The Trojan Horse principle in wound management: a challenge for upscaling

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Abstract:
Infectious diseases are a major concern and increasing threat to human health. They represent a very significant burden on healthcare systems worldwide. Infection with bacteria like MRSA often cannot be prevented by vaccination and they lack early stage diagnostic and treatment options. The present paper introduces a European effort to develop responsive polymer based materials that can detect, signal and treat infectious disease in paediatric burn wounds. A number of possible solutions using bio-responsive nano-containers coupled with polymer based drug release systems show promising solutions. The developed materials require coating technologies such as spray coating, dip coating and/or plasma coating.

Introduction:
The skin is the largest organ in the body and performs numerous vital functions, the most important being its function as a protective coating against infection. When this barrier is damaged, pathogens have a direct route to infiltrate the body, possibly resulting in infection. As an example, immediately following a thermal burn, the surface of the burn wound is free of micro-organisms. However, deep cutaneous structures that survive the initial burn injury (e.g. sweat glands, hair follicles) often contain staphyloccoci, which colonise the wound surface during the subsequent 48 hours. Over the following 5-7 days, other microbes, including gram-negative and gram-positive bacteria, colonise the wound. These potential pathogens typically come from the patients’ gastrointestinal tract, upper respiratory tract, or the hospital environment, transferring through contact with health care workers. Quite often, fungal infections develop later, either after broad-spectrum antibiotics have been administered or after wound care has been delayed.

The current state of the art in burn wound dressings includes plain wound dressings and different ‘biological’ dressings (dressings impregnated with growth factors). These are generally based on a polyethylene or polypropylene non-woven base material that is surface modified in wet chemical dip coating processes. Other materials include viscase and polyurethane or mixtures of these materials.

Alternatively to these plain wound dressings, allografts from human cadavers are applied after cleaning of the burn wound. If the burn is deeper (3rd degree), skin cover and healing can only be achieved by the grafting of the patient’s own skin. Other approaches involve the culture of human dermal fibroblasts set in a natural human collagen matrix, which mimics the structure of skin and is intended to assist the regeneration of sub epithelial cells (the dermis) such as ICX-SKN™ made by Intercytex.

Recent developments of biologically derived wound dressings containing both a collagen scaffold and growth factors, such as Biobrane™ and have helped reduce the need for grafting, especially in more superficial burn injuries (Figure 1). Unfortunately, dressings that encourage cell growth can also assist microbial growth. On the other hand, dressings such as Acticoat™, which contain silver to suppress microbial growth are also partially cytotoxic and can reduce epithelialisation and tissue growth. This, in turn, may lead to a significant increase in hospitalisation time and health care costs.
Silver containing dressings continually expose the wound to the antimicrobial agent which may increase the rate of bacteria evolving resistance.

*Figure 1 Photograph showing a paediatric burn wound treated with the biological dressing Biobrane™ (courtesy of Frenchay Hospital, NHS, Bristol, UK)*

In current clinical situations, serious burns in adults or children are cleaned, dead tissue / debris are removed and a dressing or tissue graft (as required) is applied. Depending on the type of wound or burn being treated this can be very traumatic for the patient. Furthermore, it will result in a loss of the dressing, the need for anaesthesia and the need for repeated dressing changes and readmission to hospital.

The most important pathogenic bacteria in burns infections are: Staphylococcal aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Klebsiella pneumoniae. Pathogenic bacteria cause harm to their eukaryotic hosts by secreting enzymes and toxins that damage cells and connective matrix material of the tissues. One of the most important bacteria in nosocomial infections is *Staphylococcus aureus*, which displays a large armoury of specific proteins that enable infection.\(^V\) The secretion of such proteins or virulence factors will alter the properties of the host environment and is common to all pathogenic bacteria. Their natural role is the degradation of tissue via enzymatic cleavage of tissue matrix proteins. Each of these types of enzymes attacks selectively a particular type of bond. Lipases, for instance, are known to attack and hydrolyse acyl bonds. This degradative behaviour has been exploited for the controlled release of hydrophobic drugs from polymeric carriers, such as nanoparticles, micelles or vesicles. Knowledge of the enzymes and toxins secreted by specific pathogens and their mode of attack on eukaryotic cells provides a unique opportunity for detecting the presence of pathogens and using this to combat infection.

A European group of experts in the area of materials science, biology and engineering has initiated a study into the development of a novel wound bandage that exploits these mechanisms of nature to (a) indicate infection and (b) treat infection by releasing drugs only when pathogenic bacteria are present. It involves the development of nano sized, polymer based containers filled with the desired dye or the desired drug. The outer shell of the nano-container is susceptible to lysis by the bacterial toxins and releases its payload upon command, see Figure 2.

*Figure 2: Schematic of the nano-containers attached to a fibre surface releasing their payload upon stimulation of bacterial toxins.*
Results & Discussion

Figure 3 Micrographs showing the nano-containers before and after release of their payload (courtesy of Dr. Grit Baier, MPIP)

Current state of the art technology allows for a number of different synthetic routes to be used for the production of the nano-containers. A typical example of a responsive nano-container is shown in Figure 3 before and after lysis. Irrespective of the pathways chosen to synthesise the nano-container, the key requirements for the capsules are: stability, sensitivity, bacterial selectivity, control over the chemical conjugation to polymeric wound dressings and the proven absence of cytotoxicity to healthy tissue. Figure 4 shows the proof of principle study for bacterial lysis of lipid based nano-vesicles containing a reporter dye molecule which is released and dispersed upon bacterial toxin attack. Non-pathogenic bacteria were not able to “crack” the nano-vesicle shell to release the dye.

Fluorescence Microscopy of Vesicle Lysis by bacteria

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Figure 4 Fluorescence micrographs showing the proof of principle of dye release upon exposure to pathogenic bacteria such as S. aureus and P. aeruginosa in comparison to a non-pathogenic strain of E.coli (courtesy of Dr. A.T.A. Jenkins, University of Bath, UK)

In order to constitute a viable system for industrial production, the experimental lab process must be scaled up and an evaluation must be made of the critical parameters in this process. The main factors to be considered are:
- Uniformity and density of the nanocapsule attachment process,
- Speed of the process,
- Applicability to continuous production and
- Processing costs.
Current methods to immobilise and chemically attach the nano-containers to typical wound bandage materials, i.e. non wovens, is by the use of plasma activation methods followed by dip coating or spray coating techniques. Some of the possible routes currently under investigation are shown in Figure 5 above. Optimum processing conditions are still being investigated and will be discussed.

Summary

The current paper discusses a new approach to develop “intelligent”, or bio-responsive wound dressings based on sophisticated nano-containers designed to be covalently attached to textile surfaces such as non wovens and fabrics.

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