Characterization of Single Layer Wound Healing Dressing by Using Different Techniques

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A bio-adhesive single layer wound dressing was prepared by solution casting and its characteristics were measured with the help of different techniques. Using a Universal Testing Machine, the tensile strength (TS) and the percentage elongation at break (Eb) for the single layer bandage were respectively found to be 2.49 MPa and 99.15%. We investigated the absorption behavior of this dressing into the body fluid such as water and blood serum by taking neutron radiographs at different immersion times. Furthermore, the presence of Ca, Sr, and Fe was found in the bandage using XRF technique. A scanning electron micrograph and neutron radiographs of the wound dressing disclosed its uniformity and uniform porosity, which are essential criteria for an ideal wound dressing. The solubility rate of the dressing into blood serum is lower than into water at different immersion times.

1. Introduction

The average human body is made up of 60% water, 17% fat and only 5% carbohydrate. Protein comprises a similar proportion of the body as fat at 17% and forms an essential component of all body tissue structures such as cell membranes and genetic material, and plays a major role in metabolic systems in the form of blood hormones, antibodies and enzymes [1]. All proteins in the body have a function and there is no storage pool of proteins but is for fat in the form of adipose tissue or carbohydrate in the form of glycogen. There is a large body of evidence demonstrating the essential role of nutrition in wound healing. Without adequate nutrition healing may be impaired and prolonged. Improved nutritional status enables the body to heal wounds [2] such as the accelerated wound healing seen with nutritional supplementation [3]. Nutrition is a critical factor in the wound healing process, with adequate protein intake essential to the successful healing of a wound [4].

Body protein can be divided into three functional pools, which are listed here in order of their size, with muscle mass being the largest, as: a) Muscle protein, b) Visceral (abdominal organs protein and c) Plasma proteins and Plasma amino acids. Due to various accidental cases of injured or burnt human body, there is a need for a proper dressing with effective dressing materials. An improved dressing can enhance both the rapidity of healing and its quality, including a reduction of infection, pain, and scar. An improved dressing can also reduce costs by improving the rate of wound healing and thereby reducing the duration of treatment, and thus allowing for a less frequent and simpler attention by medical professionals. Wound healing is a dynamic process, and the fruitful requirements of the dressing can improve burn/wound healing process. However, it is widely accepted that a warm, moist environment encourage rapid healing, and most modern wound care products are designed to provide these conditions [5,6]. Wound care is labor intensive and often requires frequent attention by skilled professionals. Severe wounds (injury or burning) take millions of lives each year all around the world. Severe wounds damage the epithelium or even the endothelium of skin, which is the primary defense barrier of the body [7]. People die due to severe infection and most likely due to dehydration [8,9]. Conventional wound dressing materials do not provide notable infection resistance. They also lack any water-retaining property to minimize the dehydration. But an ideal wound dressing material should control the humidity, provide bacterial resistance, and enhance the activities of the growth factors. It should have permeability for oxygen and carbon dioxide, and be able to absorb the wound

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flow out of a body through the pores, and enhance the burn/wound healing. The incorporation of collagen, poly-amino acids, hyaluronates, and dextran into synthetic wound dressings has been shown to enhance the healing process [10-12]. Gelatin is a well-characterized protein fragment obtained by partial degradation of water-insoluble collagen fiber, and it has been widely used in the biomedical field because of its merits including its biological origin, biodegradability, hydrogel properties, and commercial availability at a relatively low cost. It is also a biocompatible, and very low antigenic material. Gelatin has also been used in medicine as a plasma extender wound dressing, an adhesive, and in absorbent pads for surgical use [13]. Recently, gelatin has been demonstrated to exhibit activation of microphage [14,15] and high-hemostatic effects [16]. Consequently, it has been used in a wide variety of wound dressings and as a biomaterial in tissue engineering [17]. One of the drawbacks of gelatin for tissue engineering applications is its solubility in aqueous media; therefore, gelatin-containing structures for long-term biomedical applications need to be cross linked [18]. The neutron radiograph gives information about the internal structure as an intensity/gray value pattern representative of the internal structure of the object [19], any in-homogeneity in the object or internal defects such as voids, cracks, porosity, inclusion, corrosion, and morphological change in the plant pod seeds [20] that will show up as change in gray value/radiation intensity reaching the detector. Also migration/rising of water in various building products and building materials, physical description of water transportation in a porous matrix of the sample material, density fluctuations and porosity can be detected [21-37].

