Quality Evaluation Methods for Textile Substrates Based Wound Dressings

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Abstract—Over the past several years, great advances have been made on development of Textile substrates based wound dressing, which plays vital role in health and hygiene sector. The quality evaluation technique is essential for evaluate the performance, characteristics and properties of the wound care products. The present review highlights the quality evaluation technique for textile substrates based wound dressings.

Keywords—Bio-polymers, Drugs, Evaluation, Textile Substrates, Quality Evaluation, Wound dressing

I. INTRODUCTION

The primary function of wound dressings is to avoid strike through and to protect the wounded site from contamination and further injuries [1]. The use of standards and quality evaluation techniques to characterise the surgical/wound dressings for product performs clinically that will determine its acceptability and commercial success, well-designed laboratory tests can provide a useful performance indicator, particularly in comparative terms [2].

II. IMPORTANCE OF QUALITY EVALUATION FOR WOUND DRESSING

A. Dressing selection:

Dressing selection is challenging because of the availability of large varieties of dressings. Each product has specific actions, benefits and drawbacks and hence determining which dressing best suits the patient’s needs, is a multifaceted process. Dressing choice depends on factors such as wound type and wound appearance, exudate, presence or absence of pain and required dressing changing frequency [3]. Product true performance is analyzed by specific/widely accepted standards and thus make user / buyer selecting the superior product among various wound care products in market.

B. Compare the performance of selective wound dressing by international standards.

C. To facilitate comparisons with competitive products.

D. To analyze the shelf life and adversely side effect of the product, etc.

This review focus on following essential quality evaluation technique for textile substrates based wound dressing:

- Physical characterization
- Antimicrobial characteristics
- Resistance of wound dressing to penetration by odour
- Drug release study
- Cytotoxicity
- Field trails
  i. Skin irritation study
  ii. Wound healing ability

III. QUALITY EVALUATION METHODS

A. Physical characterization

Physical characterization of wound dressing [4] is dressing mass and thickness, absorbency, dehydration rate, dispersion characteristics, vertical wicking, air permeability, pH and Moisture Vapour Transmission Rate (MVTR) [5]. Prior to all the testing, the dressing specimens are conditioned for 48 hours in 65% relative humidity and 20°C atmosphere.

i. Dressing Mass and Thickness

Dressing mass (g m⁻²), thickness (mm) and number of layers are determined in accordance with BS EN 12127:1998 and BS EN ISO 9073-2:1997[6&7]. The mass of five specimens of each dressing is measured individually and the mean mass per unit area of the specimens is calculated. The specimen sizes is 5cm ×5cm for determination of mass and these are converted to g m⁻². The thicknesses of specimens are normally determined by using a thickness tester. Five specimens for each dressing are tested and the mean value is calculated in mm.

ii. Absorbency of Wound Dressing

The absorbency of wound dressings is determined by using BS EN 13726-1:2002 section 3.2 [5] free swell absorptive capacities. For this test, 5cm x 5cm dressing specimens were prepared.
The test solution A (2.298g sodium chloride, 0.368g calcium chloride dihydrate are added to 1 litre of de-ionised water) is prepared for the testing. All the prepared dressing specimens are weighed and recorded before testing and placed in Petri dishes.

The solution A is warmed to 37±1°C and 40 times the mass equivalent of the specimen is dispensed slowly and gently onto the specimens in the Petri dishes. The Petri dishes are then placed in an incubator for 30 minutes at 37±1°C (body temperature). After 30 minutes of conditioning the dishes are removed from the incubator and suspended by one corner by using tweezers to allow excessive solution to drip off for 30 seconds and reweighed for wet mass. The calculation of results; 

Mass loss upon drying (%) = (B-A) ×100/B

Where;
B is the mass of specimens before testing and A is the mass of specimens after testing

The dehydration behaviour was determined by measuring the difference between the mass of wet and dry specimens. The specimens are dried in an incubator for 24 hours at 37±1°C. The mass of dry specimens is determined before submerging them in an excess volume of solution A at 37±1°C for 30 minutes. The specimens are taken out from fluid and suspended by a corner for 30 seconds for free drainage. After draining they were re-weighed and put into Petri dishes and kept in an incubator for 24 hours at 37±1°C.

