Introduction

Skin is the largest organ in the human body. It plays a crucial role as a protective barrier from the external environment; prevent external noxious agents such as all type of microbes from getting inside the body. It also maintains the internal environment by the regulation of water and electrolyte balance and thermoregulation. It is crucial to keep the skin intact so that the designated functions of skin are uncompromised. When the skin is damaged, due to any cause (mechanical injuries, ulcers, burns, neoplasm, or surgical trauma), there are chances of it getting infected. In that case the body resources are wasted in fighting them.

In the old approach of wound management, wounds were traditionally managed by the concept of wet to dry. The olden dressings were supposed to dry the wounds completely. This has changed into wet to moist dressings now. With the advanced progress of technology, the old understanding has shifted to become a new one. New

Study of hydrocellular functional material as microbicidal wound dressing for diabetic wound healing

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Abstract
A hydrocellular functional material as a wound dressing is developed and it is found to be superior in its efficacy as compared to some of the comparator controls in diabetic wound healing studies. A study on wound contraction and Histopathological analysis is done in rats. The efficacy of the dressing is comparable to the established wound dressings like Carboxymethyl cellulose alginate dressings and autolytic enzyme based hydrogel. It is found to be superior to Polyhexamethylene biguanide dressing used as reference controls in this study.

The reason for good wound healing performance of the dressing can be attributed to a combined property of effective exudates management and broad spectrum antimicrobial effect. The concept of functional hydro cellular material has shown good results due to the excellent balance of exudates pickup and drying it out. This ensures moist wound healing conditions on the wound. Because of its porous nature it allows good air flow and gaseous exchange in the structure.

The cationic sites created on the surface of the dressing ensure a good antimicrobial action on the exudates in the dressing. It reduces the infection load on the wound. The nonleaching property of the dressing also helps in preventing the generation of more resistant and mutant strains of the microbes.

The developed dressing can be used as a relatively durable long lasting dressing for wound management in diabetic wounds. The need of repetitive wound dressing changes can be brought down with this concept of dressing. It is not only cost effective in terms of its material cost but also is a cost effective solution when entire wound management cost is considered. Such novel wound dressing material can change the quality of life of diabetic wound patients especially in developing world, where access to functional advanced wound care dressings is limited.

Keywords
Hydrocellular, wound dressing, microbicidal, diabetic wound, exudates

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approach emphasizes on need of moist wound for better healing. Cotton gauze dressings as the universal surgical dressings used previously are being replaced by advanced wound dressings. These are engineered to specific wound handling to suit the dynamic process of healing. This is true especially in regards of various levels of exudates and infection management. A film of moisture on wound, prevents the formation of dry scab, and facilitates epithelialization of wound. If the wound is without moisture and scab develops from desiccation, the vascular plexuses in the dermis will be eroded and lost. Further water soluble essential substances such as amino acids, sugar, and electrolytes, are available for new cells formation only when the wound is moist. The transport of mediators will also run normally, and it can provide ideal condition for immune defense response only in moist wound conditions. The concept of moist wound healing has greatly facilitated chronic wound management in recent times. This has led to numerous dressing materials to control surface moisture in optimal amount.²

This moisture concept has been elaborated in the principals of managing wound to prepare its bed. It is called as TIME which refer to T for the removal of unhealthy and non-viable tissue, I for the control of infection and reduction of microbial load, M for the maintenance of moisture balance, and E for advancing the wound edge.³ In terms of moisture balance, the wound should be kept moist but not highly wet and flooded with exudates. High level of exudates built up lead to maceration of the wound. Maceration causes erosion of the healthy skin boundary of the wound well and hampers the wound healing and wound closure.

However, the moist and warm dressing may act as favorable conditions for the microbial proliferation. Bacteria and other organisms in the exudates have been studied to have an adverse effect on wound healing process.⁴ Microbial proliferation may lead to prolong inflammatory stage and cause infection. It may even lead to acute wounds getting converted to hard-to-heal chronic wounds.

Wound healing gets complicated further, where the body is under immune compromised condition. The body conditions like diabetics hinder the normal healing of wound. This lead to the formation of chronic wound, which is highly infected and difficult to heal. Such infected wounds are breeding ground for pathogens that build a colony in the wound site with the strong protein film called biofilm.⁵ Biofilm is very difficult to be eliminated by the use of topical or systemic antiseptics and antibiotics. Such wound may lead to bone infections that progresses rapidly in the bone marrow and if unattended, may ultimately lead to amputation of body part.

An acute wound is simply recognized as a wound that has occurred within the past 3 weeks or in some literature it may be to 4–5 weeks. Wounds that persist until more than 3–4 weeks are considered as chronic wounds. It mostly happens on diabetic foot ulcers and gangrene, pressure sores, ulcers due to chronic vein insufficiency or occlusive arterial diseases, radiation injury.⁶

Classic signs and symptoms of highly infected wounds include fever, warmth, edema, swelling, pain, erythema, and purulent drainage.⁷ Infected wounds will usually have a density of >10⁵ colony-forming bacterial units per cubic millimeter of tissue.²

The available and most widely used substrates like gauze materials, mostly cotton are too ineffective to manage the exudates load of chronic wounds. They get loaded with exudates soon. Nonwoven pads or sponge are prone to have basic disadvantage of keeping the reservoir of exudates on wound bed that leads to the wound bed getting wider on account of degradation of skin of wound well. Frequent dressing changes causes frequent traumatization of wound by peeling the newly formed wound surface and also increases the cost of wound management.⁶

Any significant delay in replacing dressing laden with exudates is seen to have negative effect on wound healing⁷

It is learned from the study of existing dressings that a base material is needed that has to be porous enough to soak and keep exudates in it and at the same time be stiff enough to avoid squeeze back when pressure from body movements are applied on it.⁸ Material is needed to be breathable for the gaseous exchange from the wound and help in gradual drying of the load of exudates over period of time. If the material could maintain a near equilibrium in terms of loading and drying, there would be a constant moistness on the wound, but not a flooding wetness. This is said to be the ideal condition of the wound dressing.⁹ The material is needed to be economical, easily available and at the same time proven non toxic and widely accepted by the medical regulators as safe material for the wound contact.