The mechanical properties, namely the tensile strength (TS) and the percentage elongation at break (Eb) of the membranes were determined by using a Universal Testing Machine (INSTRON, model 1011, UK) with cross-head speed of 10 mm/min and gauge length of 20 mm. The load capacity was 500 N and the efficiency was within ±1%. The pH values of blood serum and demineralized water were measured using established pH meter, Thermo Scientific Orion Star^{TM} and Star plus series meter (USA).

Neutron radiography is a powerful non-destructive testing (NDT) technique for internal evaluation of materials, such as voids/cavity, cracks, homogeneity, water absorption behavior, and so on. It involves attenuation of a neutron beam by an object to be radiographed and thus to make the registration of the attenuation process (as an image) on a film or video. Thermal neutron radiography facility installed at the tangential beam port of 3MW TRIGA MARK-II reactor is used in this study**.**

In the present investigation, various well-known and established methods such as Neutron Radiography (NR) technique, SEM technique, X-RF technique, and PH and TS measurement technique are being used to measure various characteristics of the single layer wound dressing (collagen) bandage.

2. Experimental Details

2.1.1. Preparation of wound dressing bandage

Gelatin granules (10g) were dissolved in demonized water (100 ml) with continuous stirring at 60ºC to make a viscous solution (the final volume was 50 ml). The solution was autoclaved for 15 min at 121ºC for sterilization. Then the solution was casted at room temperature. The films (membrane) were formed after 48 hours of casting. The membranes of gelatin were collected, and then subjected to further drying in vacuum desiccators for 2 days. Then the membranes were stored in desiccators prior to testing. The membranes of gelatin/PEG were also prepared by solution casting. The thickness of the single layer bandage was 0.05 mm.

2.1.2. Immersion procedure of the wound dressing into blood serum/water

The dressing material was placed perpendicularly in a plastic container and a constant 2.54 cm height of blood serum and water level was maintained. The blood serum/water level was observed very carefully and extra blood serum/water was added to maintain water level at 2.54 cm during the immersion times. After the time of interest (TOI) (in between 1sec − 4 min) samples were taken out from the different containers.

2.2. Analysis Techniques

2.2.1. Analytical technique

An energy dispersive X-ray fluorescence (EDXRF) [38] spectrometer was used to analyze the elemental compositions of the wound dressing. The EDXRF analysis employs detectors that directly measure the energy as well as the intensity of the x-rays by collecting ionization produced in a suitable detecting medium. The EDXRF analysis based on Cd-109 radioisotope (emitting Ag-K x-rays) excitation are being used for the present study. The EDXRF system consists of a primary x-ray source (Cd-109), sample holder, an x-ray detector, a multichannel analyzer (MCA) and associated NIM (Nuclear Instrument Module) electronics for data acquisition and processing. The EDXRF spectra were processed and quantified using the Qualitative X-ray Analysis System (QXAS), and the Analysis of X-ray spectra by Iterative Least-square fitting (AXIL) [38]. All samples were analyzed using a life time of 3000 sec. The detection limits of used spectrometer for various elements were different such as 10.2 for Ca, 1.07 for Fe and 1.2 for Sr in units of ppm.

2.2.2. Scanning electron microscope (SEM) technique for image analysis

The SEM photographs were taken with a Philips scanning electron microscope (XL 30, Philips, UK) at a magnification of 40,000× at room temperature. The working distance was maintained between 15.4 and 16.4 mm, and the acceleration voltage was 5 kV, with the electron beam directed to the surface at a 90º angle and a secondary electron imaging (SEI) detector. In that case a bandage sheet (5 mm × 5 mm) was deposited on an aluminum holder and sputtered with gold–platinum (coating thickness, 150–180 Å) in a Hummer IV sputter coater.

