Evaluating the Mode of Action of the Components within Antimicrobial Wound Dressings in Relation to their Antimicrobial and Antibiofilm Properties

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INTRODUCTION

Wound management can be challenging within the healthcare system, in particular antimicrobial dressings, and considering the vast amount available, it can be difficult to make a clinically informed choice. Antimicrobial agents within wound dressings are used to reduce or inhibit microbial bioburden which in turn promotes wound healing, reducing the need for systemic antibiotics.

It is widely documented that PHMB and Silver offer broad spectrum antimicrobial and antibiofilm activity and are widely used in wound care as topical dressings to manage infection. Aside from the antimicrobial agent, wound dressings may contain other excipients e.g. chelating agents, surfactants, which can further enhance the performance of the product, making them a suitable choice for chronically infected wounds.

The purpose of this investigation aims to evaluate two commercially available carboxymethylcellulose (CMC) fibre dressings, one is a PHMB + EDTA CMC gelling fibre dressing (Fig 1) and the other a Silver CMC dressing containing, EDTA and Benzethonium chloride (BC) (Fig 2), to determine their mode of action in relation to their antimicrobial and antibiofilm activity.

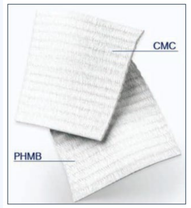

Fig 1 – CMC PHMB + EDTA fibre dressing

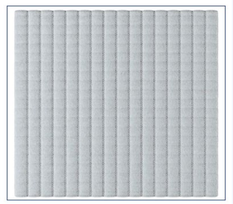

Fig 2 – Silver CMC, EDTA and BC fibre dressing

OBJECTIVE

Using a structured literature review, the study will focus on three mechanistic domains: **(1)** direct antimicrobial effects, **(2)** chelation and disruption of the extracellular polymeric substance (EPS), and **(3)** surfactant-mediated or charge-mediated effects on biofilm architecture and bacterial adhesion.

The scientific review will assist in understanding the specific mechanisms and synergistic interactions involved to combat infection that each dressing is engineered to be effective at.

REVIEW

Direct Antimicrobial Effects:

Polyhexamethylene biguanide (PHMB) – is a broad-spectrum antiseptic, effective against Gram positive / Gram negative bacteria and fungi and has a low risk of resistance developing[1].
PHMB is a positively charged polymer that can electrostatically bind to the negatively charged phospholipid of bacterial cell walls / membranes. This binding causes disruption of the cell membrane, by displacing the calcium and magnesium ions that normally stabilise the membrane. This creates gaps that allow the PHMB molecules to leak into the cytoplasm, hence PHMB is primarily a membrane-disruptor[2] (Fig 3).

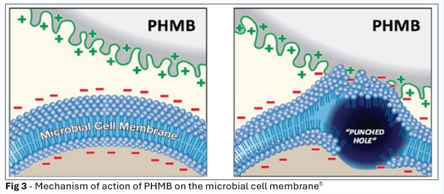

Fig 3 - Mechanism of action of PHMB on the microbial cell membrane[5]

Research has then gone on to highlight the intracellular damage, whereby PHMB also binds to DNA and other nucleic acids, damaging or inactivating them. By binding so tightly to the nucleic acids, PHMB causes the DNA/RNA to aggregate or precipitate. This effectively "shuts down" the cell's ability to replicate or transcribe its genetic material, leading to cell death[3, 4] (Fig 4).

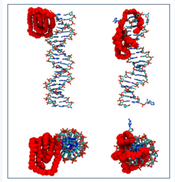

Fig 4 – PHMB and DNA interaction[6]

Silver – has been widely recognised for its broad-spectrum antimicrobial properties for centuries. It provides an antimicrobial effect via a multi-targeted approach, whereby the positively charged silver ions are electrostatically attracted to the negatively charged bacterial cell wall, and following disruption to the cytoplasmic membrane, leakage of the key cellular components , such as potassium ions, proteins, and ATP occurs, causing the cell to burst[7] (Fig 5).

Silver ions transported into the cell disrupt cell function by binding to proteins and interfering with energy production, enzyme function and cell replication, which are all vital for survival[7].
The interaction between silver and the cell, triggers the production of ROS (Reactive Oxygen Species Generation) causing oxidative stress that damages cellular proteins and structures[8].