We have developed a 3 dimensional (3D) hydrocellular knitted antimicrobial functional material (fabric) which acts as an advanced foam dressing. It can be categorized as hydrocellular dressing; wherein the porous spaces in fabric structure facilities quick absorption of exudates by capillary action. It is a flexible and stretchable structure. The material being polyester, is highly biologically neutral, cheap and easily available and also accepted by medical regulators across the globe as a safe material to contact the wound surface.

The designed base material is a hydrocellular structure for physical excellence of permeability and moisture management. It was very vital to ensure the microbial load on and in the wound is taken care by suitable antimicrobial technology. There are many compounds and substances that are used for such purpose. Compounds containing silver, zinc, copper, chitosan, Polyhexamethylene biguanide (PHMB) and many active drugs like fucidic acid, povidone Iodine and silver sulfadiazine are used that act as antimicrobial on wound. The basic disadvantage with above listed every substance here is that the active
component is leached from the substrate and acts on the wound. Hence there is gradual reduction in the concentration of the active component that acts on the wound. This reduction leads to the reduced effectiveness of the antimicrobial effect in the wound over period of time. Also leaching of ingredients has varying degree of cell toxicity that needs to be managed by the healing body by overworking on the cells. Leaching chemistry is been documented to cause lot of microbial resistance built-up and hence it is a major challenge to ensure the microbes are killed but are not eventually getting more and more resistant to the antimicrobial agent and evolving in more dangerous strains. The current threat posed by evolution of drug resistant superbugs is a major challenge to the medical industry.

Considering these facts, in the construction of the functional dressing material in this study, we selected an antimicrobial agent that would not leach from the surface of the base material. It is bound to the surface of the substrate. Quaternary ammonium salts (QAS) have promising future as antimicrobial agents for textiles as they can be immobilized on the surface of textiles and have been said to cause no bacterial resistance built up due to the unique nature of its kill which is attributed to physical rupture of the cell wall of microbes. It is caused by the positively charged long aliphatic chains (cations). This is known to be the most efficacious antimicrobial agent as it disrupts the cell membrane of the microbes. The efficacy of dressing is long as the active ingredient is not utilized and unexhausted like traditional drug based dressings.

The functional material is characterized and it has been evaluated for its efficacy in preclinical studies on the induced diabetic wounds on albino Swiss mice. The functional dressing is also compared with commercially available advanced dressings like Carboxymethyl cellulose alginate dressing, Polyhexamethylene biguanide (PHMB) based foam dressing and Enzyme based autolytic hydrogel. The authors believed in the efficacy of QAS in various disinfectant activities as mentioned in various literatures constructed with the use of Polyethylene terephthalate [PET] and polyurethane yarn in the composition of 90% Polyethylene terephthalate and 10% Polyurethane. The fabric is produced on 24 gauge interlock knitting machine by spacer knitting technology. The structure has three distinct surfaces. The face is knitted by the cylinder needles with Polyethylene terephthalate yarn of 150/108 D and the back is knitted by the dial needles with Polyethylene terephthalate yarn of 150/108 D. The middle layer is made up of 40 D Monofilament Polyethylene terephthalate yarns that connect the front layer (cylinder loops) with the back layer (dial loops). 40 D polyurethane yarn is used as the elastomeric yarn to impart stretch to the structure. The Cylinder to dial height on the machine decides the space between the face and back layers of the fabric and hence it is optimally set to have 1.8 mm height of the fabric. The material is three dimensional (3D) in its construction unlike the conventional gauze dressings.

It must be noted that Polyethylene terephthalate is a known biocompatible material. Further it is a very stable material against bio fluids of the wound and it does not get deteriorated in long term usages.

Figure 1 shows the image of dressing along with the cross sectional view of the material. Table 1 enlists the constructional details of material.

Quaternary Ammonium Salt (QAS) are widely studied third and fourth generation antimicrobial agents in recent years.

The authors believed in the efficacy of QAS in various disinfectant activities as mentioned in various literatures.

**Experiment**

**Material**

The functional material for wound dressing is produced by technique of Weft knitted spacer fabric Production. It is

| Fabric content | 90% PET/10% polyurethane |
|----------------|--------------------------|
| Yarn at the face of fabric | 150/108D polyester filament yarns |
| Separator yarn at the center | 40D polyester, monofilament yarn |
| Yarn at the back of fabric | 150/108 polyester filament yarns |
| Polyurethane yarn | 40D |
| GSM of fabric | 300 |
| Fabric thickness | 1.8 mm |

Table 1. Construction details of hydrocellular material.
and chosen it to crosslink it on textile substrate (3D hydrocellular material) and study its efficacy.\(^{11}\)

The hydrocellular fabric is impregnated in a padding bath containing 15 grams per liter (gpl) solution in purified water of Quaternary Ammonium Salt (QAS) called dimethyltetradecyl[(3-trimethoxysilyl)propyl]ammonium chloride. The fabric is squeezed under the stenter mangles at around 6 bar pressure and then it is dried and cured in stenter frame at 180°C for around 35 s residence time. After treatment, the fabric is washed at 40°C with wetting agent and dried at 140°C. The fabric after treatment is an active and functional antimicrobial material. The active component of QAS is supposed to be cross linked on the surface of the hydrocellular fabric imparting it the antimicrobial property. The mechanism of kill is documented in literature as physical rupture of the pathogens cell wall by long aliphatic chain of the cross linked chemical. Figure 2. shows the schematic representation of the manufacturing process.

As per literature the antimicrobial compound is bond by the silanol (a hydrolyzed silane) and it is covalently bonded to receptive surfaces (chemisorption). This bonding is then made even more durable by the silanol functionalitity, which homopolymerises (bonds to its neighboring molecule). After the molecule has homopolymerised, it becomes an integral and permanent part of the product even on materials with which it cannot react covalently\(^ {11}\). Figure 3. shows the schematic representation of the manufacturing process.

