Antimicrobial Functionalization of Oxine Forestomach Matrix with Ionic Silver


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Background & Objective

The wound environment is characterized by contamination with microorganisms. Should contamination progress unchecked to critical colonization or infection, this may adversely affect wound healing outcomes, patient safety and the economy of care. dressings with antimicrobial functionality to prevent microbial colonization of the dressing are a useful tool to mitigate such complications of microbiologically infected wounds.

The deacetylated extracellular matrix biomaterial Oxine Forestomach Matrix™ (OFM) is an established scaffold for use in wound management and tissue repair indications. An OFM variant incorporating ionic silver, termed OFM-Ag, has recently been developed as a dressing to combine the benefits of an extracellular matrix scaffold with antimicrobial functionality.

This study sought to characterize the functional properties of OFM-Ag in relation to its intended use as an antimicrobial wound dressing. The antimicrobial effectiveness spectrum and wear time of the dressing were assessed, in addition to dressing silver concentration, kinetics of silver release and dressing cytotoxicity.

Methods

Antimicrobial Effectiveness

The ISO 20743 absorbance method including ASTM E1654 validated neutralization procedure was used to assess OFM-Ag dressing antimicrobial effectiveness. Antimicrobial spectrum determination utilized a panel of clinically relevant wound colonizing species (including drug-resistant strains) with antimicrobial exposure times assessed at 1, 3 and 7 days after microbial challenge and incuclation under physiological conditions.

Silver Quantiﬁcation and Elution Kinetics

Silver quantification was performed by atomic absorption spectrometry (AAS). Dressings were hydrated in concentrated nitric acid and digested anaerobically with hydrogen peroxide (H2O2). Silver elution kinetics were characterized by elution using pure water (0.29 mL/cm²/day) at 37°C over a 7 day time course, with water replaced and replaced daily. At the 1, 3 and 7 day time points dressings were removed from elution, lyophilized, digested with nitric acid and silver quantified by AAS.

Results & Discussion

Antimicrobial Effectiveness Spectrum & Wear Time

OFM-Ag dressings demonstrated a high degree of antimicrobial effectiveness across a spectrum of 11 microbial species of relevance to wound care including representatives of gram positive bacteria, gram negative bacteria, yeast and mold. The sustained effectiveness of OFM-Ag over a 7-day wear time period indicated prolonged antimicrobial protection of the dressing, rather than a transient initial inoculation.

Antimicrobial Effectiveness of OFM-Ag Dressings

<table>
<thead>
<tr>
<th>Microbial Species</th>
<th>Log Reduction 1 Day</th>
<th>Log Reduction 3 Days</th>
<th>Log Reduction 7 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin Resistant Staphylococcus aureus (MRSA)</td>
<td>7.0</td>
<td>8.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Streptococcus pneumonia (Group A. hemolytic)</td>
<td>8.3</td>
<td>8.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt;7.6</td>
<td>&gt;7.6</td>
<td>&gt;7.6</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>7.5</td>
<td>7.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>&gt;9.0</td>
<td>&gt;9.0</td>
<td>&gt;9.0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>6.1</td>
<td>8.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>6.6</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>7.3</td>
<td>&gt;7.6</td>
<td>&gt;7.6</td>
</tr>
</tbody>
</table>

Note: indicate growth but no visible colonies remained on any test plate (minimum log reduction calculated for this assay or “complete kill”)

Cytotoxicity

Cytotoxicity testing was performed via the ISO 10993-5:2009 test method using murine fibroblasts (3T3 cell line). A common collagen/ORC-silver dressing was also included. Triplicate assays were performed, where dressings were hydrated in saline (per respective PU) and extracted in cell culture media according to ISO 10993-12. All cell culture experiments were conducted in 96-well plates with 90% cell confluence and incubated at 37°C for 24 hours. MTT solution was added and cell viability quantified via MTT reduction measured by optical density at 570 nm.

Collagen Structure

Integrity of dressing collagen structure was assessed using differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). For comparative purposes, DSC and SEM analysis included functional collagen/ORC-silver wound dressing and OFM-dressed OFM and unprocessed oxine forestomach tissue (OF). For DSC, samples were hydrated in phosphate buffered saline (5 minutes) and 5.31 mg total collagen sealed in aluminum crucibles. The temperature was equilibrated (10°C) before ramping to 120°C (6°C/min). Data analysis was used to calculate onset melt temperature. For SEM, 2x5 mm samples were mounted on aluminum stubs and viewed using a Hikari TM1350 (University of Auckland, New Zealand) with 15 kV accelerating voltage.

Conclusions

Characterization studies have determined the following properties of OFM-Ag:

- Broad spectrum antimicrobial effectiveness over a 7 day wear time.
- Contains 0.30% (w/w) silver, equivalent to 12 μg/cm².
- Maintains antimicrobial effectiveness over 7 days of elution.
- Well tolerated by mammalian cells, exhibiting no cytotoxic response.
- Retains the native extracellular matrix structure of OFM.

Cytotoxicity

Silver is a well-known antimicrobial, however depending on concentration, form and presentation silver may impart cytotoxic effects detrimental to wound healing. Therefore it is vital to balance the antimicrobial effects of silver while maintaining dressing biocompatibility.

Response of Mammalian Cells to Silver Dressings

OFM-Ag dressings were well tolerated by mammalian cells, exhibiting no statistically significant difference in cell viability relative to the media control (p>0.05). In contrast, collagen/ORC-silver dressings exhibited a marked cytotoxic response. As measured in these standards, MTT viability experiments, OFM-Ag dressings were ~60% less cytotoxic compared to collagen/ORC-silver.

Collagen Structure

The intact collagen structure of extracellular matrices is known to confer benefits to wound healing. Collagen matrix integrity was measured by melt onset temperature (T_m) via DSC. For reference, human dermis has a melt onset of ~60°C, whereas degraded collagen can be identified by decreased melt onset temperature.

SEM of Wound Dressings

Silver Wound Dressing DSC

There was no significant difference (p>0.05) in T_m between OFM and OFM-Ag, demonstrating the inclusion of ionic silver does not damage the collagen matrix. The T_m of collagen/ORC-silver was notably lower, indicative of degraded collagen (i.e. gelatin) without native matrix structure. SEM imaging concurred with DSC results, showing collagen/ORC-silver to have a very porous structure matched with cellulose strands but no fibrillar collagen present. OFM-Ag exhibited an innate architecture of interwoven heterogeneous collagen fibrils characteristic of a native extracellular matrix.

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