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A new carbohydrate-based wound dressing fibre with superior absorption and antimicrobial potency

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ABSTRACT

Heavily exudating wounds can lead to infection and unnecessary trauma if they are not adequately managed. Manufacturers involved in production and marketing of high absorption silver dressings, besides emphasising high absorptions of their dressings are keen to highlight potent antimicrobial abilities of their products against all kinds of pathogens including MRSA. However, there are little or no credible reports on minimal but potent quantities of silver needed in a dressing to eliminate bacteria spread and growth or how effectively the silver within a dressing is released over time. This paper introduces a new hybrid biomaterial fibre made from polysaccharide-based polymers with inbuilt ability to gel and absorb large quantities of pseudo exudates. Furthermore, it will be reported that the new fibre carries up to six times less silver than it is conventionally used in silver dressings and displays a very slow rate of release whilst maintaining full potency over time against known Gram positive, Gram negative micro-organisms including MRSA. The paper concludes that the developed hybrid fibre has long lasting antimicrobial and gelling properties comparable, if not better, than Acticoat AA and Aquacel Ag, two commercially available silver dressings.

1. Introduction

Maceration or spread of the exudate to vicinity of the wound is due to poor management of the wound and is one of the prime causes of infection. Primary objective of any wound dressing therefore is to keep wound environment uncontaminated from any kind of microbial agents whilst providing the right conditions for the healing process to take its course (Cooper, 2004; Ryan & Ray, 2003; White, 2005). Antimicrobials play a major role in reducing the risk of wound infection and improve the endurance of patients to infection concurrent with good exudate management (Landis, 2008; Ryan & Ray, 2003).

Silver, amongst other antimicrobial agents, is the most common and well-studied types of antimicrobial due to its wide spread antimicrobial spectrum. Silver is reported to be effective against a wide range of aerobic, anaerobic, Gram positive, Gram negative, fungus and viruses (Dunn & Edwards-Jones, 2004; Howe & Dobson, 2002). It is also believed to be active at very low concentrations (Bowler, Jones, Walker, & Parsons, 2004; Burd et al., 2007; Burrell, 2003; Edwards-Jones & Foster, 2002; Thomas, 2004).

Traditional coatings, hydrogels, material compounds and nanostructured technologies are being used for application of silver as antimicrobial and these technologies are found to be effective, to a large extent, in reducing the risk of wound infection and further complications. In order to maximise the effectiveness of silver for longer periods, slow and sustained release of silver is very important (Alloway, 1995; Kalsen, 2000; Smith, 1947). The natural polymeric materials, especially alginates have been found to be instrumental in achieving successful and long lasting healing effects from silver antimicrobials (Keong & Halim, 2009; Samadra & Esther, 1997). Alginate is a major polysaccharide found in brown algae and is biocompatible, biodegradable material. It is non-toxic to human body and is used as moist healing material in wound management industry (De & Robinson, 2003; Fan, Du, Zhang, Yang, & Zhou, 2006).

Calcium/sodium ion exchange at the wound surface helps alginate to gel and function more effectively as a primary wound dressing. Exudate absorption of alginate can be as high as 12 g/g thus helping to reduce the spread of bacteria due to its gel forming ability whilst dispensing silver (Qin, 2004; Stashak, 2004). However, in heavily exudating wounds, alginate struggles to

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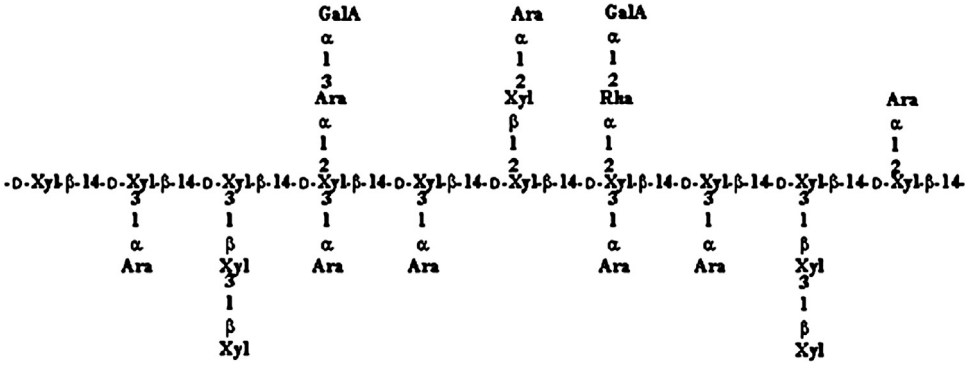
Table 1
Physical properties of control, Psyllginate and Psyllginate Ag fibres.