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As this agent is positively charged (cations) and microbes are negatively charged, in close proximity the microbes are drawn into active surface of antimicrobial agent and killed. Active component responsible for the microbial kill is the edge that blows the microbes, the long molecular chain acts like sword that pierces the cell membrane of all microbes that come in contact with it.\(^ {12}\)

This stabbing and an “electrocution” of the anionic biochemical in the membrane of pathogens resulting from the positive charge means that the antimicrobial will be fully effective as long as the surface of the treated substrate remains intact. Since it is not consumed and does not dissipate, the antimicrobials active portion is not depleted and continues to control microbial growth. The mechanism of action is a physical control, unlike chemical controls as seen in case of leaching biocides, and drugs.\(^ {10}\) Figure 3. shows the schematic diagram of the mechanism of pathogen kill.

The textile material thus formed is a functionalized active material for wound dressing and is evaluated for various physical properties as listed in the Table 2. It can be cut into any dimensions and used on the wound as per the shape and size of the wound.

The prepared wound dressing material was used to evaluate the efficacy in diabetic wounds in rats. The dressing was evaluated along with available best technologies used in hospital setup to treat diabetic wounds in humans. Comparators were sourced from the healthcare market with the advice of endocrinologist and podiatrist. Table 3 lists the comparator technologies of advanced wound care treatments used in the experiment. The items are coded based on the product technology and initials of company that produced the products and compared with the developed new dressing (ND).

**Test system**

The study focuses on excision wounds on clinically induced diabetics in rats. Animals used for the study were male rats of strain Sprague Dawley. Various test conditions as advised by the CPCSEA guidelines\(^ {13–15}\) pertaining to animals were followed during the course of experiments. The rats were injected with Streptozocin (STZ) solution, so that the pancreas of the rats are damaged and the blood sugar levels are elevated to induce diabetes.\(^ {16}\) These studies were done after the clearance of ethics committee by following the due process of ethical studies on animals. Appropriate clearances from Institutional Animals Ethics Committee (IAEC) were taken and the studies were done.
Figure 3. Mechanism of pathogen kill.

Table 2. Physical properties of the dressing material.

| Property                        | Instrument used                  | Method/standard used     | Value          |
|---------------------------------|----------------------------------|--------------------------|----------------|
| Weight                          | GSM cutter- Weigh Scale          | GSM                      | 298            |
| Air permeability                | Air permeability tester          | ASTM D 737               | 110 cm³/cm²/s  |
| Elasticity                      | Extension relaxation method      | British Pharmacopeia (BP)| 43%            |
| Stiffness (Bending length)      | Stiffness tester                 | BS 3356                  | 4.3 cm         |
| Overall moisture management capacity | Moisture management tester     | AATCC 195                | 0.6033         |
| Water vapor permeability        | Water vapor permeability tester  | ASTM E 96- 95 Option B   | 2203 Gm/Met ²/24 h |
| Water vapor resistance          | Sweating hot plate method        | ISO 11092                | 4.7423 M² Pa/W |
| Water holding capacity          | Gravimetric                      | On weight method (OWF)   | 360%           |
| Blood holding capacity          | Gravimetric                      | On weight method (OWF)   | 538%           |
| Bursting strength               | Bursting strength tester         | ASTM D 3746              | 183 PSI        |
| Tearing strength                | Elmendorf tester                 | ASTM D 1424              | 14.1 lbf       |

Table 3. Comparator technologies of advanced wound care treatments used in the study.

| Test item code | Technology                                      | Material form                |
|----------------|-----------------------------------------------|------------------------------|
| CMC-ME         | Carboxymethyl Cellulose(CMC) alginate dressing | Nonwoven Fabric              |
| PHMB-KD        | Polyhexamethylene biguanide,(PHMB) Impregnated Foam | Foam                        |
| HG-PG          | Hydrogel for autolytic debridement            | Gel                          |
| ND             | QAS dressing                                   | Knitted hydrocellular fabric|
Test item preparation

All the test item were prepared by cutting the material in sizes of 3 cm × 3 cm. The dressings were placed on the excision wound, and crepe bandage was applied over it, and the ends of the bandages were secured with adhesive tape. HG-PG gel was applied on the excision wound area post wound creation. Figure 4 shows different dressings applied as per the experimental plan.

Experimental design

Table 4 below lists the details of experimental design followed. The test control ND was kept throughout the study to know the efficacy till the end of study period. The other dressings were replaced periodically as per the indications of the dressings and the guidance of usage given by the manufacturer.

Diabetes induction

Preparation of Streptozocin (STZ) solution. STZ-Na citrate solution was prepared immediately prior to dosing to avoid degradation of STZ. 8.4 g of citric acid was dissolved in 200 ml distilled water to get 0.1 M citric acid solution. 2.94 g trisodium citrate was dissolved in 100 ml distilled water to get 0.1 M trisodium citrate solution. 150 ml of 0.1 M citric acid solution was mixed with 90 ml of 0.1 M trisodium citrate solution to obtain 0.1 M Na citrate buffer of pH 4.5. 1.5 g of STZ was weighed and dissolved in 150 ml of 0.1 M cold citrate buffer to get a concentration of 10 mg/ml and the pH of the solution was adjusted to 4.5, and was maintained on ice prior to use.17

Induction of diabetes. Animals were fasted overnight. Following overnight fasting, diabetes was induced in animals by intraperitoneal injections of STZ at a dose 50 mg/kg. Feed was given immediately after STZ injection. 3 h post STZ injection; animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. On the morning of the following day the 5% glucose solution was replaced with normal drinking water.17,18

Animals were checked for fasting blood glucose post 72 h, of STZ administration, to check diabetes induction

Table 4. Experimental design.

