Methods to test bacterial/viral barrier properties of dressings

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Introduction

A good wound dressing will possess a range of desirable qualities such as maintaining an environment that promotes healing (Sood et al., 2014), protecting the wound from external pathogens, and preventing infection in the wound from contaminating the external environment in order to protect patients and staff. To achieve these requirements, some dressings are designed to stop bacteria entering a wound (Mertz et al.,1985), whilst others possess 'anti-bacterial' properties (such as dressings impregnated with silver) which are designed to destroy bacteria in an infected wound (Toy and Macera 2011).

Viruses may contaminate wounds, and can be a source of contamination to staff and patients. There are cases where live vaccinations have led to outbreaks of viral infection, most probably from transmission of the virus from the vaccination site (for example Vaccinia virus inoculation as a vaccination against small pox) (Talbot et al., 2006, Savona et al., 2007). Patients with blood borne virus infections (such as HIV and Hepatitis B) who sustain wounds may also pose an infection risk to others.

Manufacturers of wound care products often wish to demonstrate the viral/bacterial barrier properties of their dressings. The test method presented here has been developed by SMTL and this paper aims to describe the methodology devised to test the viral and/or bacteria barrier properties of dressings.

Methods

Note that all tests are carried out in triplicate.

The test dressing is placed between the 2 glass chambers as shown in the diagram below (fig 1). The apparatus is then clamped into place using a metal frame (seen in fig. 2).

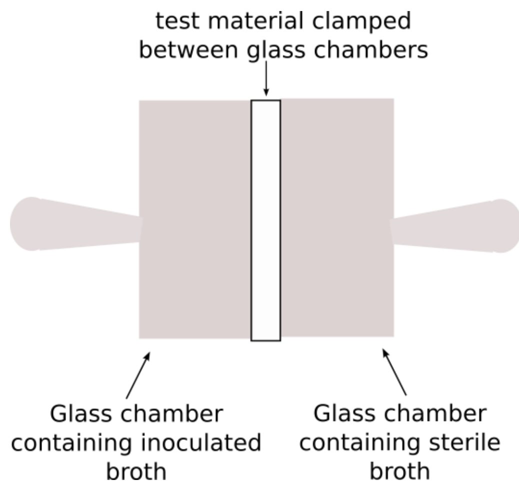

Figure 1 - schematic of the test chamber.

Figure 2 - photograph of test apparatus in use

Viral barrier testing

Once the chamber has been assembled, sterile bacteriophage nutrient broth is added to both sides, and one side is inoculated with at least $1x10^7$ pfu/ml (plaque-forming units) of bacteriophage (viruses that infect bacteria). The apparatus is left at room temperature for 7 days. Unlike the bacterial barrier test (see below), this experimental set up is not pre-conditioned for 2 days beforehand. The reason for this is because testing takes place at room temperature, there is less opportunity for pathogens that could contaminate the sample to thrive. Controls are used to ensure that it is the challenge organism that is being detected rather than another organism which may have contaminated the sample. The bacteriophage used for the experiments is the only virus known to infect the particular strain of bacteria used on the agar plates.

After 7 days, samples from both the inoculated and non-inoculated sides of the chamber are serially diluted and plated out onto agar plates containing host bacteria, grown as a mono-layer (or lawns, see fig. 3) across the plate surface.

Unlike bacteria, bacteriophage are not visible on agar plates after incubation. The detection method used is therefore to grow a 'lawn' of bacteria on an agar plate, and then add the bacteriophage solution to this plate.   Each phage within the sample will infect and lyse a host bacteria, and then replicate and continue to infect surrounding bacteria over time. This forms a plaque of dead bacteria. After incubation, the plaques of lysed bacteria are clearly visible against a background of live bacteria (see fig. 4). As each plaque originates from a single bacteriophage, plaques can be counted and bacteriophage concentrations calculated.

Fig. 3 shows a mono-layer of host bacteria covering the surface of the agar plate and fig. 4 shows a mono-layer after it has been infected with phage, forming clearly visible plaques.