The calculation of results; 

Dehydration rate (g/min) = W-D/T

Where;
W is the wet mass of specimens, D is the dry mass of specimens, T the test period in minutes

In order to evaluate the rate of absorption, drops of solution A are dropped onto the specimen using an eye dropper on the wound contact layer surface of each dressing and are allowed to fully absorb and the time of absorption was recorded in seconds. 20 drops are dropped onto each dressing and the mean times are calculated.

The vertical wicking is one of the important properties for fibrous dressings. This test can be applied only for the fibrous dressings due to the nature of test procedure. The test specimens are prepared to 15mm width and 100mm length.

Eosin B was added into the solution A. The specimens are slowly immersed into the solution vertically up to 10mm length and left for 60 seconds. Vertical wicking of dressings is determined in mm.

Dispersion characteristics of the dressings were determined in accordance with BS EN 13726-2:2001[8], section 3.6. For this testing, 5cm x5cm dressing specimens are prepared and placed into a 250ml conical flask into which 50 ± 1ml solution A was added. The flask was slowly and gently swirled for 60 seconds. Following which, the specimens are removed and the dispersion is determined visually. The results are expressed as to whether there is dispersion or not in accordance with the standard.

The specimens were suspended into de-ionised water at a ratio of 1:100 (w/v) and are kept at room temperature for 24 hours. The pH is measured by using pH meter. Two measurements are carried out for first after 3 hours and second after 24 hours. pH meter is calibrated to pH 7.

Air Permeability

Air Permeability is evaluated as per ASTM D737-96 standards [9]. A powerful, muffled vacuum pump draws air through an interchangeable test head with a circular opening. For measurement, the test head appropriate for the selected test standard is mounted to the instrument. The specimen is clamped over the test head opening by pressing down the clamping arm which automatically starts the vacuum pump.

Skin is a viscoelastic material which is permanently subjected to slight stress. The tensile strength of a human skin is reported to be in the region of 1.8 (N/mm²) [10]. Any wound dressing developed ought to have tensile strength higher than this value. Otherwise, even with slight stress at the near vicinity of the wound, the dressing is likely to get ruptured [11].

Moisture vapour transmission rate (MVTR) testing

The MVTR test is important for wound dressing material, the liquid formed inside the wound layer are changes to vapour and transport to atmosphere. This moisture vapour transmission is helps to heal the wound; otherwise the wound dressing material is not allowing the moisture vapour to atmosphere will create wound infection [11].
MVTR as per BS EN 13726 – 2: 2002 [5] method. The test sample size of 40 mm diameter and fixes it in a test area of 35.7 mm inner diameter container, containing 20 ml distilled water. The test sample container is weighted and 0th hour readings ($W_1$) are noted and test starts. The testing condition of MVTR test is 37 ± 1 °C at RH 20%. Test run for 24 hour, than the test container is taken and weighted with helps of balance and this weight is note down as 24th hour reading ($W_2$). Now, the difference between 0th hour and 24th hour is note and result is calculated based on formula:

$$X = (W_1 – W_2) \times 1000 \times 24/T$$

Where,

$X$ is MVTR (g/m²/24 hour)

$W_1$ is the mass of the container, sample and liquid in grams

$W_2$ is the mass of the container, sample and liquid in grams after test period and

$T$ is the test period in hours

B. Antimicrobial Characteristics

i. Need for antimicrobial incorporated wound dressing

Wound is made by accident, deices, injury, foreign object penetration in the body or medical devices (ie: sutures, metal plate, surgical knife, etc) introduced in the body. The reason for infection lies in the formation and growing of bacterial biofilm on the surface of surgical devises. Once biofilm is formed, it is extremely difficult to eradicate even with vigorous antibiotics treatment. It has been observed that a higher dosage level of antibiotics to that of 50-5000 times is required to kill the biofilm bound bacteria [12].

Some wound dressings has ability to kill the bacteria or reduce the bacterial infection and dressing contain agents that have intrinsic antimicrobial activity such as antibiotics, antiseptics, silver ions or materials which possess a significant osmotic pressure capable of inhibiting bacterial growth [2]. Evaluate the antimicrobial performance of wound dressings is necessary for specialized dressings which contain antimicrobial agent or inherent antimicrobial activity.

ii. Methods for antimicrobial evaluation

Most popular method for evaluation of Antimicrobial characteristics for textile based wound dressings [13] are,

1. Quantitative method:

   ✓ American Association of Textile Chemists and Colorists (AATCC) – 100 [14]

2. Qualitative method:

   ✓ Determination of anti-bacterial property of test specimen : AATCC test method 100 – 2004

   Swatches of test and control specimens are inoculated with the organisms (S. Aureus : 1.5 x 10^8 Cfu/ml and K. Pneumoniae : 1.5 x 10^8 Cfu/ml). After inoculation, the specimens are incubated for 18 hours. After incubation, the bacteria are eluted from the specimen swatches by shaking in known amounts of neutralizing solution.