| Group | Group ID | Treatment | Treatment frequency | No. of animals/time points (Day 1, 7, 14, and 28) | Total no of animals |
|-------|----------|-----------|---------------------|-----------------------------------------------|--------------------|
| 1     | Control  | No treatment | -                   | 5                                             | 20                 |
| 2     | Reference standard I | CMC-ME | Freshly applied once weekly | 5                                             | 20                 |
| 3     | Reference standard-II | PHMB-KD | Freshly applied once weekly | 5                                             | 20                 |
| 4     | Reference standard-III | HG-PG | Freshly applied once in three days | 5                                             | 20                 |
| 5     | Test control | ND | Applied once and maintained through the study | 5                                             | 20                 |

Figure 4. Different dressings applied as per the experimental plan.
(Blood glucose ≥ 250 mg/dl). To check for fasting glucose, animals were fasted for 6 h and glucose was checked using One Touch Ultra® by tail prick method. Five days post diabetes induction, animals that showed fasting blood glucose more than 250 mg/dl were considered as diabetic and selected for the study.\textsuperscript{18}

**Excision wound creation**

The dorsal skin of the diabetic animals was shaved using a pet trimmer.\textsuperscript{17–19} This area ensures that the dressing is beyond the reach of the rodent and it will not bite and try to remove it. Animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) + xylazine (10 mg/kg) cocktail. Depth of anesthesia was checked by tail pinch. A wound of about 2 cm × 2 cm area was made on the depilated dorsal thoracic region of the animal. Under aseptic conditions, a predetermined area of 2 cm × 2 cm skin in its full thickness was excised using autoclaved surgical instruments under anesthesia. Post excision, 5 ml/kg ketoprofen was administered subcutaneously to reduce the pain and stress. Following wound creation, animals were housed individually in polycarbonate cages with species specific enrichment. On the day of, and following wound creation, the wound area was measured by tracing the wound boundaries on a transparent paper. Animals were treated for 28 days post wound creation with respective reference and test item as specified in experimental design. Group 2 and Group 3 animals were treated weekly once by applying dressings cut in size 3 cm × 3 cm; Group 4 animals treatment interval was 3 days. Group 5 animals were treated, applied once in size 3 cm × 3 cm and maintained throughout the treatment period of 28 days as specified in experimental design (Table 4).

**Observations**

Following characterization and observations were recorded and studied.

**FESM micrographs**

FESM (Field emission scanning microscopy) was conducted at various magnification levels of the hydrocellular structure to obtain micrographs of the structure. The images were obtained for the hydrocellular structure before and after cross linking of QAS on the surface of the structure.

**Liquid management properties**

The dressing material was evaluated for liquid management properties to quantify various liquid management properties as per AATCC 195 method. This method accesses the various aspects of moisture movement in and on the material and summarizes it with a common index for ease of comparison.

**Antimicrobial assessment of wound dressing**

The dressing was evaluated for quantitative values of antimicrobial activity by ASTM E2315 for 3 gram positive bacteria, 3 gram negative bacteria and yeast.\textsuperscript{20} Various contact periods were used to understand the broad spectrum effect spread over a long period of time.

**Repetitive tensile load and elongation testing**

**ASTM D4964**

In this study the material is subjected to three repetitive loading cycles with the constant rate of extension principle. The speed of loading cycle is 300 mm/min. The instrument used is TBI/KMM the maximum load applied is 100 N which is the maximum load a wound dressing is expected to face during usage. The dressing was tested for both ways that is, horizontally (course way) and vertically (wale way).

**Leachability of the QAS**

Exhaustive leaching conditions were employed as per ISO 10993-12 to extract the QAS from the wound dressing and the leachet was analyzed based on UV-Vis spectrophotometer data. Leaching conditions followed were pertaining to the use conditions of the dressing on wound.

**Weight of animals**

Body weights were recorded on the first day of acclimatization, before, and after randomization and weekly during treatment period.

**Mortality of animals**

Animals were observed for mortality twice a day from the first day of acclimatization till termination of experiment.

**Clinical signs**

Animals were observed for clinical signs once a day during the entire experimental period. The signs include dehydration, abnormal licking, scratching, aggressiveness, back arching, and decrease in urine and fecal output.

**Percent wound contraction**

The size of wound from each animal was measured by tracing on a transparent paper on days 0, 1, 7, 14, 21, and 28.\textsuperscript{21} The tracing was then transferred to \textsuperscript{1 mm²} graphsheet, from which the wound surface area was estimated. The surface area was then employed to calculate the percentage wound contraction by using the following equation.
% Wound Contraction = \frac{\text{Initial (day 0) Wound Size} - \text{Specific Day Wound Size}}{\text{Initial Wound Size}} \times 100

**Epithelization period**

The period of epithelization was calculated as the number of days required after wound creation for the eschar to fall off leaving no raw wound behind.

**Histopathology**

After measurement of wound size on Day 1, 7, 14, and 28, animals were euthanized using CO₂, and the skin samples were collected from the wound and preserved in neutral buffered formalin. The samples were processed by the standard paraffin embedding technique. The prepared blocks were sectioned into 3–5 µ thickness using a rotatory microtome and mounted on clean glass slides. The prepared slides were stained by hematoxylin-eosin stain and observed under a light microscope at 4× magnification.

GraphPad Prism 5.0 was used for statistical analysis. Column statistics were estimated and a two tailed t-test was used to check the significance of percent wound contraction of the treatments relative to control. A p level < 0.05 was considered to be significant.

**Biocompatibility**

In vitro cytotoxicity was tested by direct contact method by using BALB/c 3T3 Cells. Skin sensitization and intracutaneous reactivity was studied by using the extract of the wound dressing. Acute systemic toxicity was also studied in swiss albino mice.

**Result and discussions**

**FESM micrographs**

The micrographs at the magnification levels of 20 and 2 µm are shown in Figure 5. Figure shows the hydrocellular structure before and after QAS cross linking on the surface of the structure. The micrographs in Figure 5, A and B corresponds to before QAS cross linking and C and D represents the cross linked structure. It can be seen that C and D shows QAS as cross bonded on the surface of the hydrocellular structure and represents a layer that is immobilized on the surface. It is this layer that contains cationic sites which are responsible for the antimicrobial property of the material. In the
micrographs A and C of the figure, it can be seen that the mono filament constituent yarn is embedded within the multifilament yarn. It is locked on the face and goes inside the structure to form the height of the structure. The structure is seen as porous enough for the gases to exchange from one side to the other side of the dressing. The overall structure also is seen to have a high amount of surface area and capillary spaces which makes it a hydrocellular structure that would facilitate in the excellent exudates management properties of the material. The excellent moisture management property of the dressing can be attributed to the wicking action of the moisture due to this structure.