2.2.3. Neutron radiography (NR) technique

To obtain neutron radiographic images at different conditions (dry/immersed in blood serum and in water), the experimental neutron radiography facility installed at the tangential beam port of 3 MW TRIGA Mark II Reactor (located at the Institute of Nuclear Science and Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh) was used. The bismuth filter, cylindrical divergent collimator, lead shutter, beam stopper, sample and camera holder table, beam catcher, biological shielding house was also used as a part of the experimental facility. At first, a thin converter (gadolinium metal foil of 25 µm thickness) was placed at the back of the X-ray industrial film. Then the sample was placed in close contact with the NR cassette and directly on the sample holder table. The NR cassette was placed on the cassette holder table. Both of NR cassette and sample were placed in front of the neutron beam having 30 cm in beam diameter. After that the neutron beam was disclosed by removing the wooden plug, lead plug and beam stopper from the front side of the collimator. Each sample was then irradiated for the optimum time (3:30 min) one by one at dry and wet (in blood serum/water) conditions for various immersion times. Finally, the procedure of developing, washing in water and then fixing was followed and final washing was completed at 20^{o}C for 05, 01, 05 and 15 minutes, respectively. After final washing, the films were dried by clipping in a hanger at fresh air/or in a drying cabinet and obtained radiographic images (Fig. 2) of the required bandage sample at dry and wet (different immersion times) situation and then to visualize this image it was transferred to the PC using high resolution camera and was viewed in the computer screen by the image analysis software Image J.

2.2.4. Blood serum/water uptake behavior

The neutron intensity before reaching the dry/wet wound dressing materials (object) was different from the intensity of the neutron after passing through it. The relationship between these two intensities can be expressed through the following equation [32]

$$I = I_0 \, e^{-\mu x} \tag{1}$$

Where, x is the thickness of an object, µ is the linear neutron attenuation coefficient, I is the neutron intensity after passing through the dry/wet object, and I_0 is the neutron intensity incident on the object.

The mathematical expression for the neutron intensity ratio/optical density [33] at a point of the film/NR image, D is given by

$$D = \ln (A_0/A) \tag{2}$$

Here, A_0 is the response of densitometer without the sample image and A is the response of densitometer with the sample image.

Actually, optical density is the darkness or opaqueness of a transparency film and is produced by film exposure and chemical processing. An image contains areas with different densities that are viewed as various shades of gray. Now, these values are directly measured by using the international standard image J-software [39] from the radiographic images of the D-S layer wound healing dressing materials.

3. Results and Discussions

Tensile strength, porosity, homogeneity, and body fluid absorption are the basic major physical properties of the wound dressing that are good predictors of sample's ability to protect from bacteria and to heal. The mechanical properties, namely the tensile strength (TS) and percentage elongation at break (Eb) for the single layer bandage were found to be 2.49 MPa and 99.15%, respectively by using a Universal Testing Machine. The amount of Ca, Sr, and Fe was 634.03, 1.52, and 12.58 ppm in the bandage. The pH of the blood serum and demineralized water was found to be 6.92, 6.70, respectively at room temperature 27˚C, using usual pH meter.

At the time of any wound, different bacteria attack in and around the wounded place. From this point of view an antibiotic agent (sterile injectable ciprofloxacin) was incorporated into the gelatin/PEG membrane because of its effectiveness as an antibiotic agent against air-borne bacteria as well as enterobac-teriaceae [40,41]. A drug release study of the membrane was performed by placing small disks of both antibiotic-added gelatin/PEG membrane and control (gelatin/PEG) membrane on a bacterial lawn. This type of bandage has very small homogeneously arranged pores as shown in Fig. 1. Fig. 1 shows the homogeneity measurement data curve obtained from the radiographic image of the single layer wound dressing. Fig. 1 shows that the intensity ratio fluctuations at some places are irregular and at some places are regular against the pixel distance plotted in x-axis. Intensity fluctuation complains the presence of the porosity as well as the homogeneity of any text subjects. The pH of the gelatin/PEG solution was determined using a digital pH meter (Philip, PW-9409, UK) with an efficiency level of ±0.3. The pH of the blood serum and water was 6.917, 6.702 respectively, which is very close to the blood pH (7.4 ± 0.04) [42].

