Investigation of the Use of Microencapsulated Sodium Fusidate for Antibacterialwound Dressings

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Abstract

This research was done with the aim of investigating the use of microencapsulated Sodium Fusidate to achieve antibacterial property in a textile based wound dressing. The sterile cotton gauze was selected as the substrate and was improved into an antibacterial wound dressing that particularly targets at treating traumatic wounds, by incorporating microcapsules with antibacterial properties into the gauze structure. The process of microencapsulation was carried out via electro-spraying and the microcapsules thus produced contained Sodium Fusidate dissolved in ethanol as the active substance (core) and the Calcium alginate as the wall material (shell). The obtained microcapsules were characterized by FTIR spectrometry and SEM and the results showed that the microcapsules have been produced in the size ranges of 50-200µm, having Sodium Fusidate as the core material. To attach these microcapsules onto the gaze, they were loaded into a 2% solution of Chitosan and the sterile cotton gauze fabric was impregnated in this dispersion. The antibacterial property of samples taken from this treated gauze fabric was tested against both gram-positive and gram-negative bacteria and the samples produced an average inhibition zone with a diameter of 32 mm for gram-positive bacteria Staphylococcus aureus, which is the most common type of bacteria in traumatic wounds.

Keywords: wound healing, wound dressing, traumatic wounds, antibacterial, microencapsulation, electro-spraying

1. Introduction

A disruption in the epithelial integrity of the tissues is defined as a wound (Ather & Harding, 2009). Wound classification can be done based on the duration of wound healing (acute or chronic), the cause (pressure ulcers, venous leg ulcers, diabetic ulcers), the depth of tissue involvement and the infection method (exogenous wound infections and endogenous wound infections) or other characteristics such as closure (primary or secondary intention) (Bowler et al., 2001; Bryant & Nix, 2015; Sirijatuphat et al., 2013). Traumatic wounds form the most common wound type due to their high frequency of occurrence. Lacerations, penetration wounds and abrasions also fall into this wound category (Bryant & Nix, 2015; Sirijatuphat et al., 2013). The most prevailing bacteria that grow on traumatic wounds are Staphylococcus aureus, Streptococcus pyogenes, pneumococcus, and Enterococci and Pseudomonas aeruginosa (Bowler et al., 2001).

Wound healing is the process in which the body replaces the destroyed tissues by living tissues. During healing, a wound passes through four phases namely haemostasis, inflammation, proliferation, and tissue remodelling (Ather & Harding, 2009). A wound dressing is responsible for providing protection to the wound from external sources and

assisting fast recovery (Thomas & Uzun, 2019). There are seven types of dressings as gauze, films, hydrogels, foams, alginates, composites, hydrocolloids, and interactive dressings, based on the materials that they are made of (Ather & Harding, 2009).

The sterile cotton gauze is one of the most common and widely used textile based wound dressing type as it is available in many shapes and sizes and being applicable to a wide variety of wound types. It is used both as a primary and secondary dressing. However, there are certain drawbacks of conventional sterile simple gauze such as the sticking nature to the wound, and the inherent properties of the gauze which provide favourable conditions for microbial growth thus causing the risk of infection and a delay in healing (Hajimirzababa et al., 2017).

Modifying the textile based wound dressings so as to minimize the undesirable features in them and improving the functionality is of higher concern to medical and biomedical researchers (Bowler et al., 2001; Sarheed et al., 2016; Hansson, 1997). At present, antibacterial wound dressings have been produced by incorporating either antibiotics or antiseptic agents (Bowle et al., 2001; Sarheed et al., 2016; Morais, 2016) into these sterile gauzes. An ideal antimicrobial wound dressing should possess antimicrobial properties belonging to a wide spectrum, against major types of microorganisms. Furthermore, it should be non-toxic and non-allergic to host cells and should be able to release drugs in a sustained manner.

Microencapsulation is a novel technique that has been identified as an effective way to impart antimicrobial or antibacterial properties to textile substrates in which micro-sized solid particles, liquid droplets or gasses are enclosed in shell structures called microcapsules within the range from 0.2 to 5,000µm (Salaün, n.d.; Dubey & Rao, 2009; Bakry et al., 2015; Keyan et al., 2012; Singh et al., 2010; Ngwuluka, 2010; Silva et al.,2014). It is considered as one of the most versatile techniques of releasing an active substance as it ensures encapsulated material reaching the required area of action and releasing at the proper time.