**Liquid management properties of the dressing as per AATCC 195 method**

AATCC 195 method uses moisture management tester. This test method is for the measurement, evaluation and classification of liquid moisture management properties of textile fabrics. The test method produces objective measurements of liquid moisture management properties of knitted, woven, and nonwoven textile fabrics.

The results obtained with this test method are based on water resistance, water repellency and water absorption characteristics of the fabric structure, including the fabric's geometric and internal structure and the wicking characteristics of its fibers and yarns. The test method gives a combined index of testing called as overall moisture management capability (OMMC) of fabric.

It was found that OMMC of the developed fabric was 0.6033 which is graded as excellent (Grade 4 as per the grading standards described in the test Protocol. Higher grade means far superior the property of moisture management). Table 5 shows the comparative value of the OMMC index of other dressings. OMMC testing is not applicable for gel material as that has no defined physical structure.

Thus it can be seen that the material effectively acts as a hydro cellular structure like foam, and it is breathable so the evaporation of exudates keeps happening continuously. This helps in maintaining sufficient moistness on the wound but not keeping it flooded. This material is suitable to take the exudates away from the wound and maintain moist condition on wound.

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### Quantitative evaluation of antimicrobial activity

ASTM E 2315 method of quantitative assessment of antimicrobial activity for 3 gram positive strains, 3 gram negative strains and yeast were done. The positive control used was sterile nonwoven polyester viscose wipes saturated with Neolone P.E. Phenoxyethanol and negative control was untreated sterile nonwoven polyester viscose wipes.

In this method a control tube using nutrients was prepared without inoculation of micro-organisms. The bacterial culture is added in 10ml sterile nutrient broth and vortex and let to stand still for 15–20 min. This was then diluted with suitable medium to 1 × 10^6 organisms (CFU/ml).

The dressing material (ND) is cut to size such that it is capable of absorbing 1.0 ± 0.1 ml standard innoculum. The positive and negative controls are also made ready. The test item (ND), positive control and negative control were placed inside sterile screw cap bottles. The test innoculum (1 ml) was added on the surfaces of the items using Micro pipette. The contact time of 0 hour, 1, 4, 24, and 48 h was used in the study. After the study time, the specimen was transferred into 250 ml culture bottle and 100 ± 1 ml of 0.05% neutralizing solution was added and shaken vigorously for 1 min. Serial dilutions (10^0, 10^1, 10^2, and 10^3) was done with sterile water. The dilution is plated on suitable nutrient agar plate for bacteria and fungus. The Incubation of 36 h at 37°C for bacteria and 5–7 days at 25°C for fungus was done. After incubation the number of colonies were counted and noted for different contact times. Evaluation was done by reporting the number of microbes recovered on 0th contact time and required contact time.

The percentage reduction of microbes was counted as per below formula.

\[
R = 100 \left( \frac{B - A}{B} \right)
\]

Where,

R Percentage reduction
A The number of microbes recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period.
B The number of microbes recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at “0” contact time).

Time based kill results for different strains shows that the kill rate is at least 4 log reduction within 1 h except...
Table 6. Quantitative assessment of antimicrobial activity of wound dressing material.

| S.NO | Test organisms                      | % of Reduction |
|------|------------------------------------|----------------|
|      |                                    | 1 h | 4 h | 24 h | 48 h |
| 1.   | Staphylococcus aureus ATCC 6538     | 99.99 | 99.99 | 99.9999 | 99.9999 |
| 2.   | Listeria monocytogenes ATCC 19115   | 99.99 | 99.99 | 99.9999 | 99.9999 |
| 3.   | Enterococcus faecalis ATCC 29212    | 99.99 | 99.99 | 99.9999 | 99.9999 |
| 4.   | Escherichia coli ATCC 25922         | 99.99 | 99.99 | 99.9999 | 99.9999 |
| 5.   | Pseudomonas aeruginosa ATCC 15442   | 99.99 | 99.99 | 99.9999 | 99.9999 |
| 6.   | Klebsiella pneumoniae ATCC 4352      | 99.99 | 99.99 | 99.9999 | 99.9999 |
| 7.   | Candida albican ATCC 10231          | 90   | 90   | 99.9999 | 99.9999 |

Figure 6. Load versus elongation coarse way.

Candida albican yeast which as per literature needs long duration contact for effective kill.

Table 6 shows that hydrocellular wound dressing material has excellent broad spectrum antimicrobial activity. As seen in the literature survey it can be evidenced that the antimicrobial action of the QAS is attributed to its surface action after it is immobilized on the surface of textile. Irrespective of the class of pathogen, the mechanism of kill is effective in physical lyses of the pathogen. The study shows relatively less percentage of kill in short interval of time for yeast called Candida albicans, as it needs more incubation times for the yeast, which is a known medical fact.

In general the infected chronic wound will be composed of many type of pathogens. When this dressing is used on such wounds, it would mean, irrespective of the nature of microbial load in chronic wound, the efficacy of dressing would be intact.

**Repetitive tensile load and elongation testing**

**ASTM D4964**

ASTM D4964-96(2020) was the test method employed to check the stress properties of the dressing. It is very essential that the dressing material is tested for very similar conditions of stretching repetitively as seen on wounds. In real wound conditions the skin on which the dressing is placed is likely to be stretched, flexed, twisted, and released as per the movement of the patient. This loading happens multiple times and repetitively. The dressing is expected to behave uniformly in it’s lengthways and width way behaviors. The dressing is not expected to restrict the minimal movements of patient and also must not be displaced with slight movements, but must conform to the area of wounds properly. The material must perform equally in both the directions that is, horizontally (Course way) and vertically (wale way).

