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Epithelial Interleukin-8 Responses to Oral Bacterial Biofilms

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An in vitro model of bacterial biofilms on rigid gas-permeable contact lenses (RGPLs) was developed to challenge oral epithelial cells. This novel model provided seminal data on oral biofilm-host cell interactions, and with selected bacteria, the biofilms were more effective than their planktonic counterparts at stimulating host cell responses.

The organization of multispecies biofilms found in the supra- and subgingival regions in the oral cavity demonstrates structural features reflecting the pattern of accretion of individual species (12). These patterns are likely related to interrelationships of species in the multicellular structures that presumably reflect both specific cognate interactions leading to coaggregation (19) and symbiotic nutritional partnerships (5). Responses of oral host cells, e.g., epithelial cells, fibroblasts, lymphocytes, macrophages, and osteoclasts, following challenge with oral bacterial species have been routinely studied with planktonic bacteria and carried out under aerobic conditions (10). However, it is clear that the apical aspect of the subgingival sulcus at sites of periodontal lesions is characterized by an anaerobic microenvironmet (3). Still remaining ill defined are the patterns of host cell responses that occur when biofilms interface with these host cells in this type of altered local environment. This report describes a model system using rigid gas-permeable contact lenses (RGPLs) to create bacterial biofilms and the use of this model in examining the production of a biomarker of host inflammatory responses, interleukin 8 (IL-8), by epithelial cells.

Porphymonas gingivalis FDC381, Fusobacterium nucleatum ATCC 25586, Actinomyces naeslundii ATCC 49840, Streptococcus sanguinis ATCC 10556, Streptococcus oralis ATCC 10557, and Streptococcus gordonii ATCC 10558, representing common members of the subgingival plaque (17), were cultured in broth for a further 24 h. The metabolic activity assessed using the cell proliferation reagent WST-1 (Roche Diagnostics Corp., Indianapolis, IN) demonstrated a negligible effect of anaerobic conditions. Planktonic bacteria, biofilms, and control challenges were carried each out in 6 wells of a 48-well plate containing fresh cell culture medium and incubated for 6 h under anaerobic conditions (1, 6). IL-8 was initially targeted for assessment in this model due to its significant elevations in inflamed gingival tissues and gingival crevicular fluid (4) in periodontitis and its critical role as a chemotactant for polymorphonuclear leukocytes, a hallmark of periodontal inflammation (16).

An immortalized epithelial cell line, OKF4 (14), was cultured in keratinocyte serum-free medium (KSM) (Invitrogen, Carlsbad, CA) and seeded into 48-well tissue culture plates (Costar, Cambridge, MA) at a density of 105 cells per well in a 1-ml volume and allowed to adhere overnight in a 5% CO2 chamber at 37°C (aerobic conditions) to form a confluent monolayer. The RGPL was overlaid on the adherent OKF4 cells and maintained under anaerobic and aerobic conditions for a further 24 h. The metabolic activity assessed using the cell proliferation reagent WST-1 (Roche Diagnostics Corp., Indianapolis, IN) demonstrated a negligible effect of anaerobic conditions and cultivation under RGPLs on the metabolism of this substrate (Fig. 1). RNA was extracted, using a PicoPure RNA isolation kit (Arcturus Bioscience, California), from OKF4 cells that were lysed (Purelink RNA minikit; Invitrogen, California) for 15 min and stored at −80°C. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH), human β-actin, RPL19F, and 18S (Integrated DNA Technologies, Indiana, and Operon, Alabama) housekeeping genes (Table 1) were analyzed using real-time PCR (11). The mean crossing point value of duplicate determinations for GAPDH (mean ± standard deviation [SD], 17.42 ± 1.12 and 16.77 ± 0.74 under aerobic and anaerobic conditions, respectively), for β-actin (17.07 ± 1.73 and 17.32 ± 1.04), for RPL19F (18.65 ± 1.53 and 18.16 ± 0.44), and for 18S (17.37 ± 1.92 and 16.70 ± 0.58) were determined. Thus, all 4 housekeeping genes were expressed similarly under both aerobic and anaerobic growth conditions.

Planktonic bacteria, biofilms, and control challenges were each carried out in 6 wells of a 48-well plate containing fresh cell culture medium and incubated for 6 h under anaerobic conditions. Planktonic bacterial challenge (108, 107, and 106 cells/well) corresponding to a multiplicity of infection (MOI) at 1,000:1, 100:1, and 10:1 and 3-day-old biofilms grown on and adherent to the RGPLs were used to stimulate the OKF4 cells for 6 h. We estimated that an MOI of 10:1 to 50:1 bacterial cells in the biofilms were in contact with the epithelial cells (13). Both planktonic cells and biofilms were washed twice to remove metabolic by-products and incubated in KSM medium for challenge of the epithelial cells. OKF4 cells with or without

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overlaid RGPLs (minus biofilms) and phorbol 12-myristate 13-acetate (PMA) (10 nm)-treated OKF4 cells were used as controls. OKF4 cell supernatants from each of two wells were pooled and stored at $-80^\circ\text{C}$ for IL-8 determination.

