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Development of a novel antimicrobial seaweed extract-based hydrogel wound dressing

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ABSTRACT

The objective of this study was to develop a novel antimicrobial seaweed wound dressing. The seaweed extract was active against nine clinically-relevant wound pathogens. A hydrogel formulation was prepared using polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP), followed by addition of 1% seaweed extract. The antimicrobial properties of the novel dressing were tested using agar diffusion assays, with release-profiles examined using gel leaching and gel transfer assays. The dressing was found to be effective against the same microbial strains as the seaweed extract, with similar efficacy to the commonly used silver-based dressing, Acticoat®. Antimicrobial release-profile assays revealed that the dressing was effective in inhibiting 70–90% of the bacterial population within the first 30 min, followed by a long, sustained released up to 97 h, without leaving a residue following five subsequent transfers of the dressing. Antimicrobial activity was stable for up to 6 months of storage at 4 °C, but activity was reduced slightly after 15 weeks. Following autoclave sterilization, the dressing displayed a slower release profile compared to a non-autoclaved counterpart. Hence, the seaweed dressing may have commercial applications, potentially competing with silver-based dressings at a lower cost per-application. This is the first report of development of a seaweed-based antimicrobial dressing.

1. Introduction

Staphylococcus aureus is commonly found on the skin, but once this bacterium is transferred to a vulnerable site such as an open wound, it will cause infection. The development of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) and the more virulent vancomycin-resistant *S. aureus* (VRSA) have made the control of these infections difficult. Treatment of MRSA-infected wounds presents a great challenge, as there are relatively few antimicrobial wound dressings currently available on the market that specifically treat MRSA infections. Silver-based wound dressings are one option for the management and treatment of MRSA-infected wounds, but these are expensive, and there are concerns about their toxicity (Edwards-Jones et al., 2004). There are also side effects associated with their use, the most common being blackening of the area exposed to the silver, a condition known as argyria (Lansdown and Williams, 2004). In addition, there are cases, though limited, of increased levels of silver (above the normal range) in blood and urine (Trop et al., 2006); increased levels of liver enzymes (Baldi et al., 1988; Trop et al., 2006); damage to organs such as liver and kidneys; irritation to eyes, skin, respiratory and intestinal tracts; and effects on white blood cell counts (Drake and Hazelwood, 2005; Lansdown and Williams, 2004). It has also been shown that silver nanoparticles cause mitochondrial damage *in vitro* and result in the generation of reactive oxidative species which, in turn lead to DNA damage, even at concentrations as low as 25–50 μL (AshaRani et al., 2008).

Therefore, in recent years, natural/holistic remedies have become more attractive for wound management. For instance, honey is reported to have antimicrobial activity against a wide range of skin pathogens, including MRSA, and is beneficial for wound healing (Davis and Perez, 2009; Molan, 2006). Apart from honey, there are many plants with reported anti-MRSA activity. However, only a few studies have attempted to incorporate either honey or plant extracts into wound dressings. These studies include, for example, those that have incorporated essential oils from tea tree, patchouli, geranium and lavender into commonly used gauze dressings (Edwards-Jones et al., 2004) and honey into a chitosan/gelatin hydrogel-based dressing (Wang et al., 2011). Both plant- and honey-based dressings were active against wound pathogens such as *S. aureus* and MRSA (Edwards-Jones

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Table 1
Microbial strains used and their culture conditions.