From the Figures 6 and 7 it can be seen that the dressing was almost identical in its behavior in both the directions of testing. It exhibited same behavior which can be attributed to its 3D structure which is not biased and fairly isotropic in its construction. Hence there is less likelihood of the dressing material to be deformed in any specific directions during its usage.

**Leachability of QAS**

The QAS is cross linked on the surface of the structure and in the manufacturing process it is prewashed to remove any unbounded chemical from the surface after bonding.
This ensures that the chemical is immobilized inside the fabric and on the surface of fabric in the form of an active matrix that cannot leach.\textsuperscript{11}

ISO 10993-12 is a standard protocol for leaching that was used on the dressing. Here a 6 cm\textsuperscript{2} of the dressing was cut and added to the flask which has extraction solvent. The mixture of dressing and solvent was agitated for 72 h at temperature of 37°C and 50°C with constant agitation at 250 rpm.

The solvents used for extraction were, polar and non-polar in nature. Polar Solvent – 0.9% Nacl solution & Non-Polar Solvent – Cotton seed oil was used. Since the wound content may have components that vary in its polarity, as per clinical guidelines it is essential to include polar solvent and non-polar solvent in the extraction conditions.

The conditions were exhaustive for the intended purpose of a wound dressing applied on body where a normal body temperature does not exceed 40°C.

The leachet was collected at end of 72 h and analyzed with UV–Vis Spectrophotometer. The QAS used in the study was seen to have peak absorption at 205.9 nm and that was used as the characteristic property graph of QAS in the analysis.

The base graph of actual QAS was used for analysis (Figure 8). The results obtained are tabulated in Table 7. No any presence of QAS was found in leachet at different temperatures under polar and non-polar solvents. It is concluded that the wound dressing is not leaching any QAS that acts on the wound. It is the cationic sites created in and on the surface of the dressing, that helps in keeping the microbial growth under control.

**Mortality and clinical signs**

No mortality occurred during the course of experiment. No clinical signs were observed in any of the animals during the course of experiment except for minor discomfort during the first week of the experiment, which could be

![Figure 7](image1.png)

**Figure 7.** Load versus elongation wales way.

![Figure 8](image2.png)

**Figure 8.** UV–Vis spectrophotograph of the QAS used in the study.
ascribed to wound creation. Thus the dressings were seen to be safe and causing no any major discomfort and hamper the normal growth of the animals or possess any hindrance in the normal wound healing cycle of the body.

Body weight

Body weight was checked at periodic intervals. This was done to understand the wellness of the animals during the experimentation period. There was no significant change in body weights for all the animals throughout the experiment period (Table 8).

Body weight (g) (Mean ± SEM)

| Group No. | Group ID | Treatment           | Day 0       | Day 7       | Day 14      | Day 21      | Day 28       |
|-----------|----------|---------------------|-------------|-------------|-------------|-------------|--------------|
| 1         | Control  | No treatment        | 175.25 ± 3.93 | 168.67 ± 4.15 | 167.00 ± 5.59 | 176.00 ± 6.96 | 180.00 ± 8.22 |
| 2         | Reference control-I | CMC-ME            | 174.20 ± 4.15 | 168.00 ± 4.85 | 171.00 ± 5.62 | 187.00 ± 9.3  | 192.00 ± 10.56|
| 3         | Reference control-II | PHMB-KD           | 171.50 ± 2.43 | 161.67 ± 3.47 | 158.50 ± 3.5  | 159.00 ± 8.28 | 162.00 ± 8.15 |
| 4         | Reference control-III | HG-PG            | 183.70 ± 3.48 | 174.33 ± 3.87 | 175.00 ± 4.08 | 183.00 ± 5.15 | 192.00 ± 5.61 |
| 5         | Test control | ND                | 180.15 ± 3.28 | 174.80 ± 4.21 | 171.50 ± 3.17 | 176.00 ± 1.87 | 186.00 ± 2.92 |

Blood glucose levels

In general healthy animals would have blood glucose levels of 70–110 mg/dl. The animals induced with the diabetes showed more than three times the normal glucose compared of a healthy animals. Diabetes was maintained through the study in all the treatment groups (Table 9). There was no significant change in fasting blood glucose for all the animals throughout the period of experimentation. The observations suggest that the animals had desired effect of the induced drugs and they were diabetic throughout the study, which has hampered the systemic normal wound healing mechanism of otherwise healthy animals.

Wound contraction

The results of efficacy of the different treatments on excision wound healing are shown in Table 10 and corresponding Figure 9 shown in the form of graphs for comparison.23

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**Table 7.** UV-Visible spectrophotometric analysis of Wound dressing leachet.

| S. No | Sample particulars | Incubation | Presence of QAS in leachate (PPM) |
|-------|--------------------|------------|----------------------------------|
|       |                    | Time (h)   | Temperature (°C)                |
|       |                    | Agitation (rpm) |                       |
| 1.    | 0.9% NaCl          | 72         | 37                               | —                       |
| 2.    | Cotton seed oil (CSO) |          |                                  | —                       |
| 3.    | 6 cm² dressing + 0.9% NaCl | 0         | PPM                              |
| 4.    | 6 cm² dressing + CSO |            |                                  | 0 PPM                   |
| 5.    | 0.9% NaCl          | 72         | 50                               | —                       |
| 6.    | Cotton seed oil (CSO) |          |                                  | —                       |
| 7.    | 6 cm² dressing + 0.9% NaCl | 0         | PPM                              |
| 8.    | 6 cm² dressing + CSO |            |                                  | 0 PPM                   |