Universal Probe Library (UPL) probes (Roche, Indiana) were used to enhance the specificity of gene expression for each species. Extensive literature has reported biomarker of response capacity of epithelial cells to individual species or their components. Various investigations of host responses to oral bacteria have targeted IL-8 product or message levels as a comparative biomarker of response capacity of epithelial cells to individual species or their components. Extensive literature has reported a range of responses from host cell types (e.g., epithelial cells, fibroblasts, and immune cells) when challenged in vitro with individual planktonic bacteria or components isolated from individual species (10). However, in vivo, it would be expected

![FIG. 1. Metabolic activity of OKF4 epithelial cells under aerobic (5% CO$_2$ and air) and anaerobic (85% N$_2$, 5% CO$_2$, and 10% H$_2$) conditions. At each time point, the mean and standard deviation of triplicate determinations of WST-1 colorimetric change in OKF4 cells alone (control) or OKF4 cells cultivated under RGPLs is shown.](http://cvi.asm.org/)

<table>
<thead>
<tr>
<th>Probe</th>
<th>Gene or product</th>
<th>Primers*</th>
<th>$T_m$ (°C)</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
</table>
| SYBR green | GAPDH | F: 5'-AAAGTCTACTTCCCTGAGCTGAA-3'  
R: 5'-TGCTAGGACCATACTCGTG-3' | 59.80 | 310 |
|       | β-Actin | F: 5'-AGAGCTAGCTACGGCTGCAG-3'  
R: 5'-AGTACTTGCCTCAGAGGAGG-3' | 59.96 | 300 |
|       | RPL19 | F: 5'-AGATCCGGAAAGCCTATCAA-3'  
R: 5'-TCAATGGAATTCCGCTTGT-3' | 59.77 | 301 |
|       | 18S | F: 5'-ATAGCCCTTGGCCATACCGTCCC-3'  
R: 5'-AGAACTGACGCAGCCCTCTCA-3' | 60.24 | 304 |
| UPL probe 9 | GAPDH | F: 5'-GGAAGCTGTGACATCTGAGA-3'  
R: 5'-TGGACTCTAGCTACTCA-3' | 60 | 102 |
| UPL probe 72 | IL-8 | F: 5'-AGACACAGAGACACACACAGC-3'  
R: 5'-ATGGGTCTCCCTCCGAGGT-3' | 59 | 62 |

* F. forward; R. reverse.
that numerous host cells, particularly epithelial cells, interact with the oral bacteria growing as complex biofilms. Our findings clearly demonstrate that certain oral microbial species, as biofilms, significantly increase the induction of both IL-8 product and mRNA from oral epithelial cells. These findings provide a basis for utilization of this RGPl biofilm model system to explore multispecies biofilms (13) and their capacity to trigger a portfolio of host response molecules from epithelial cells. This model system can also be used to examine other host cell responses to mono- or multispecies biofilms targeting gene and product expression levels related to inflammation, innate immunity, and even adaptive immunity. The system is also amenable to evaluating the response of the bacteria to these interactions, either affected by direct host cell contact or in response to a milieu of host-derived factors that could alter the microbial physiology and/or cell communications, e.g., quorum sensing, between the various bacterial species. Moreover, the system will enable the assessment of how modifications within the biofilm-host cell environment could alter the host response profiles.

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**REFERENCES**


**TABLE 2. Expression of IL-8 mRNA following challenge of OKF4 cells for 6 h**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No challenge</th>
<th>F. nucleatum</th>
<th>P. gingivalis</th>
<th>A. naeundii</th>
<th>S. gordoni</th>
<th>S. oralis</th>
<th>S. sanguinis</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGPl Biofilm</td>
<td>1.47 ± 0.72</td>
<td>11.97 ± 5.84</td>
<td>0.35 ± 0.11</td>
<td>5.88 ± 1.33</td>
<td>4.81 ± 2.43</td>
<td>4.91 ± 2.98</td>
<td>1.56 ± 0.64</td>
</tr>
<tr>
<td>Planktonic (cells/well)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁸</td>
<td>1.94 ± 0.88</td>
<td>2.56 ± 1.03</td>
<td>1.00 ± 0.29</td>
<td>0.98 ± 0.14</td>
<td>0.65 ± 0.30</td>
<td>0.39 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>10⁷</td>
<td>1.75 ± 0.80</td>
<td>1.82 ± 0.67</td>
<td>0.46 ± 0.06</td>
<td>1.39 ± 0.45</td>
<td>0.45 ± 0.24</td>
<td>0.48 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>10⁶</td>
<td>0.84 ± 0.32</td>
<td>1.01 ± 0.42</td>
<td>0.41 ± 0.03</td>
<td>2.56 ± 0.87</td>
<td>1.59 ± 0.74</td>
<td>0.56 ± 0.17</td>
<td></td>
</tr>
</tbody>
</table>

*The values represent mean fold change ± 1 SEM compared to results for cultures of untreated epithelial cells in triplicate determinations from 2 to 6 independent experiments.*