterial strains and resistance profile

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Isolation site</th>
<th>Resistant to</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. marcescens ATCC 13880</td>
<td>Pond water</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Drug-resistant organisms

P. aeruginosa 31: Microbial keratitis: GEN, TOB, PRL, NOR, OFX, MXF, CIP
P. aeruginosa 37: Microbial keratitis: GEN, TOB, PRL, NOR, OFX, MXF, CIP
S. aureus 60: Hospital strain: GEN, ERY, CIP
S. aureus 110: Microbial keratitis: MET, TOB, ERY, CIP

Table 2: Bacterial strains and MIC/MBC

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (nmol/ml)</th>
<th>MBC (nmol/ml)</th>
</tr>
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<tbody>
<tr>
<td>S. marcescens</td>
<td>1056</td>
<td>2113</td>
</tr>
<tr>
<td>P. aeruginosa 31</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>P. aeruginosa 37</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>S. aureus 60</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>S. aureus 110</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

RESULTS
- Table 2 lists the MIC and MBC for all the bacteria tested. Highest MIC and MBC were determined for S. marcescens ATCC 13880.
- The Mel4-coated lenses showed more than 1.5 log inhibition of adhesion for P. aeruginosa and S. aureus (Figure 1).
- All participants successfully completed the trial, 8 male and 9 female, with an average age of 22.5 ± 1.4 years.
- No significant difference in fluorescein staining in any of the five corneal areas were observed between control and Mel4 coated lenses during this study (Figure 2; p> 0.05).

Table 3: Questionnaire scores for comfort, dryness, lens and edge awareness over one week of wear. Data is represented as a box plot showing the median and 25th and 75th percentile ranges

CONCLUSION
Mel4 has high antimicrobial activity against drug resistant bacteria in addition to presenting no adverse effects for human eyes as a contact lens coating, offering excellent potential for development as an antimicrobial agent and contact lens coating.

REFERENCES
3. Hancock RE. Hancock Laboratory Methods 1999.

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