   The number of bacteria present in this liquid is determined and the percentage reduction by the specimens is calculated.

   Percent reduction (R) of bacteria by the specimen treatment

$$R = 100 \frac{(C–A)}{C}$$

Where,

$A$ = The number of bacteria recovered from the inoculated test specimen swatches incubated over the desired contact period

$C$ = The number of bacteria recovered from the inoculated control specimen swatches immediately after inoculation.

✓ JIS L 1902–Absorption method :

   The JIS L 1902–Absorption method as follows: first, an inoculum was prepared in 20±0.1 ml of NB and incubated for 24 h at 37±1°C. Then, bacteria concentration is adjusted to 3×108 cells ml−1, by absorbance reading and using the respective calibration curves. A volume of 400 μl from the previous suspension is added to 20 ml of NB and incubated for 3 h at 37±1°C. The bacteria concentration is measured again and diluted in NB 20× (in distilled water) to 3×105 cells ml–1 and 200 μl of this inoculum are added to each sample. The samples are incubated for 24 h at 37±1°C.

   Then, 20 ml of physiological saline solution (8.5 g of NaCl and 2.0 g of non-ionic surfactant) is added to samples which are vortexed. In order to achieve the number of living bacteria, a serial dilution plate count method is performed (JIS L 1902 2008).
2. **Qualitative Method**

   ✓ **Determination of anti-bacterial property of test specimen: Agar diffusion method (SN 195920)**

   Agar diffusion method is used determine the effectiveness antimicrobial agents and performance of the test specimens evaluated against pathogenic bacteria. A 100 IL aliquot of bacteria reconstituted in nutrient broth and previously subcultured spread onto an agar plate. Test specimens are cut into circular discs (15 mm in diameter) and placed on the top of the agar plate. The plates were incubated at 37°C for 24 h. If inhibitory concentrations is reached, there would be no growth of the microbes, which could be seen as a clear zone around the disc specimens. The zone is then recorded as an indication of inhibition against the microbial species.

   ✓ **AATCC 147-2004**

   In order to perform the AATCC 147 method, an inoculum is prepared as follows: 1.0±0.1 ml of a 24 h culture in nutrient broth (NB) is transferred into 9.0± 0.1 ml of sterile distilled water. With an inoculating loop, five streaks of the diluted inoculum are made over a standard Petri dish with nutrient agar (NA), without refilling the loop. The samples are placed over the streaks, ensuring intimate contact with the agar surface. The Petri dishes are incubated for 24 h at 37±2°C (AATCC 147 2004).

   ✓ **ISO 20645 method**

   In the ISO 20645 method, the textile samples are placed between two agar layers. The lower layer contained 10±0.1 ml of NA and the upper layer had 5±0.1 ml of NA with 6.7×10⁵ cells ml⁻¹ of the bacteria. The bacteria came from a previous NB inoculum incubated for 24 h at 37±2°C (ISO 20645 2004).

   ✓ **JIS L 1902–Halo Method**

   The protocol from JIS L 1902–Halo Method is performed as follows. A previous NB inoculum is incubated for 24 h at 37±2°C. Then, 1.0±0.1 ml from the inoculum with 1×10⁷ cells ml⁻¹ is added to 15 ml of NA warmed at 45–46°C. This solution is disposed in a sterilized Petri dish. After agar solidification, the samples are placed over the agar, and incubated for 24 h at 37±2°C (JIS L 1902 2008).

   **C. Resistance Of Wound Dressing To Penetration By Odour**

   Repulsive malodour of wounds is generally considered as an evidence of poor hygiene and cleanliness[11]. The objective of the test is to assess the resistance of wound dressing to penetration by odour.

   The sample is tested as per BS EN 13726 – 6: 2003 method. In this method, control sample and test sample is required for testing. The stainless steel container of 35.7 mm diameter and 40 mm height is used as both bottom part and top part. In between the two parts, the test sample is inserted with help of PTFE hollow gasket. In control preparation, the two pieces of container is heated for 1 hour at 105 °C. Place a gasket between the two parts of the container and purge the container with nitrogen gas through sampling port on top and seal with appropriate septum. Inject 0.5 µL of pure diethylamine into the container through septum and place into a 37°C oven for 20 minutes. Remove 250 µL sample of gas by gas tight syringe and inject it into GC (Gas chromatography) for testing.