In this research, the principle of microencapsulation has been used to improve the functionality of the simplest textile based wound dressing; cotton gauze in order to treat the most common wound type; traumatic wound. The proposed anti-bacterial gauze fabric was manufactured by incorporating Sodium Fusidate (core material) encapsulated in Sodium Alginate (shell material) onto a commercially available sterile cotton gauze fabric.

Sodium Fusidate is a topical antibacterial agent used for treating bacterial infections and is active against Staphylococcus aureus, the most common bacteria found in traumatic wounds. Sodium Fusidate has been a widely used antibiotic agent for treating traumatic wounds, as it prevents the translocation of the translocation of the elongation factor G (EF-G) from the ribosome and interferes with bacterial protein synthesis (Ahmed et al., 2015).

Sodium alginate which was used as the shell material is a biopolymer with properties that promotes wound healing, as it is biocompatible and non-toxic and has been used in various novel wound healing applications ("Alginate in Wound Dressings", 2018; Torres, 2013). The microencapsulation method utilized was electro-spraying which happens based on the theory of charged particles; the interface of a liquid droplet exiting a capillary can be deformed when an electric field is applied on to it (Bock et al, 2012).

2. Materials and Methods

The section given below explains materials and methods used in the manufacturing of the proposed antibacterial gauze fabric.

2.1 Preparation of microcapsules

The preparation of microcapsules was experimented under four different conditions: by changing the injection rate of the electro-spinning machine, and the availability of a dispersing agent in the medium. The experiments were conducted at the room temperature. Table 1 gives details of the four experimental conditions and the chemical compounds. The injection rate of the electro-spinning was changed as 20ml/hour, 40ml/hour and 80ml/hour to check the harnessing efficiency of the microcapsules while the masses of chemicals used in all four instances were kept approximately equal.

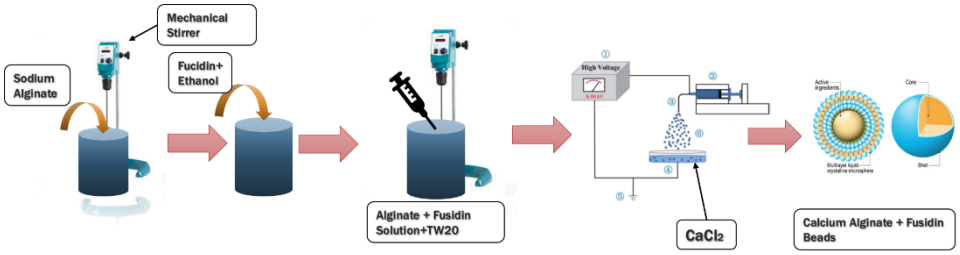
Table 1: Parameters used in encapsulation experiments with electro-spraying

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Sodium Alginate mass (in 100 ml of solution)	2.5289g	2.5344g	2.5344 g	2.5344g
Sodium Fusidate mass (in 100 ml of solution)	0.2165g	0.2178g	0.2178g	0.2178g
CaCl2 (in 100 ml of solution)	7.4322g	7.5288g	7.5288g	2.5288g
Dispersing agent	-	Tween 20	Tween 20	Tween 20
Injection rate	20	20	40	80

As given in Table 1, Sodium Alginate solution was prepared by dissolving Sodium Alginate (GLORCHEM Enterprise, 216.12 g/mol) in water and stirring at 400 rpm for 10 minutes using a mechanical stirrer. Tween 20 (Sigma Aldrich, 1,227.54 g/mol) was added to this solution and stirring was continued further for 5 minutes. Sodium Fusidate was dissolved in 10 ml of Ethanol and that was added to the prepared Sodium Alginate solution. The resulting solution was stirred further using a mechanical stirrer for 15 minutes at 400 rpm. Then it was left to cool down to the room temperature for another 30 minutes.