Fig. 2(a), (b) show the neutron radiographic images of single layer collagen bandage after immersion into serum and water for immersion times: (a) (i) 0.5 (ii) 1 and (iii) 2 minutes; (b) (i) 1, (ii) 2 and (iii) 4 seconds. The graphs for neutron intensity ratio versus pixel distance (mm) are shown in Figs. 3, 4 and 5.

Figs. 3 and 4 imply serum absorption curves for the single layer wound dressing at different immersion times: from 30 seconds to 2 minutes and from 1 to 4 seconds, respectively. From Fig. 3 it is seen that the neutron intensity ratio for 1minute is greater than that for 30 seconds and the neutron intensity ratio for 2 minutes is greater than that for 30 seconds and 1 minute. Fig. 4 shows that the neutron intensity ratio for 4 seconds is greater than that for 1 and 2 seconds. This means that the neutron intensity ratio in dressing increases with an increase of immersion time. It is also said that that the serum absorption rate increases with increase in immersion time and after certain time (> 2min.) the dressing was entirely dissolved into the serum, observed in the laboratory. The radiographic image (Fig. 2(a)) also proves this characteristic behavior.

The water absorption curve of a single layer wound dressing is shown in Fig 5. From this figure it is seen that the neutron intensity ratio for 4 seconds is greater than that for 1 and 2 seconds. This means that the neutron intensity ratio in dressing increases with time and also means that the water absorbtion rate increases with immersion time. Absorption curves are very near within 1- 4 seconds and after that it dissolved into water. Fig. 2(b) accords with this characteristic.

From the above observations, we can summarize that the single layer wound dressing dissolves into the body fluid, flowing out from the wound, rapidly (Fig. 2).

From the radiographic images (Fig. 2) it is seen that the insolubility power of the bandage into serum is higher than that into water. Since water and serum flows out from the body wound simultaneously, so it can also be interpreted that the dressing dissolved within the fluid very short time in case of large wound/burn.

Therefore, the bacterial resistance of the bandage will be low. Thus the dressing will not be so effective in case of large burn/injury to protect bacteria and other germs and to heal the injury.

A collagen dressing is a type of wound dressing that is made with a form of collagen to aid in the body's healing processes. Collagen is a category of structural proteins that are naturally present in almost every part of the human body and are especially common in connective tissue. In a wound, the application of these proteins protects the body's own collagen from degradation because of the over-activity of enzymes so that body has the building materials needed to repair the damage. Collagen is a biomaterial that encourages wound healing through deposition and organization of freshly formed fibers and granulation tissue in the wound bed thus creating a good environment for wound healing [43]. Collagen sheets, when applied to a wound, not only promote angiogenesis, but also enhance body’s repair mechanisms [44,45]. While acting as a mechanical support these reduce edema and loss of fluids from the wound site, along with facilitation of migration of fibroblasts into the wound and enhancing the metabolic activity of the granulation tissue [44,46,47]. Moreover, it is easy to apply and has the additional advantage of stopping the bleeding process [48].