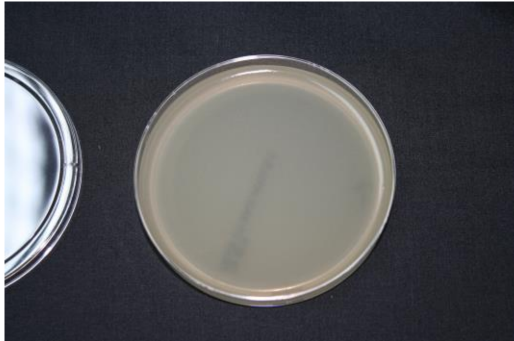

Figure 3 - agar plate with lawn of bacteria

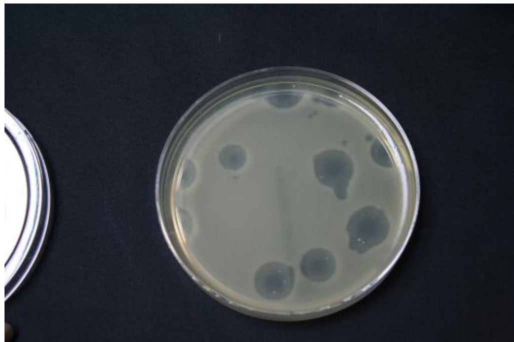

Figure 4 - agar plate showing plaques, indicating presence of phage.

Bacterial barrier testing

Once the dressing has been clamped between the two chambers as shown above, nutrient broth is added to both sides and the apparatus pre-incubated for 2 days at 37°C to ensure there is no contamination of the broth. Following this, the chamber on one side is inoculated with bacteria (*Serratia marcescens* is commonly used as it is small in size and easily distinguished under the microscope) and incubated at 37°C. The non-inoculated sides are examined on a daily basis for signs of turbidity which indicates the presence of bacteria. When passage of bacteria is suspected, samples are taken from that chamber, and spread onto plates alongside samples from the inoculated chambers. After incubation and histological staining, the bacteria can be compared to clarify whether the challenge bacteria penetrated the dressing or whether the turbidity indicates contamination of another type of organism.

Controls

Positive and negative controls are key elements of viral and bacterial barrier testing. A complete barrier product is used as the negative control, which must ensure that no organisms penetrate to the non-inoculated side. If the experiment has been set up appropriately, the non-inoculated side of the negative control dressing should remain free from challenge organisms. In contrast, a positive control film possesses holes large enough for organisms to pass through, resulting in both sides of the chamber containing challenge organisms. SMTL have sourced a positive control material from a manufacturer of woundcare films which contains holes in the range 0.03-0.05mm diameter. An example of the ranges seen is given in table 1 below, which shows the diameters of 10 holes randomly selected from a 10 cm^2 surface of the positive control film.

Size of holes in mm
0.038
0.042
0.046
0.050
0.035
0.033
0.041
0.033
0.040
0.041

Table 1-Positive control hole sizes

Results

The following 2 tables display the results of viral barrier testing on two different types of dressing (A and B) performed by SMTL for a dressings manufacturer. A negative result means that the non-inoculated broth remained sterile. Positive and negative control results are also included.

Sample	**Contamination of sterile broth +/-**	
	24h	**Day 7**
1	-	-
2	-	-

3	-	-
+ve control	+	+
-ve control	NA	-

Table 2 - Dressing A

Sample	**Contamination of sterile broth +/-**	
	48h	**Day 7**
1	+	+
2	-	-
3	-	-
+ve control	+	+
-ve control	NA	-

Table 3 - Dressing B

The results above show that one of the 3 samples of dressing B was permeable to bacteriophage within the first 48 hours, whilst all other samples were not (table 3). The samples were randomly sampled from 3 different boxes of dressings. A failure of one of the triplicate samples means that particular dressing fails the test.

When the same 2 dressings were tested as a barrier to bacteria (as described above) no bacteria were detected on the non-inoculated side of the dressing on any occasion, indicating that both dressings were impermeable to the test bacterium, and that dressing B was permeable to phage.

Discussion

This paper describes a test method devised by SMTL to assess whether different materials such as wound dressings are permeable to bacteria or viruses. In the experimental set up, the dressing can be orientated so that the external or internal surface faces the inoculated broth in order to assess permeability of organisms in either direction. Future developments of this test could include methods to test viral permeability when the external surface of the dressing is dry for some of the time. This would more accurately mimic use of such dressings where the outside remains dry for most of the time but may become wet due to washing and showering. This system could be used to provide useful information to dressing manufacturers and evidence for the selection of products by health care providers and workers.

Bibliography

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