Calcium Chloride (E.Merck (India) Limited, 99%, 110.98 g/mol) solution was prepared using the mass and volume fractions given in Table1. A syringe with a 16-gauge needle was loaded with 30 ml of the solution containing Sodium Alginate and Sodium Fusidate. It was fixed to the syringe pump (NLS20, Nanolab Instruments Sdn. Bhd., Malaysia) of the Electro-spinning machine. A piece of Aluminium foil was deposited in a Petri dish and 40 ml of Calcium Chloride was poured on to it. The Aluminium foil was used to ground the Calcium Chloride bath in order to produce microcapsules via electro-spraying. The Petri dish was placed in front of the electro-spinning machine. A high electric field of 10 kV was applied to the set up using high voltage power supply (PS-35, Nanolab Instruments Sdn. Bhd., Malaysia). Microencapsulation took place and microcapsules thus produced were collected by filtering with a clean piece of poly-cotton blended cloth (30 GSM) and were washed in water.

Figure 1: Preparation of microcapsules via in-situ polymerization using electro-spraying set up



2.2 Characterization of the microcapsules

The mean particle size and distribution of prepared microcapsules were studied using Scanning Electron Microscopy (SEM) (EVO18 Research, Zeiss, Germany). The functional groups of the samples and their changes were investigated with a Fourier transform infrared spectroscope (FTIR) (Alpha, Bruker, Germany) in order to clarify the constituents of the prepared microcapsules.

2.3 Incorporation of microcapsules into the gauze fabric

Chitosan was selected as the binding agent as it has previously shown good binding properties and due to its biocompatibility (Hajimirzababa et al., 2017). Chitosan has shown effective performance in various wound healing applications and different derivatives and formulations have been tested in-vitro and in-vivo (St Denis, 2012).

5 ml of diluted Chitosan (1%, Sigma Aldrich) was taken into a Petri dish and the microcapsules were introduced into it. A 5cm x 5cm square fabric piece was cut from the sterile cotton gauze fabric (40's x 40's), and impregnated into this Chitosan solution which contained the microcapsules. It was left for 60 minutes to promote the binding reaction, and then they were taken out and left to dry in the oven for another 20 minutes at 35°C.

2.4 Evaluation of the antibacterial behaviour of the improved gauze fabric

After incorporating the microcapsules into the sterile cotton gauze fabric, its effectiveness was evaluated in terms of antibacterial activity by using the agar diffusion (SN 195920)test method ("Quality Evaluation Methods for Textile Substrates Based Wound Dressings", 2014). Gauze samples were tested against the two main types of bacteria, gram-positive (S. aureus, AATCC 6538) and gram-negative (Escherichia coli, AATCC 11303). Three samples of developed antibacterial gauze fabric, one untreated sterile gauze fabric sample (negative control) and one sample of commercially available Fucidin tulle (positive control) were used for the antibacterial tests. These samples were placed on the top of the previously sub-cultured agar plates with the respective bacteria. Then these plates were incubated at 37ºC temperature for 24 hrs. Inhibitory zones were identified and inhibition zone diameters of the three samples were recorded. Then the average inhibition zone diameter was calculated.

On the wound site the ion exchange that happens with the wound exudate would cause the dissolution of the Calcium Alginate shell releasing the encapsulated active substance (Thomas, 2000). As the dressing was tested in a culture medium instead of on an actual wound an external force was needed to rupture the microcapsules to get the core substance released. Therefore, prior to being placed in the culture medium, samples of

microencapsulated gauze were thrusted between two glass plates by applying a 3N force was applied for about 20 minutes to rupture the embedded microcapsules.

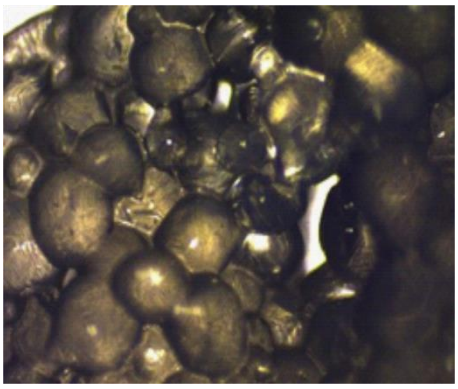
3. Results and Discussion

The following results were obtained from the experimental procedures that were described under the section of materials and methods.

