Abstract

Benzanthonium chloride (BTC) is a quaternary ammonium compound (QAC) that is used as an antibacterial in several applications. BTC can be complexed with laponite clay (Bioclay) particles, making it capable of being applied and retained topically. Once complexed, BTC cannot be easily leached out of the clay by diffusion due to strong interactions with the clay surface. Bioclay has a wide range of potential applications for imparting antimicrobial activity in medical, industrial, and household applications. Bioclay has been previously shown to be effective against methicillin resistant Staphylococcus aureus (MRSA), and has been developed in cream form for topical application as an antiseptic cream. This study investigates the hypothesis that BTC, when incorporated into a pharmaceutical grade clay, can enhance the process of wound healing. This is done using an in vitro wound healing model utilizing a variety of cell lines. WI38 cells are normal diploid human fibroblasts. TM12 and TM12T are immortalized mammary epithelial cell lines. TM12T is a derivative of TM12 and has undergone epithelial to mesenchymal transition. This study utilized the TM12 cells which were epithelial in phenotype and when confluent looked cuboidal and tightly joined. The cells tended to maintain cell to cell connections after being scraped in order to induce the wound and as an attempt to prevent this flap of cells from falling back into the wound well was washed with PBS before the different media were added. In this study an induced ‘wound’ on a confluent well is treated with media or media treated with either BTC or Bioclay. The wound was measured after 24 hours to compare the width of the wounds between the control and treatment groups. A viability assay was run in parallel, to control for any effects of BTC compounds on total viable cell number. No effect was seen on wound healing at any of the concentrations tested, in either fibroblast, epithelial or EMT epithelial cell lines. Lack of effect was not due to loss of viability. Although no effect was seen of BTC compounds on these purified cell lines, wound healing may still be affected, due to the coordinated participation of several cell types, including granulocytes, macrophages, fibroblasts, and epithelial cells.

Wound beds are complex environments in which death, excise, and the bacterial biofilm interact in a complicated manner among themselves as well as the host. In these situations, bacteria tend to organize into biofilms, reducing their susceptibility to elimination as well as impeding healing. The cleaning of the wound is one of the basics of standard care and helps to achieve a chronic wound. Published literature shows that cleaning improves the wound environment and accelerates healing. In previous studies, BTC has proven to be effective against a number of bacteria including (but not limited to) E. coli, H. influenzae, S. pyogenes, S. auresus, and S. pneumonae. Anecdotal evidence from topical use of Bioclay has indicated that BTC complexed with laponite can have a positive effect on wound healing. This is a first attempt to understand the mechanism(s) by which this can occur.

Methods

Wound healing assays Cell lines epithelialized in 24 well plates at 8x10^4 cells/well grown to confluence. The confluent layer of cells in triplicate wells was then scratched from the top of the well down using a micropipette tip. Wells were rinsed with sterile PBS to remove detached cells. Cells were then fed with media containing 0, 1.25, 2.5, or 5 µg/ml BTC or Bioclay. These concentrations were selected to be below concentrations previously used in a cream formulation (50 µg/ml) and based on literature that assessed the toxicity of BTC. At 24 hours, images were taken using a phase contrast inverted microscope. Wound diameter was measured at the time point of 0 (D0) and assay 6% wound closure at 24h using the formula:

\[ \text{Percentage of Wound Healed} = \frac{\text{D0} - \text{D24}}{\text{D0}} \times 100 \]

Results

% Wound Closure

<table>
<thead>
<tr>
<th>Concentration</th>
<th>BTC</th>
<th>Bioclay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg/ml</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1.25 µg/ml</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>2.5 µg/ml</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>5.0 µg/ml</td>
<td>20.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

![Figure 1](https://example.com/figure1.png)

![Figure 2](https://example.com/figure2.png)

![Figure 3](https://example.com/figure3.png)

![Figure 4](https://example.com/figure4.png)

![Figure 5](https://example.com/figure5.png)

![Figure 6](https://example.com/figure6.png)

Discussion

- There was no enhancement of wound healing responses in either WI38, TM12, or TM12T cells by BTC or Bioclay.
- There was no difference in wound healing between the BTC and Bioclay groups for any of the cell lines used.
- The TM12 cell line was more sensitive to the BTC and Bioclay than the TM12T cell line.
- WI38 fibroblast viability was more sensitive to Bioclay than BTC alone as seen in the viability assay.
- At higher concentrations of BTC there was a small loss of cell viability in TM12 cells, but TM12T cells were less sensitive to BTC which might be contributed to the cell’s more tumorigenic properties.
- The use of the TM12 and TM12T cells as an in vitro wound healing model demonstrated that BTC and Bioclay do not inhibit wound healing in concentrations as high as 5.0 µg/ml. There was also minimal effects on cytotoxicity at these levels.
- There are limitations using a single cell model because of the lack of cell to cell interactions between the many different cells in the wound bed, that will require the use of an in vivo model to observe.

![Figure 7](https://example.com/figure7.png)

References