**Table 8.** Body weights of animals during the course of the study.

| Group No. | Group ID | Treatment       | Day 0       | Day 7       | Day 14      | Day 21      | Day 28       |
|-----------|----------|-----------------|-------------|-------------|-------------|-------------|--------------|
| 1         | Control  | No treatment    | 175.25 ± 3.93 | 168.67 ± 4.15 | 167.00 ± 5.59 | 176.00 ± 6.96 | 180.00 ± 8.22 |
| 2         | Reference control-I | CMC-ME        | 174.20 ± 4.15 | 168.00 ± 4.85 | 171.00 ± 5.62 | 187.00 ± 9.3  | 192.00 ± 10.56|
| 3         | Reference control-II | PHMB-KD       | 171.50 ± 2.43 | 161.67 ± 3.47 | 158.50 ± 3.5  | 159.00 ± 8.28 | 162.00 ± 8.15 |
| 4         | Reference control-III | HG-PG        | 183.70 ± 3.48 | 174.33 ± 3.87 | 175.00 ± 4.08 | 183.00 ± 5.15 | 192.00 ± 5.61 |
| 5         | Test control | ND             | 180.15 ± 3.28 | 174.80 ± 4.21 | 171.50 ± 3.17 | 176.00 ± 1.87 | 186.00 ± 2.92 |

**Table 9.** Blood glucose readings in rats through the period of the study.

| Group No. | Group ID | Treatment       | Day 0       | Day 7       | Day 14      | Day 21      | Day 28       |
|-----------|----------|-----------------|-------------|-------------|-------------|-------------|--------------|
| 1         | Control  | No treatment    | 329.55 ± 12.82 | 332.47 ± 12.88 | 354.20 ± 13.35 | 344.40 ± 14.93 | 354.80 ± 17.13 |
| 2         | Reference control-I | CMC-ME      | 328.20 ± 11.53 | 354.53 ± 14.49 | 350.50 ± 16.03 | 354.80 ± 12.35 | 338.80 ± 19.62 |
| 3         | Reference control-II | PHMB-KD    | 329.80 ± 11.06 | 359.53 ± 16.45 | 363.60 ± 15.49 | 359.20 ± 19.83 | 357.60 ± 14.05 |
| 4         | Reference control-III | HG-PG       | 329.60 ± 11.24 | 352.27 ± 15.08 | 353.90 ± 16.32 | 368.20 ± 25.71 | 360.00 ± 26.36 |
| 5         | Test control | ND            | 328.50 ± 11.22 | 355.47 ± 13.72 | 346.30 ± 13.14 | 336.20 ± 21.26 | 339.40 ± 16.87 |
Test control (ND) with ~12% wound contraction, Reference standard I with ~25% wound contraction and Reference standard III with ~29% wound contraction were significantly effective as compared to control group on day 7 post wound creation.

On Day 14 All treated groups were significantly effective for wound contraction (ND ~49%, Reference standard I~54%, Reference standard II~24%, and Reference standard III ~57% wound contraction ) as compared to control group.

On Day 21 All treated groups were significantly effective for wound contraction (ND ~83%, Reference standard I~77%, Reference standard II~39%, and Reference standard III ~96% wound contraction) as compared to control group.

On Day 28 All treated groups were significantly effective for wound contraction (ND ~98%, Reference standard

Table 10. Effect of different treatments on wound contraction.

| Group No. | Group ID | Treatment   | Day 7 (Mean ± SEM) | Day 14 (Mean ± SEM) | Day 21 (Mean ± SEM) | Day 28 (Mean ± SEM) |
|-----------|---------|-------------|--------------------|---------------------|---------------------|---------------------|
| 1         | Control | No treatment | 4.59 ± 0.51       | 17.24 ± 3.10       | 29.71 ± 2.29       | 54.99 ± 3.28       |
| 2         | Reference control-I | CMC-ME | 25.49 ± 1.79*     | 54.18 ± 1.18*      | 77.25 ± 3.70*      | 99.10 ± 2.01*      |
| 3         | Reference control-II | PHMB-KD | 4.46 ± 0.53       | 24.07 ± 1.09*      | 39.64 ± 2.34*      | 80.76 ± 4.62*      |
| 4         | Reference control-III | HG-PG | 29.06 ± 0.73*     | 57.42 ± 1.04*      | 96.07 ± 2.81*      | 100.00 ± 0*        |
| 5         | Test control | ND | 12.16 ± 0.72*     | 49.71 ± 2.31*      | 83.37 ± 3.14*      | 98.78 ± 1.19*      |

*Significant at p < 0.01 compared to control.

Figure 9. Wound contraction on day 7, 14, 21, and 28 of experiment.
I–99%, Reference standard II–80%, and Reference standard III ~100% wound contraction) as compared to control group.

The study shows that the wound dressing material picks up in the property of effectiveness of wound healing, gradually, and catches up with the best results of CMC alginate dressing and autolytic enzyme based hydrogel within 21–28 days. The dressing developed is significantly better when compared with PHMB based foam wound dressing. Figures 10 to 13 shows the actual wound pictures to depict the healing in terms of wound contraction.

The combined effect of excellent management of exudates and at the same time keeping very low infection load on the wound bed has resulted in favorable conditions for wound healing. The moist wound healing concept has worked in tandem with effective infection control. The body is not burdened heavily with fighting the infection load and hence the wound healing happens faster. The study also shows that the wound dressings employing alginate and autolytic debridement hydrogel have faster response to the wound healing with respect to wound contraction due to the active component of the dressing going into the wound and facilitating the wound healing. The active components of these comparators do act on the microbial load but does not have effective hydrocellular activity as seen with 3D hydrocellular dressing material. This causes the comparator dressings to be loaded with the exudates and replacements periodically every 3–4 days is needed. The hydrocellular dressing was not changed throughout the study time of 28 days. This is extreme condition stress test. The periodic changes of all the dressings except the 3D hydrocellular dressing proves the property of longer usage of the dressing. The longer usage property of dressing will lead to reduced frequency of dressing changes in real life. Reduced frequency would mean reduced hospital visits and reduction in related costs. The overall cost of wound management will be reduced when the developed hydrocellular dressing is compared with other advanced wound dressings. The developed dressing ensured optimal amount of infection control mechanism in place and helped to reduce the healing load on the body as compared to the untreated control.