   In test sample, the two pieces of container is heated for 1 hour at 105°C. After heating, the 1.3 % w/v solution of diethylamine in 20 mL of water is added to bottom container. Purge top half with nitrogen gas through sampling port, remove the purge line and seal with septum. Remove initial sample of 250 µL sample for GC analysis and place it in 37°C oven. Removing samples with appropriate intervals and note down the time to achieve 6 ppm with an accuracy of 10%. Repeat until the peak area of the control is exceeded.

   **D. Drug Release Study**

   In a test tube, 10 ml of distilled water is taken. The drug loaded substrate is immersed and kept in the water for 24 hours at room temperature. After 24 hours, the supernatant in the test tube is tested for the quantum of drug present in it using UV visible spectrophotometer. In the second day, 10 ml of distilled water is taken in another tube and the same drug loaded substrate (which is tested on the first day by water immersion) is immersed in the tube. After 24 hours, the supernatant is tested for the quantum of drug present. This procedure is repeated for till drug is exhausted from the substrate.

   **D. Cytotoxicity Test**

   Some wound dressings are biodegradable, incorporated with an antimicrobial agents or special finishes / coatings and produce by different techniques for specific purpose. In order to analyze the toxic compound or particles are present in the dressing, which may contaminate a wound, disturbing the healing pattern or adverse side effects to tissue growth, not supporting for cells growth etc. Cytotoxicity test method is a key to analyze and evaluate the wound dressing performance. ISO 10993-5 method [19] for an *in vitro* cytotoxicity test using direct contact method.
After incubation at $37 \pm 1^\circ C$ for 24 to 26 h, cell medium (normally culture medium of L-929 monolayer is used as a medium) is examined microscopically for the response around the test samples. The reactivity are graded as 0, 1, 2, 3 and 4 based on zone of lysis, vacuolization, detachment and membrane disintegration as per the Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Grade</th>
<th>Reactivity</th>
<th>Description of reactivity zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>No detectable zone around or under sample</td>
</tr>
<tr>
<td>1</td>
<td>Slightly</td>
<td>Some malformed or degenerated cells under specimen</td>
</tr>
<tr>
<td>2</td>
<td>Mild</td>
<td>Zone limited to area under specimen</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Zone extending specimen size up to 0.33 cm</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Zone extended farther than 0.33 cm beyond specimen</td>
</tr>
</tbody>
</table>

As per the ISO 10993 – 5 the achievement of numerical grade greater than 2 is considered as cytotoxicity.

It should be noted that whilst laboratory tests provide a means of comparing the performance of dressings, they cannot always predict how the dressing will perform in vivo. Table 2 indicates the testing standards applicable to the dressing categories for some conventional as well as advanced wound dressing included in this review.

### Table 2

<table>
<thead>
<tr>
<th>Dressing</th>
<th>Main functions</th>
<th>Key performance indicators</th>
<th>Testing standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gauze</td>
<td>Dressings can stick to the wound surface and disrupt the wound bed when removed. Use for minor wounds or as secondary dressings</td>
<td>Secondary dressing</td>
<td>BS EN 12127:1998  BS EN ISO 9073-2:1997</td>
</tr>
<tr>
<td>Alginate</td>
<td>Absorbs 815xudates to form moist gel on wound surface to regulate moisture at wound bed.</td>
<td>Absorbency. Gelling. Fluid handling.</td>
<td>BS EN 13726-1:2002</td>
</tr>
<tr>
<td>Antimicrobial (Honey, Silver, Iodine)</td>
<td>Control bio-burden, applied at infected wound bed.</td>
<td>Bacterial barrier properties</td>
<td>BS EN 13726-5</td>
</tr>
<tr>
<td>charcoal</td>
<td>Used as a primary dressing (when wound not exudative) or as a secondary dressing (over primary dressing) to suppress odour. Moisture inactivates charcoal.</td>
<td>Odour control.</td>
<td>BS EN 13726-6:2003</td>
</tr>
<tr>
<td>Film</td>
<td>Polurethane or co-polymer with porous adhesive layer which allows oxygen to pass through the membrane and moisture vapour to escape.</td>
<td>Moisture vapour transmission rate (MVTR).</td>
<td>BS EN 13726-2:2002</td>
</tr>
<tr>
<td>Hydrocolloid fibrous</td>
<td>Hydrocolloid fibres (sodium carboxymethylcellulose) absorb 815xudates and form a gel.</td>
<td>Absorbency.</td>
<td>BS EN 13726-1:2002</td>
</tr>
</tbody>
</table>
Hydrogel sheet
Cross linked polymers form thin sheet. High moisture content maintains moist interface which facilitates cell migration and reduces dressing adherence.