3.1 Production of the microcapsules

As shown in figure 2 microcapsules within a size range of 50µm-200 µm were obtained from three experimental conditions (experiments 2,3 and 4) explained under section 2.1 out of the four experimental conditions.

Figure 2: Light microscope image of sodium alginate-Fucidin micro capsules (4/0.25 magnification)*

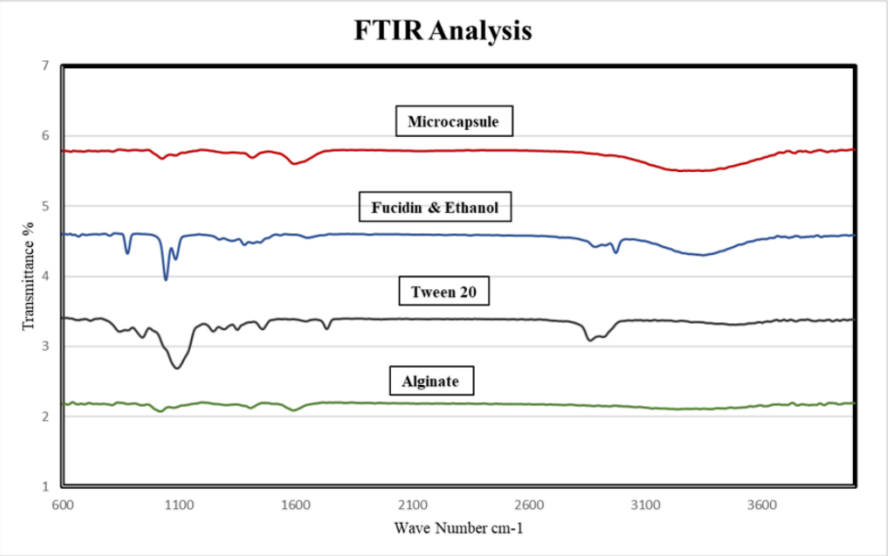


In all four cases approximately equal masses of Sodium Fusidate (0.2178g) and Sodium Alginate (2.5344g) were used and the applied voltage was also kept constant. The flow rate (injection rate) was from 20ml/hr to 80ml/hr and the number of microcapsules obtained also increased accordingly demonstrating a directly proportional relationship between the injection rate and the harnessing efficiency of microcapsules. The maximum amount of microcapsules was obtained when the injection rate was 80 ml/hr. The use of surfactant Tween 20 facilitated the reduction of the size of the capsules.

3.2 FTIR results

FTIR analysis was carried out to confirm the constituents of the produced capsules. Fig 3 shows the FTIR graphs obtained. The blue colour graph was obtained for Sodium Fusidate dissolved in ethanol, the grey colour graph was obtained for Tween 20 and the green colour graph was obtained for Sodium Alginate. The red colour graph was obtained for the microcapsules that were crushed in order to get the core substance released.

Figure 3: FTIR analysis of Sodium Alginate (green curve), Sodium Fusidate dissolved in ethanol (blue curve), Tween 20 (black curve) and the microcapsules (red curve)



Spectrum of sodium alginate showednoticeable peaks at 2947 cm^{-1}, 1597 cm^{-1}, 1412 cm^{-1}, 1082 cm^{-1} and a broad peak within the range 3000–3500 cm^{-1}. These peaks represent stretching vibrations of aliphatic C–H, stretching vibrations of carboxylate salt ion, O-H bending of the carboxyl group, C–O stretching vibration of pyranosyl ring in alginate and stretching vibrations of O–H bonds of alginate respectively. Graph of sodium fusidate dissolved in ethanol showedpeaks at 2973 cm^{-1} and 2888 cm^{-1}, 1668 cm^{-1}, 1384 cm^{-1} and 1366 cm^{-1}, 1087 cm^{-1}, 1045 cm^{-1} and a broader peak within the range 3100–3500 cm^{-1}. These peaks represent the stretching vibrations of aliphatic C–H, weak stretching of C=C bond in sodium fusidate, the bending of O-H bond in ethanol, C-O stretching in ethanol and stretching vibrations of O–H bonds in both ethanol and carboxyl groups respectively. Spectrum of Tween 20 shows peaks at 3499 cm^{-1}, 2924 cm^{-1} and 2867 cm^{-1}, 1460 cm^{-1} and 1250 cm^{-1}. These peaks represent stretching of O-H bond, stretching of O-H bond, bending of -CH2 and stretching of C-O in ester respectively. The graph of the capsules shows peaks at 2935 cm^{-1}, 1594 cm^{-1}, 1441 cm^{-1}, 1417 cm^{-1}, 1089 $cm^{-1}$and 1032cm^{-1} and these peaks corresponds to bonds that are also shown by the graphs of the constituents at close wavelength values. The FTIR analysis verified the constituents of the capsule and confirmed that they have been produced in the expected manner i.e.; Calcium Alginate as the shell material and Sodium Fusidate dissolved in ethanol as the core material.

