A comparative study of single and multi-species biofilm removal from the wall of a simulated root canal model by passive or active sodium hypochlorite irrigant.

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Introduction
A multitude of studies have identified the resistance of bacterial biofilms to the antimicrobial agents used in the root canal treatment (1-4). Although this has been ascribed to the complex anatomy of the root canal system that interferes with an effective irrigant penetration (4,5), there is increasing concern that the cooperative behaviour of a multi-species biofilm enables persistence after exposure to an antimicrobial agent.

Aims
The aim of this study was to compare the difference between the residual single and multi-species biofilms on the wall of the root canal system during passive or active (manual, sonic and ultrasonic) NaOCl irrigation protocols.

Materials and methods

- A total of eighty root canal models (n = 10 per group) of an 18 mm length root canal with apical size 30 and taper 0.06 were manufactured using 3D printing.
- Biofilms of single species (Enterococcus faecalis) or multispecies (Streptococcus mutans, Enterococcus faecalis, Fusobacterium nucleatum, and Prevotella intermedia) were grown on the apical 3 mm of sterilised models for 10 days. Biofilms were stained using crystal violet for visualisation.
- The biofilms on the surface of the root canal models were observed using scanning electron microscopy (SEM) (Figure 1):

![SEM images of biofilms](image)

- Following 60 seconds of 9 mL of 2.5% NaOCl irrigation using syringe and needle, the irrigant was either left stagnant in the canal or activated using manual, sonic or ultrasonic methods for 30 seconds. The removal of biofilm by NaOCl irrigant was observed under a fluorescence microscope. Images were then captured every second using a camera;
- The residual biofilm percentages were measured using image analysis software (Figure 2);
- The data were analysed using generalized linear mixed model analysis at a level of significance p ≤ 0.05.

![Image illustration of the set-up of the equipment](image)

Results
Results indicated a conclusive evidence of the effect of biofilm type (single, multispecies) on the efficacy of NaOCl irrigation. In all irrigation protocols, the residual multi-species biofilms were greater than the residual single species biofilm. The difference was statistically significant (p ≤ 0.001) (Table 1).

<table>
<thead>
<tr>
<th>Irrigation protocols</th>
<th>Comparable groups</th>
<th>*Coefficient (±SD)</th>
<th>95% CI for *Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe irrigation + passive irrigation</td>
<td>Single vs multispecies biofilm</td>
<td>36.42(±3.7)</td>
<td>38.71, 24.13</td>
<td>0.001</td>
</tr>
<tr>
<td>Syringe irrigation + GP agitation</td>
<td>Single vs multispecies biofilm</td>
<td>43.75(±1.5)</td>
<td>47.32, 40.18</td>
<td>0.001</td>
</tr>
<tr>
<td>Syringe irrigation + sonic agitation</td>
<td>Single vs multispecies biofilm</td>
<td>31.89(±2.9)</td>
<td>37.61, 26.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Syringe irrigation + ultrasonic agitation</td>
<td>Single vs multispecies biofilm</td>
<td>36.31(±3.2)</td>
<td>42.60, 30.02</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Coefficient for the residual biofilm, SE: standard error.

Conclusions
Within the limitation of the present study, Passive and active irrigation protocols (manual, sonic, ultrasonic) using 2.5% NaOCl were less efficient against the multi-species biofilms than single species biofilms.

References

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