Absorbency.
BS EN 13726-1:2002

Collagens[21]
Dressings come in pads, gels or particles. Promote the deposit of newly formed collagen in the wound bed. Absorb exudate and provide a moist environment

Absorbency, MVTR, Conformability
BS EN 13726-1:2002
BS EN 13726-2:2002
BS EN 13726-4:2003

Soft silicone dressings[21]
Dressing coated with soft silicone as an adhesive or a wound contact layer
Suitable for almost all indications where it is important to prevent trauma to the wound and the surrounding skin

Moisture vapour transmission rate (MVTR).
BS EN 13726-2:2002

Composite wound dressings[21]
Combine the properties of hydrocolloids and polyurethane foams, making the dressing easy to apply and remove and last longer than any of these products used alone

Absorbency, MVTR, Conformability. Fluid handling.
BS EN 13726-1:2002
BS EN 13726-2:2002
BS EN 13726-4:2003

E. Field Trails / Animal Trails
A. Standard test method for assessing skin irritation (ASTM F 719 – 81 standard) [22]

The wound dressing materials are evaluated for potential skin irritation when they are used for covering the wound. The evaluation was as per ASTM F 719-81 standards. Exposure of skin to the test material is accomplished by means of a patch test technique employing two intact sites on the back of each of six albino rabbits. The skin is clipped free of hair one day prior to testing. The test substance is applied using 0.5 ml for liquids, 0.5 g for solids or semisolids and a 2.5 by 2.5 cm square patch for films. After application, each test site is covered with a 2.5 by 2.5 cm gauze flat and the entire trunk is occluded with a polyethylene sleeve. After 24 hours the sleeve, flat and test material are removed and test sites are evaluated for erythema and edema.

Scoring Method
Using the criteria given in Table 3, the test sites are scored for Erythema (ER) and Edema (ED)

Test sites can also be scored for erythema and edema at 48 hours as well as 72 hours after removal (as per the usage requirement) using the criteria given in Table 3.
B. Wound healing rate

The extent of wound healing of wound dressing is evaluated using the method proposed by Morton & Malone [23]. As per this method, healthy rats are employed for the experimentation. Excision of wounds was made on the rats as per the method suggested by Morton & Malone. The rats were anaesthetized with anaesthetic ether and placed in operation table in their natural position. A square wound of about 1.5 cm (width) x 0.2 cm (depth) is made on depilated ethanol-sterilized dorsal thoracic region of rats. Infection is made on wounds by staphylococc aureus. The dressings are applied on the wounds of the rats every day till the epithelialization is complete. The extent of wound contraction is studied by tracing the raw wound area in a tracing paper on 6th day, 12th day, 18th day and 24th day.

✓ Determination of wound healing rate

The weight of the traced portions of the wounded area of rats subjected to wound dressings is measured using electronic balance. Based on the difference in weight, the superiority or otherwise of a particular wound dressing is determined.

IV. CONCLUSION

The content of this article has been focused on quality evaluation methods for textile based wound dressing. There are many other quality evaluation methods available for textile based wound dressings like in-house testing methods, national/international testing standards. This paper highlights the major/popular/simple testing methods for textile based wound dressing. Details of quality evaluation methods for physical characterization of textile substrates based wound dressings have been discussed. Importance of antimicrobial activity assessment and its methods of evaluation for wound dressings are also reported. Biological evaluation, drug release study and odour control efficiency have been reported. Wound dressing category and standards applicable for some advanced wound care dressings also discussed. Field trials (ie animal trials) for textile based wound dressings are skin irritation and wound healing ability have been reported.

Acknowledgements

Authors express their sincere thanks to Dr. Prakash Vasudevan, Director, SITRA for his keen interest in this study.

REFERENCES


[16] SN 195920, Determination of the Antibacterial Activity -Agar Diffusion Plate Test


[22] ASTM F 719 – 81 Standard test method for assessing skin irritation