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

Karen F. Novak  
*University of Kentucky*, knova2@uky.edu

William J. Diamond  
*University of Kentucky*

Sreenatha S. Kirakodu  
*University of Kentucky*, sreenatha.kirakodu@uky.edu

Rebecca Peyyala  
*University of Kentucky*

Kimberly W. Anderson  
*University of Kentucky*, kimberly.anderson@uky.edu

See next page for additional authors

Follow this and additional works at: [https://uknowledge.uky.edu/cohr_facpub](https://uknowledge.uky.edu/cohr_facpub)

Part of the [Oral Biology and Oral Pathology Commons](https://uknowledge.uky.edu/cohr_facpub)

**Repository Citation**
[https://uknowledge.uky.edu/cohr_facpub/3](https://uknowledge.uky.edu/cohr_facpub/3)

This Article is brought to you for free and open access by the Oral Health Research at UKnowledge. It has been accepted for inclusion in Center for Oral Health Research Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
Authors
Karen F. Novak, William J. Diamond, Sreenatha S. Kirakodu, Rebecca Peyyala, Kimberly W. Anderson, Ronald C. Montelaro, and Timothy A. Mietzner

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

Notes/Citation Information
Published in Antimicrobial Agents and Chemotherapy, v. 51, no. 5, p. 1837–1839.

Copyright © 2007, American Society for Microbiology. All Rights Reserved.
The copyright holders have granted the permission for posting the article here.

Digital Object Identifier (DOI)
http://dx.doi.org/10.1128/AAC.00924-06

This article is available at UKnowledge: https://uknowledge.uky.edu/cohr_facpub/3
Efficacy of the De Novo-Derived Antimicrobial Peptide WLBU2 against Oral Bacteria

Karen F. Novak,1* William J. Diamond,1 Sreenatha Kirakodu,1 Rebecca Peyyala,1 Kimberly W. Anderson,2 Ronald C. Montelaro,3 and Timothy A. Mietzner3

Center for Oral Health Research, College of Dentistry,1 and Department of Chemical and Materials Engineering, College of Engineering,2 University of Kentucky, Lexington, Kentucky, and Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania3

Received 26 July 2006/Returned for modification 6 October 2006/Accepted 12 February 2007

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 microbial bioburden (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 dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).

The use of topical chemotherapeutic agents that inhibit plaque formation is an important adjunct in the prevention of oral diseases such as dental caries and periodontal disease. Therefore, the development of novel antimicrobial compounds that are effective against oral microorganisms is an important aspect of both basic science and commercial oral health research. The lentivirus lytic peptide (dental plaque) is the primary etiologic factor associated with dental caries and periodontal disease. Dental plaque biofilm formation is a sequential process initiated by adherence of gram-positive early colonizers to the tooth surface, followed by a shift to a predominance of more pathogenic gram-negative anaerobic species (late colonizers) as the biofilm matures. Importantly, Fusobacterium nucleatum serves as an important co-aggregation bridge between early and late colonizers (9).
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 W83 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 far 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* material (unpublished data). Replicate RGPLs served as controls. Treated and control biofilms were released from the RGPL into PBS with a sterile cell scraper and disrupted by vortexing. Serial dilutions were spread onto blood agar plates in triplicate for determination of CFU.

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

### 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 BAA0308/W83</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>

### FIG. 1. Dose-dependent killing of oral bacteria and *P. aeruginosa* (positive control) by WLBU2. Bacterial cultures (0.5 × 10⁸ to 1 × 10⁹ 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 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><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Planktonic</td>
<td>PBS</td>
<td>(1.29 ± 0.25) × 10⁸</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.00 ± 0.00) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(1.35 ± 0.41) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(1.22 ± 0.11) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td>Biofilm</td>
<td>PBS</td>
<td>(2.39 ± 0.08) × 10⁷</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.21 ± 0.02) × 10⁷</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(1.63 ± 0.11) × 10⁷</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(1.8 ± 0.14) × 10⁷</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><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Planktonic</td>
<td>PBS</td>
<td>(1.04 ± 0.08) × 10⁷</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.00 ± 0.00) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(1.00 ± 0.15) × 10⁷</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(1.02 ± 0.07) × 10⁷</td>
<td>NS</td>
</tr>
<tr>
<td>Biofilm</td>
<td>PBS</td>
<td>(1.75 ± 0.13) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WLBU2</td>
<td>(0.20 ± 0.27) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>(2.75 ± 0.07) × 10⁸</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>(2.86 ± 0.57) × 10⁸</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.

This work was supported by a University of Kentucky research support grant and by Kentucky Science and Technology Corporation/Kentucky Science and Engineering Foundation grant KSEF-47-RDE-004.

We thank Jeff Mattingly for valuable technical assistance with this project, Malini Bharadwaj for assistance with manuscript preparation, and Kazi Islam at the University of Pittsburgh Molecular Medicine Institute Peptide Synthesis Facility for assistance in peptide preparation.

REFERENCES