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Efficacy of the De Novo-Derived Antimicrobial Peptide WLBU2 against Oral Bacteria

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The efficacy of a novel synthetic antimicrobial peptide (WLBU2) was evaluated against three oral microorganisms (grown planktonically): Streptococcus gordonii, Fusobacterium nucleatum, and Porphyromonas gingivalis. WLBU2 killed all three species, with F. nucleatum being the most susceptible. WLBU2 also reduced the bacterial burden of S. gordonii and F. nucleatum biofilms.

With increasing evidence for links between infectious burden and systemic diseases such as coronary artery disease (6, 15, 18), the oral cavity has been shown to be an important contributor to this systemic microbioburden (4, 5, 7, 8, 12). The microcosm found in the oral cavity comprises more than 700 species of microorganisms (1) arranged as both single-species and multispecies biofilms. The tooth-associated biofilm (dental plaque) is the primary etiologic factor associated with multiple forms of periodontal disease (9, 14). P. gingivalis is a late colonizer associated with multiple forms of periodontal disease (9, 14). P. aeruginosa was used as a positive bacterial control. Bacterial cultures were propagated in liquid media under appropriate growth conditions (Table 1) to mid-log phase, washed with phosphate buffer (PB), and suspended in PB such that upon dilution, 10^5 to 10^6 CFU/ml was tested in the bacterial killing assay. The bacteria were incubated with twofold dilutions of the peptide (100 μM to 0.39 μM) in 96-well plates in PB at 37°C under appropriate growth conditions (Table 1). Although WLBU2 reduces viable counts of P. aeruginosa in seconds in PB (17), a minimum of 20 min is required for killing in serum (3). Since the subgingival environment contains a serum transudate, a standard 30-min incubation of bacterial survival post-peptide exposure was evaluated using serial 10-fold dilutions of control and test wells. Bacterial colonies were counted at 24 h for S. gordonii and F. nucleatum and at 48 h for F. nucleatum and P. gingivalis and were compared to counts of non-peptide-treated controls to determine the amount of WLBU2 that reduced the bacterial counts by 3 orders of magnitude. This level of killing defined the minimum bactericidal concentration (MBC), assessed in micromolar concentrations of peptide. The results were expressed as averages of MBCs obtained from three independent experiments.

All three species of planktonic oral bacteria were killed by WLBU2 (Fig. 1). The MBC was between 1 and 2 μM for P. aeruginosa and F. nucleatum, between 12.5 and 25 μM for S. gordonii, and between 50 and 100 μM for P. gingivalis. Viable counts also were assessed for S. gordonii and F. nucleatum following exposure to comparable micromolar concentrations of amoxicillin or metronidazole, antibiotics frequently used in treating periodontal disease (16). There were no significant

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decreases in the viable cell counts of either bacterium following these treatments (Tables 2 and 3).

The high concentration required for killing P. gingivalis strain W85 may be due to the encapsulated nature of this strain or the presence of the numerous proteolytic enzymes released by this bacterium. This will be assessed in future studies. However, the MBCs for two different strains of F. nucleatum were between 1 and 2 µM, consistent with results evaluating the specificity of WLBU2 against numerous P. aeruginosa strains (2). Interestingly, both strains of F. nucleatum were more susceptible to killing by this novel peptide than either S. gordonii or P. gingivalis when grown as planktonic cells. Because F. nucleatum is recognized as an important “bridging” bacterium between the less pathogenic early colonizers and the more pathogenic late colonizers of the plaque biofilm, antimicrobial agents disrupting this bridge before the development of a mature biofilm could interfere with plaque maturation.

The effectiveness of WLBU2 was assessed against single-species oral biofilms developed on individual rigid gas-permeable lens (RGPL) material (unpublished data). Replicate RGPLs were coated with fetal bovine serum, washed with sterile phosphate-buffered saline (PBS), and incubated with overnight cultures of F. nucleatum (25586) material (unpublished data). Replicate RGPLs were coated with fetal bovine serum, washed with sterile phosphate-buffered saline (PBS), and incubated with overnight cultures of F. nucleatum (25586) material (unpublished data). Replicate RGPLs were coated with fetal bovine serum, washed with sterile phosphate-buffered saline (PBS), and incubated with overnight cultures of F. nucleatum (25586) material (unpublished data).