Hydrocellular dressing material had a combination of excellent moist exudates management conditions along with the infection control activity that ensured the pathogenic load on the wound to be consistently low. Though the active components did not leave the dressing and acted on the wound, it did ensured that the dressing material and the exudates that is captured in the dressing is low on the pathogenic load. Hence the wound healing was seen better than the control wound. The relatively slow response on the wound contraction in case of 3D hydrocellular material could be attributed to the cause that there is no direct leaching material acting on the wound. Here a equilibrium condition of moisture and infection management takes a little more time. PHMB based dressing did not show as effective efficacy as the developed 3D hydrocellular dressing material. This may be because even though PHMB has active component leaching out for infection management, its lesser performance in case of exudates management would have played the negative role in comparison with 3D hydrocellular dressing material.

Figure 10. Wound contraction pictures on day 7 of experiment.
It must be appreciated that the single use of 3D hydrocellular dressing material could accomplish the task at par with the best of dressings available for diabetic wound, used with periodic changes. The developed dressing is effective for a long period of time. Hence frequent changes that are otherwise required for comparator dressings are not needed and this leads to lower wound management cost. It is well known that the cost of wound management is inclusive of cost of
dressing, the money and efforts spent by the patient, the time and efforts that the health care provider has to dedicate for every visit of the patient for redressing of wound. However, choice of frequency of replacement of dressings is to the discretion of medical practitioner and guided by the exudates loading in the dressing. A dressing that is not laden by exudates will be preferred by the practitioner for longer usage.

Histopathology

Various parameters of wound healing were evaluated and graded. The mean scores of the histological parameters were calculated and compared with the group 1 Control. Figure 14 shows Histopathological pictures of wounds.

Day 1- The acute inflammation (predominantly neutrophils infiltration), congestion, with no epidermal closure were observed across all the groups.

Day 7- The acute chronic inflammation (predominantly neutrophils, monocytes, plasma cells infiltration), congestion, with other wound healing parameters were noticed. The order of improvement (descending) in the wound healing is from Group 4, Group 5, Group 2, and Group 3. The degree of improvement at Group 4 is mild and Group 5 is minimal when compared to Group 1.

Day 14- The chronic inflammation (predominantly, monocytes, plasma cells, lymphocyte infiltration), with other wound healing parameters were noticed. The order of improvement (descending) in the wound healing is from Group 4, Group 3, Group 2, and Group 5. The degree of improvement at groups Group 4 & Group 3 is mild and at Group 2 & Group 5 is minimal when compared to Group 1.

Day 28- The chronic inflammation (predominantly, monocytes, plasma cells, lymphocyte infiltration), with other wound healing parameters were noticed. The order of improvement (descending) in the wound healing is from Group 4, Group 3, Group 2, and Group 5. The degree of improvement at Group 4 & Group 5 is moderate; at Group 2 is mild & at Group 3 is minimal when compared to Group 1.

Thus the Histopathological data underlines the findings of good healing in terms of re-epithelization and neovascularization as visibly seen in the wound contraction data.

Biocompatibility

It is very essential that the dressing be evaluated for biocompatibility as it is used on open skin. Any adverse reaction for prolonged usages needs to be mitigated by properly studying the cytotoxicity data of the dressing. The cytotoxicity studies on the dressing were carried out for viable cell count, skin sensitization, Intracutaneous reactivity and acute systemic toxicity.

Invitro cytotoxicity by direct contact method. BALB/C3T3 cell line was used and quantitative analysis was done to arrive at viable cell count. Cell viability assay was analyzed to
understand the viable cells. The developed 3D hydrocellular dressing material showed viability of 90.37% as compared with the negative control as referred in Table 11.

As seen from the data it can be concluded that since the cell viability is more than 80%, the developed 3D hydrocellular dressing material does not show cytotoxicity and hence is safe to be used on open wounds.

Skin Sensitization, Intracutaneous reactivity and acute systemic toxicity was evaluated by ISO 10993 test method. The developed 3D hydrocellular dressing material was subjected to extraction in polar and nonpolar solvent at 50°C for overnight by continuous agitation. The leachet was used to inject in rabbits and mice and study the clinical pathology, gross pathology and histopathology.

Study showed no adverse effect on any animal and hence it could be concluded that the developed 3D hydrocellular wound care dressing material is safe to be used on open wounds.

Conclusion

The designed hydrocellular wound dressing material was significantly superior in its efficacy as compared to the untreated control in diabetic wound healing studies. It was evidenced by wound contraction and histopathological findings. Its efficacy was comparable to the established wound dressings like Carboxymethyl cellulose alginate dressings and autolytic enzyme based hydrogel. It was
found to be superior to Polyhexamethylene biguanide dressing used as reference controls in this study.

The reason for good wound healing performance can be attributed to a combined property of effective exudates management and functionality imparted as broad spectrum antimicrobial effect. The concept of hydro cellular material has shown good results due to the excellent balance of exudates pickup and drying it out. The overall moisture management capacity of this material is better than the comparator dressings. Because of its porous nature it allows good air and gaseous exchange in the structure. It also ensures moist wound healing conditions are maintained on the wound.

The cationic sites created on the surface of the dressing ensure a good antimicrobial action on the exudates in the dressing. It reduces the infection load on the wound. As these culminates in the faster healing of the wound. The nonleaching property of the dressing also helps in preventing the generation of more resistant and mutant strains of the microbes. In short, the developed material fulfills a fine balance of exudates management, gaseous exchange, broad spectrum antimicrobial activity, moist wound healing conditions and no leaching characteristics. It is the culmination of these properties that makes it a good material for wound dressing.

The developed 3D hydrocellular dressing can be used as a relatively durable long lasting dressing for wound management in diabetic wounds. The need of repetitive wound dressing changes can be brought down with this concept of dressing. It is not only cost effective in terms of its material cost but also is a cost effective solution when entire wound management cost is considered. The developed 3D hydrocellular dressing material can be used as a long lasting dressing for wound management in diabetic wounds. Such wound dressings material can change the quality of life of diabetic wound patients especially in developing world, where cost of healthcare and access to advanced wound care dressings in beyond the reach of larger public.29

**Contributorship**

All authors have contributed equally and jointly reviewed and edited the manuscript and approved the final version of the manuscript

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