Bacteria	Code	Culture conditions	Media used
MRSA	W73365	37 °C, aerobic	MHA
	WIT 618	37 °C, aerobic	MHA
	WIT 619	37 °C, aerobic	MHA
	WIT 620	37 °C, aerobic	MHA
	WIT 621	37 °C, aerobic	MHA
Vancomycin-resistant enterococci	NCTC 12201	37 °C, aerobic	MHA
	WIT 623	37 °C, aerobic	MHA
	WIT 624	37 °C, aerobic	MHA
E. coli	ATCC 25922	37 °C, aerobic	MHA
	DSMZ 10720	37 °C, aerobic	MHA
	WIT 626	37 °C, aerobic	MHA
Enterococcus faecalis	WIT 627	37 °C, aerobic	MHA
	DPC 4928	37 °C, aerobic	MHA
Bacteriodes fragilis	WIT 628	37 °C, anaerobic	MHA, B + MHA
Acinetobacter baumanii	WIT 629	37 °C, aerobic	MHA
Enterobacter cloacae	WIT 630	37 °C, aerobic	MHA
Group A streptococci	NCTC 12696	37 °C, aerobic	MHA, B + MHA
	WIT 632	37 °C, aerobic	MHA, B + MHA
	WIT 633	37 °C, aerobic	MHA, B + MHA
Pseudomonas aeruginosa	NCTC 10662	28 °C, aerobic	MHA
	PA01	28 °C, aerobic	MHA
Proteus mirabilis	NCTC 10975	37 °C, aerobic	MHA
Clostridium perfringens	NCTC 11229	37 °C, anaerobic	MHA, B + MHA
Klebsiella pneumoniae	NCTC 700603	37 °C, aerobic	MHA
	WIT 639	37 °C, aerobic	MHA
	WIT 640	37 °C, aerobic	MHA
	WIT 641	37 °C, aerobic	MHA
S. aureus	DPC 5246	37 °C, aerobic	MHA
	NCTC 12973	37 °C, aerobic	MHA
Peptostreptococcus anaerobius	WIT 643	37 °C, anaerobic	MHA, B + MHA
Candida albicans	ATCC 3179	28 °C, aerobic	MHA

MHA – Muller Hinton Agar; B + MHA – Muller Hinton Agar supplemented with 5% sheep blood.

et al., 2004; Wang et al., 2011). In another study, a traditional Chinese medicinal plant, *Bletilla striata*, was incorporated into a novel dressing formulation, not for its antimicrobial effects but for its wound healing properties (Liu et al., 2009). However, only a few commercially-available antimicrobial dressings have been developed from natural sources. These include AmeriGel® (oak extract) (Moore and Perkins, 2010), MediHoney™, L-Mesitran® and Activon (honey) which have proven effective against a range of multidrug-resistant Gram-positive and -negative bacteria (George and Cutting, 2007; Visavadia et al., 2008).

Wound dressings developed using natural compounds have the added advantage of being cheaper than silver dressings. In a study comparing the efficacy and cost effectiveness of a number of different types of wound dressings, AmeriGel® was reported to have an advantage over silver-based dressings as it had similar antimicrobial efficacy, but at a much lower cost per-application (approximately 15 times cheaper than silver) (Moore and Perkins, 2010). This is because the antimicrobial agent contained in AmeriGel® is a natural extract derived from oak and is therefore, relatively cheap to produce compared to silver. Therefore, huge potential exists to incorporate natural antimicrobial extracts into wound dressings to replace the expensive silver formulations, which are currently used in clinical settings. Seaweeds represent one such natural source, as a wide range of antimicrobial activities has been reported from seaweed extracts (Blunt et al., 2011; Chanda et al., 2010; Ioannou and Roussis, 2009; Liu et al., 2011). Alginate, a polymer derived from seaweed, is already widely used in the manufacture of wound dressings, mainly for its ability to promote wound healing by maintaining moisture levels in the wound and to promote cell proliferation and clotting mechanisms (Boateng et al., 2008). However, alginate does not display antimicrobial properties (Boateng et al., 2008) and to date, no seaweed-derived antimicrobials have been used in wound dressings. Therefore, there is potential to develop an antimicrobial dressing incorporating antimicrobial seaweed extracts which are active against a range of antibiotic-resistant, wound-related pathogens such as MRSA.

The aim of this study was to develop a novel antimicrobial seaweed extract-impregnated hydrogel dressing. The ability of the hydrogel dressing to retain and release the antimicrobial compound(s) contained within the extract was studied *in vitro* using the agar diffusion assay against a range of clinically-relevant wound pathogens. The antimicrobial release profile of the novel seaweed dressing preparation was also examined using gel leaching and gel transfer assays. Preliminary comparison of the seaweed dressing with commonly used silver dressings was also performed.

2. Materials and methods

2.1. Cultures and culture conditions

The bacterial and yeast strains used for assessment of antimicrobial activity and their culture conditions are listed in Table 1.