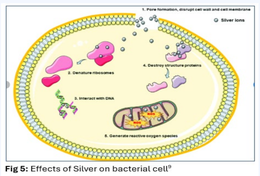

Fig 5: Effects of Silver on bacterial cell[9]

Chelation and Disruption of the Extracellular Polymeric Substance:

EDTA – a well-known chelating agent, which binds primarily to divalent cations, such as magnesium (Mg^{2+}) and calcium (Ca^{2+}), essential for maintaining bacterial cell wall integrity. EDTA destabilizes bacterial membranes by chelating vital ions, which compromises membrane integrity and increases permeability[10].

EPS (Extracellular Polymeric Substance) is the main component of a biofilm, often described as 'the slime'.
It is composed of polysaccharides, proteins, nucleic acids, and lipids, which provides a well structured and protective shield for the bacterial community within[11] (Fig 6).

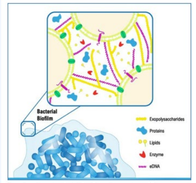

Fig 6 – Bacterial Biofilm Structure[12]

Benzethonium chloride (BC) - is a quaternary ammonium compound, that acts as a cationic surfactant; a surface-active agent with a positively charged hydrophilic head, which targets and disrupts the cell walls and membranes of bacteria, as they bind to negatively charged surfaces[13].

Surfactants help substances to mix and disperse by lowering the surface tension (the cohesive forces) between them. BC reduces the surface tension between the biofilm and the wound bed, and between components within the biofilm matrix, therefore facilitating its release, dispersal and discouraging its reformation[14].

Whilst BC has some inherent antiseptic properties, it is predominantly effective when used in combination with chelating agents (like EDTA) as it helps break down the protective exopolysaccharide (EPS) matrix, allowing increase penetration of the antimicrobial into the biofilm[15].

Surfactant-Mediated or Charge-Mediated Effects on Biofilm Architecture and Bacterial Adhesion:

EDTA – As previously discussed, EDTA disrupts EPS and bacterial adhesion mainly via its role as a divalent cation chelator.

Further to this, EDTA specifically inhibits the production and assembly of critical adhesive components like curli fibres[16] (Fig 7), which are extracellular functional amyloids that act as major factors in bacterial adhesion to surfaces and biofilm formation. EDTA reduces the ability of bacteria to adhere by directly inhibiting the production and assembly of these components[17].

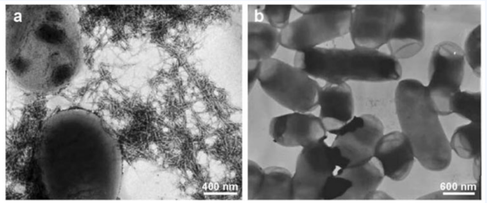

Fig 7 – Electron micrograph images of a) a bacterium-expressing curli and b) bacteria not expressing curli[18].

The information so far, provides an overview of the individual modes of action of each component. The following provides a synergistic evaluation of the following combinations, based on the 2 dressing type compositions:

PHMB and EDTA – a multi-step attack is employed, whereby the potentiation provided by EDTA, often referred to as 'a chemical drill', helps "wet" the biofilm surface by stripping away stabilizing minerals, allowing the aqueous PHMB solution to soak in rather than bead off[19].

A biofilm matrix is often negatively charged, which can "trap" positively charged molecules such as PHMB at the outer surface. By disrupting the matrix structure, EDTA ensures PHMB isn't just stuck on the outside but can migrate to the deeper, dormant layers of the colony. Another aspect of biofilms is the presence of "persister cells", which are metabolically inactive and highly resistant. EDTA's destabilisation of the matrix can "wake up" or expose these cells, making them vulnerable to PHMB's membrane-disrupting action. This is a key and pivotal attribute of the EDTA / PHMB effect when combating biofilm reformation[20].

Silver + EDTA + BC – a triple effect on biofilms [21,22]

- ❑ **EDTA** chelates metal ions, breaking down the biofilm EPS matrix.
- ❑ **BC** acts as a surfactant, reducing surface tension to allow deep penetration.
- ❑ **Silver** destroys the now-exposed, bacterial cells.

CONCLUSION

This literature-based analysis has focused on the two antimicrobial CMC wound dressings with respect to the mode of action of their antimicrobial agents and additional excipients.

Despite distinct antimicrobial chemistries, the combination of PHMB with EDTA appears to recreate the key functional elements of the Silver + EDTA + BC system i.e. broad-spectrum antimicrobial activity, matrix chelation and biofilm disruption, thereby exhibiting a multi-pronged attack.

These findings support the rationale for a PHMB + EDTA CMC dressing as a suitable, non-silver alternative, with the goal of improving treatment outcomes, whilst additionally, no bacterial resistance has been reported towards PHMB[6] and it has low cytotoxicity and high biocompatibility.

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