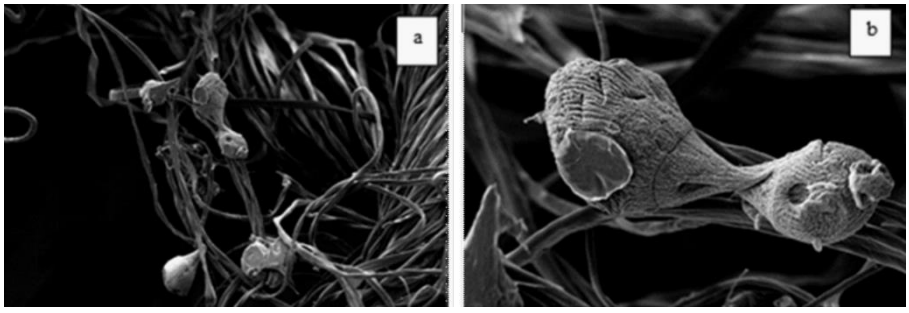
3.3 Incorporation of microcapsules to cotton gauze fabric

Chitosan, as it was capable of forming strong and stable bonds with Calcium Alginate, bonded the microcapsules well onto the gauze fabric. It is the interaction between the amino groups of chitosan and the carboxylic groups of the alginate that causes the adjacently placed chitosan and alginate to form a strong and stable chitosan/alginate complex (Hajimirzababa et al., 2017).

3.4 Size and morphological study of the developed gauze with microcapsules

SEM images were obtained under magnifications of 100×, 250×, 500×, 1000× to observe the size of microcapsules and how they have bonded with the gauze fabric. The images of 250x and 1000x magnifications are shown in Figure 4 (a) and (b) respectively. As it was observed, the microcapsules had bonded at different sites of the gauze fabric and they showed circular and irregular shaped morphologies.

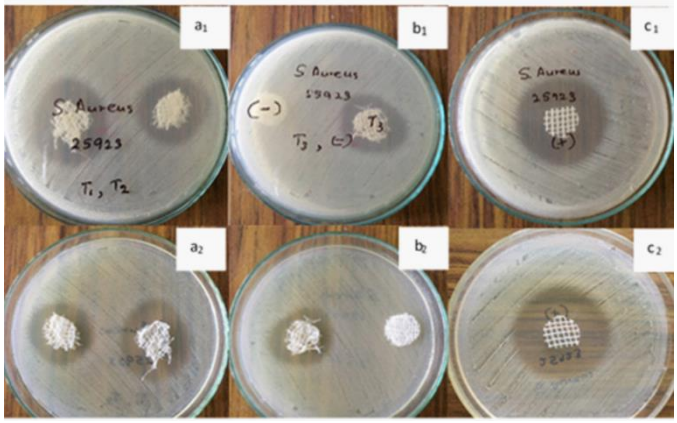
Figure 4: SEM images of micro-capsules bounded to gauze fabric using chitosan with different magnifications (a) 250x magnification (b) 1000x magnification



3.5 Qualitative analysis of the antibacterial test

The samples of the developed gauze, positive control (Fucidin Intertulle) and negative control (normal sterile gauze) didn't demonstrate noticeable antibacterial activity against the gram-negative bacteria type, E.coli therefore no zone of clearance was recorded for this type of bacteria. The reason for this is E. Coli being resistant to Sodium Fusidate which is the active substance in the developed gauze bonded with microcapsules and Fucidin Inter-Tulle, the positive control. As shown in Figure 5, a zone of inhibition was visible in the samples of the developed gauze and the commercially available Fucidin Tulle (positive control) against gram-positive S. Aureus.