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The results of three replicate experiments demonstrated that treatment of S. gordonii biofilms with WLBU2 resulted in a statistically significant decrease in mean cell counts compared to those for PBS-treated controls. Mean cell counts did not decrease significantly following treatment with either amoxicillin or metronidazole (Table 2). Similarly, treatment of F. nucleatum biofilms with these antibiotics did not decrease viable cell counts, while treatment with WLBU2 did lead to decreased counts. However, the results with WLBU2 were not statistically significant (Table 3). Taken together, these results indicate a trend toward the ability of WLBU2 to impact viable counts of both S. gordonii and F. nucleatum cells grown as biofilms.

These studies demonstrate activity of WLBU2 against early and bridging bacteria grown as planktonic cells and known to be part of oral biofilms. Our results also demonstrate that WLBU2 can potentially contribute to a lessening of the bioburden of a mature single-species biofilm. We hypothesize that the somewhat limited effect of the peptide on biofilm cells was due to inefficient penetration beyond the surface layer of bacteria in the mature biofilm. Consistent with our findings and this hypothesis, it has been demonstrated previously that mechanical disruption or adjunctive exposure to a surface-active agent

![Image](http://aac.asm.org/)

**FIG. 1.** Dose-dependent killing of oral bacteria and P. aeruginosa (positive control) by WLBU2. Bacterial cultures (0.5 x 10^6 to 1 x 10^6 CFU/ml) were treated with twofold dilutions of the peptide in phosphate buffer; the log of the number of CFU/ml remaining upon treatment is plotted as a function of peptide concentration.

### TABLE 1. Bacterial strains and growth conditions

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Medium</th>
<th>Growth conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 47085</td>
<td>Todd-Hewitt broth</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Streptococcus gordonii</em> ATCC 49818/DL1</td>
<td>Todd-Hewitt broth</td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em> ATCC 49256, ATCC 25586</td>
<td>Tryptic soy broth + 0.6% yeast extract</td>
<td>Anaerobic (5% CO₂, 10% H₂, 85% N₂)</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em> ATCC BAA038/8/W3</td>
<td>Mycoplasma broth + hemin (5 µg/ml) + menadione (1 µg/ml)</td>
<td>Anaerobic (5% CO₂, 10% H₂, 85% N₂)</td>
</tr>
</tbody>
</table>

### TABLE 2. Viable counts of untreated (PBS) versus treated *S. gordonii* cells

<table>
<thead>
<tr>
<th>Method of cell growth</th>
<th>Treatment</th>
<th>Total viable count</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planktonic</td>
<td>PBS</td>
<td>(1.29 ± 0.25) x 10^8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.00 ± 0.00) x 10^8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(1.35 ± 0.41) x 10^8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(1.22 ± 0.11) x 10^8</td>
<td>NS</td>
</tr>
<tr>
<td>Biofilm</td>
<td>PBS</td>
<td>(2.01 ± 0.08) x 10^7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.21 ± 0.02) x 10^7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(1.63 ± 0.11) x 10^7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(1.8 ± 0.14) x 10^7</td>
<td>NS</td>
</tr>
</tbody>
</table>

*All antimicrobials were used at a concentration of 25 µM.

*NS, not significant; P > 0.05 by a t test that was adjusted when necessary for unequal variance.

### TABLE 3. Viable counts of untreated (PBS) versus treated *F. nucleatum* cells

<table>
<thead>
<tr>
<th>Method of cell growth</th>
<th>Treatment</th>
<th>Total viable count</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planktonic</td>
<td>PBS</td>
<td>(1.04 ± 0.08) x 10^7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.00 ± 0.00) x 10^8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(1.00 ± 0.15) x 10^7</td>
<td>NS</td>
</tr>
<tr>
<td>Biofilm</td>
<td>PBS</td>
<td>(1.75 ± 0.13) x 10^7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.20 ± 0.27) x 10^4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(2.75 ± 0.07) x 10^4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(2.86 ± 0.57) x 10^4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*All antimicrobials were used at a concentration of 2 µM.

*NS, not significant; P > 0.05 by a t test that was adjusted when necessary for unequal variance.
can enhance peptide killing of biofilm cells (10). Therefore, future studies will evaluate the efficacy of WLBU2 in combined therapeutic strategies with a focus on the peptide’s impact on F. nucleatum as a coaggregating bridge microorganism in multispecies biofilms.

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