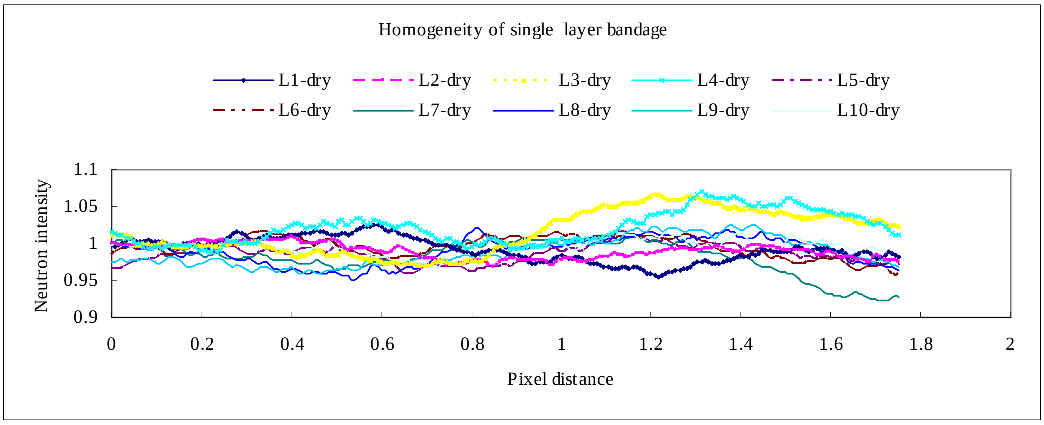
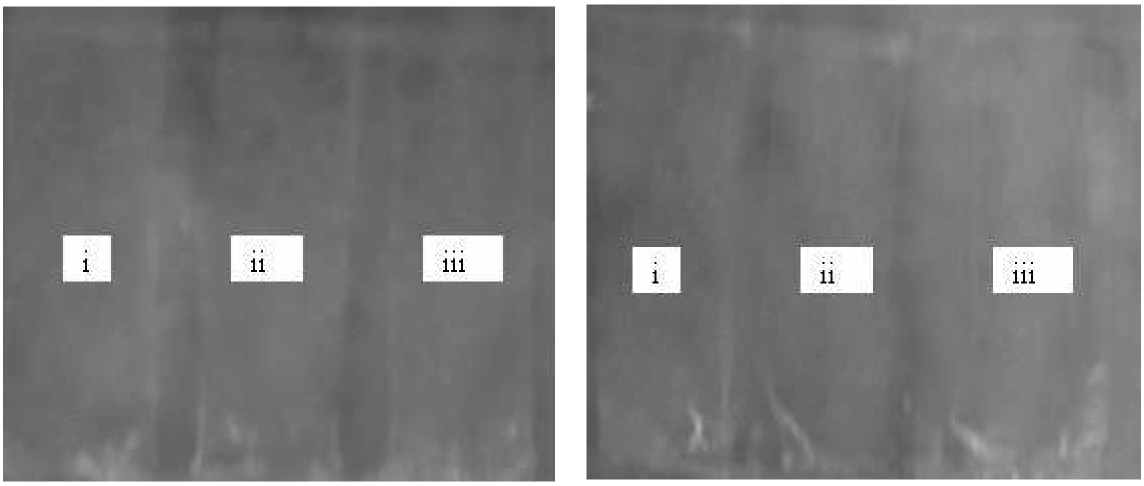


Fig.1: Homogeneity curve of single layer bandage.



(a) (b)

Fig.2: Neutron radiographic images: (a) S-layer collagen bandage immersed into serum for (i) 0.5 (ii) 1, and (iii) 2 minutes; (b) S-layer collagen bandage immersed into water for (i) 1, (ii) 2, and (iii) 4 seconds.

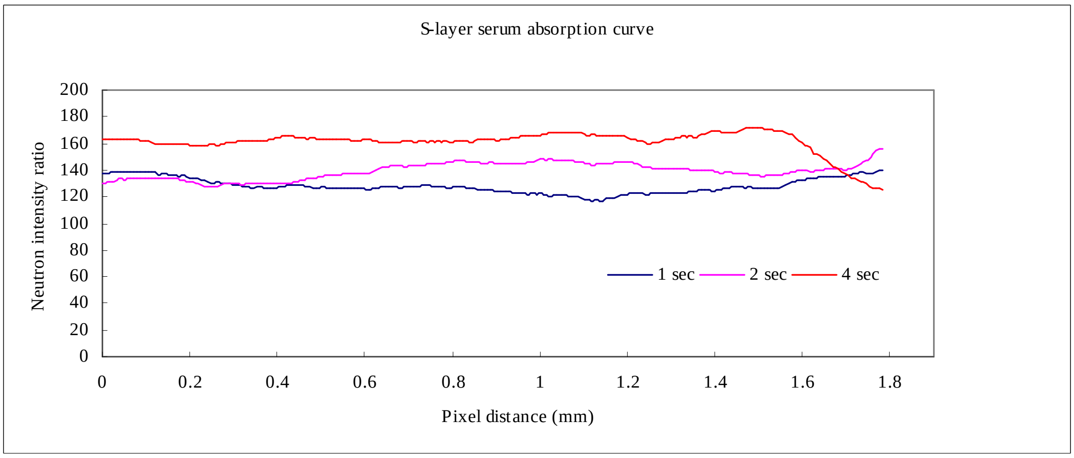


Fig.3: Serum absorption behavior of S-layer collagen bandage for 0.5, 1, and 2 minutes immersion.