Figure 5: Comparison of the growth inhibition of S. aureus bacteria by developed antibacterial gauze fabric and positive controls. a1, a2: developed antibacterial gauze fabric samples T1 and T2, b1, b2: developed antibacterial gauze fabric sample 3, and untreated sterile gauze (negative control), c1, c2: commercially available Fucidin Tulle gauze fabric (positive control)



The following calculation shows the average diameter of the zone of clearance for the developed antibacterial gauze fabric samples;

$$Davg = dT1 + dT2 + dT3 = 30 + 30 + 35 = 31.6mm$$

The diameter of the zone of clearance for the Fucidin Inter-Tulle positive control was measured as 41mm. There are three main release mechanisms in microcapsules; diffusion, dissolution and rupture (Singh et al.,2010; Ngwuluka, 2010; Silva et al., 2014). Dissolution in which the shell material dissolves and the active substance inside gets gradually released is the type of release mechanism that happens in alginate microcapsules. Sodium Alginate is water soluble and behaves like flexible coils when in a solution. Once they interact with polyvalent cations like Ca^{2+}, Ba^{2+} and Zn^{2+}, cross-linking happens and water insoluble Calcium alginate is formed. This is called the egg box structure which becomes the shell in a capsule. This shell dissolutes when the bivalent ions get replaced by the monovalent ions and then core substance gets released ("Alginate in Wound Dressings", 2018; Torres, 2013).

In the wound site Calcium Alginate comes into contact with wound fluids resulting in an ion exchange between calcium in the carboxylic group of Alginate and the mono-valent ions in the wound fluid. This causes the dissolution of the Calcium Alginate shell releasing encapsulated Sodium Fusidate. Due to the inability of performing clinical trials, encapsulated active substance was made to release by applying an external force and rupturing the shell in order to assess the antibacterial behaviour of the bounded.

The wound dressing has been able to show good bacterial resistance against Staphylococcus aureus since Sodium Fusidate was used as the active agent. The use of Sodium Alginate too has contributed towards achieving the expected antibacterial properties and performance of a better wound dressing as Alginate facilitates absorption of excess wound fluid, maintaining a moist environment and minimizing bacterial infections at the wound sites (Liakos et al, 2013; Tønnesen, 2002). Moreover, free calcium ions generated during the dissolution of microcapsules facilitate faster blood clotting and provide haemostatic properties to the wound dressing (Torres, 2013). In the traditional way of treating traumatic wounds, Sodium Fusidate in cream form is physically loaded to the wound dressing and released passively. This sometimes results in antibiotic overuse and even bacterial

resistance. Since a controlled release can be obtained through microcapsules these unfavourable conditions can also be avoided.

4. Conclusion

Wound dressings occupy a significant place among the areas of interest for research in the field of Medical Textiles. This research has focused on improving the conventional cotton gauze so as to have antibacterial properties that could promote the healing of traumatic wounds by binding Sodium Fusidate-Calcium Alginate microcapsules into the gauze structure using Chitosan as the binding agent. The process of microencapsulation was done using electro-spraying which could produce capsules with diameters within the range of 50-200 µm. The core of the microcapsules was composed of Sodium Fusidate while the wall contained Calcium Alginate. The FTIR analysis which was carried on the microcapsules confirmed the constituents of the capsules. The developed gauze fabric has shown effective antibacterial property against gram-positive S. Aureus, the main type of bacteria found in traumatic wounds. The encapsulated gauze samples gave an average inhibition zone of 32 mm compared to the commercially available Fucidin Inter-Tulle gauze fabric which gave an average inhibition zone of 42mm. Hence it can be concluded that microcapsules with Sodium Alginate as the core material and Sodium Fusidate as the shell material, produced via electro-spraying are capable of exhibiting promising antibacterial behavior against gram positive S. Aureus thus giving an antibacterial property to a conventional cotton gauze when incorporated into the gauze structure.

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