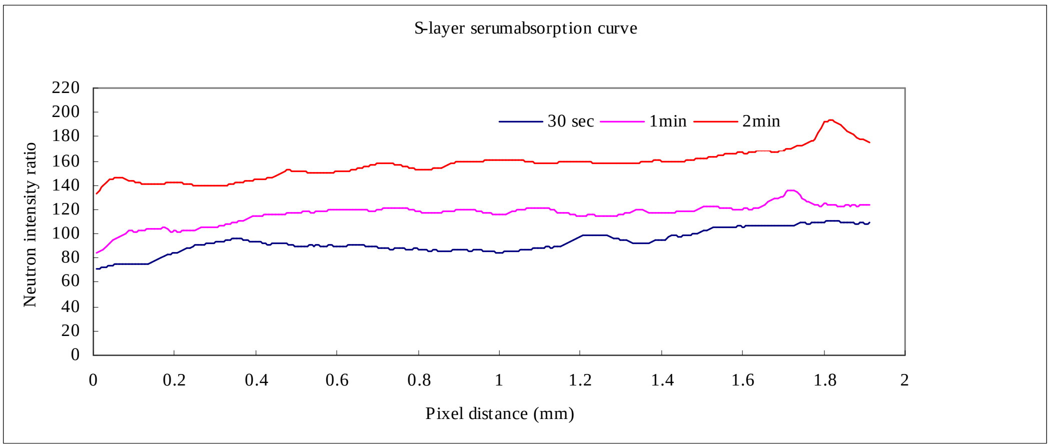


Fig.4: Serum absorption behavior of S-layer collagen bandage for 1, 2, and 4 seconds immersion.

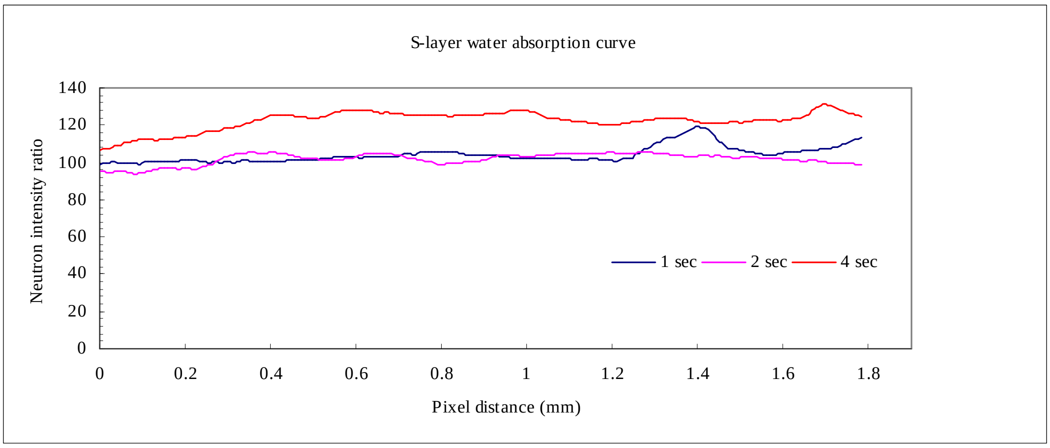


Fig.5: Water absorption behavior of S-layer collagen bandage for 1, 2, and 4 seconds immersion.

It can also be said that this dressing will not be as effective as desired in case of large injury/burn because in this case it dissolves into the body fluid within short time though it is very essential to protect bacteria and to heal injury. Due to this reason, for proper use of this collagen bandage in case of not only the small wound/burn but also the large form, it is essential to increase the effectiveness of insolubility into body fluid (blood serum, water) by upgrading this dressing into double layer which is under study.

4. Conclusions

The solubility of the prepared bandage into the body fluid must be improved or upgraded, although it is more effective in the healing of small wounds as compared to conventional wound dressing but it is not so appropriate for large wound/deep burn healing.

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