Fibres	Linear density (dtex)	Breaking force (cN)	Breaking strain (%)	Tenacity (cN/tex)	Work of rupture (cN cm)
Calcium alginate (control)	4.15	4.07	11.43	9.80 ± 1.3	0.17
Psyllginate fibre i.e. 0.75%w/v Psyllium	7.43	6.71	10.64	9.03 ± 1.0	0.35
Psyllginate Ag with 0.05% silver carbonate	7.86	7.36	8.73	9.36 ± 1.2	0.25

keep up with the absorption demands and its consequential anomalies hence consideration of other fibres perhaps with less bio-friendliness (e.g. carboxymethyl cellulose) have been replacing alginate.

Keeping in with the alginate tradition; this paper reports on introduction of a second polysaccharide component into an alginate fibre, with the prime intention of alleviating its absorption deficiencies. The second component is also polysaccharide-based and is commonly known as Psyllium. Despite its wide spread availability and its FDA approval for all kinds of oral and internal consumption, Psyllium has to date not been considered as a textile fibre or as an active constituent of another textile fibre. Psyllium is hydrophilic natural material and is abundantly available in natural habitats. It is extremely absorbent and quickly forms a gel. Its wide range of medicinal attributes are recognised in many countries around the world. Its chemistry is largely understood as being made of high percentage of hemicelluloses, highly branched acidic arabinoxylan with xylan backbone having both (1–4) and (1–3) linkages. Most residues in the xylan backbone are variously substituted at 0–2 and 0–3 with arabinose, xylose and aldobioronic acid identified as galactopyransyluronic acid (Fischer et al., 2004).



Proposed consensus structure of arabinoxylan of Psyllium (Fischer, M. H. & Yu, N.).

2. Materials and method

Food grade Psyllium husk (Qurshi Laboratories of Pakistan Limited Karachi) was purchased from local UK supermarket. Sodium alginate was provided by (Speciality Fibres and Materials (SFM), Coventry, UK), silver carbonate and calicum chloride were purchased from (Sigma–Aldrich Company Limited, USA) and acetone from (VWR International Limited, UK). Acticoat AA was obtained from Smith & Nephew and Aquacel Ag was acquired from Convatec.

2.1. Fibre production and characterisation

4% (w/v) sodium alginate solution was prepared by continuous stirring. 0.05% (w/v) silver carbonate was added to Psyllium gel solution following Psyllium extraction methodology described elsewhere (Miraftab & Masood, 2011, Miraftab & Masood, 2013 patent) and was stirred continuously to achieve adequate dispersion. The sodium alginate solution was then added to the Psyllium mixture and stirred for further 4 h. The mixture was left overnight to remove trapped air and was subsequently extruded on a pilot Wet extruder machine via 20 hole spinneret of 76 μm diameter into 1% calcium chloride coagulation bath. The coagulated fibres were drawn (1.25 DR), washed and wound up on a bobbin before drying in an increasing concentration of acetone bath (i.e. from 50/50 (v/v) to 50/75 (v/v) and then100% pure acetone). Three forms of fibres were produced, 100% calcium alginate as the control, fibres containing 0.75% Psyllium referred to as Psyllginate and samples with 0.75% Psyllium and 0.05% silver carbonate, referred to as Psyllginate Ag.

2.1.1. Tensile properties

Linear densities of each fibre were calculated based on g of fibre per 10,000 m i.e. deci-tex and subsequently subjected to tensile testing using Textechno Fafegraph M single fibre tensile tester. The average result of 20 sample tests were calculated and recorded.

2.1.2. Liquid absorption

All the fibres were tested in de-ionised water, saline (0.9% (w/v) sodium chloride) and solution A (0.8298% (w/v) sodium chloride and 0.0368% (w/v) calcium chloride). Solution A is the closest salt solution to body fluid or exudate. All samples were initially soaked for 1 h and centrifuged for 15 min at 1200 revolution per minute prior to taking measurements. Liquid absorption was calculated as the ratio of wet weight of the fibre to the weight of fibre after drying overnight at 105 °C. The following formula was used to calculate the absorption in g/g:

$$\text{Absorption(g/g)} = \frac{W_1 - W_0}{W_0}$$

where W_0 is weight of dry fibre and W_1 is the weight of wet fibre.

2.1.3. Silver content measurement

1 g of each sample fibres was dissolved in 100 ml of concentrated nitric acid and sulphuric acid solution. The solution mixture was then heated for 30 min at 60 °C for complete breakdown of the fibres. The solution was filtered using very fine nylon mesh. The filtered solution was further diluted (1/10) using de-ionized water to enable quantification of total silver in the fibres. Quantification of silver content was carried out by atomic absorption method.

Table 2
liquid absorption of the developed fibre, Psyllginate Ag and the commercial dressing fibres.

Type of fibre	Water (g/g)	Saline (g/g)	Solution A (g/g)
Calcium alginate (control)	11.78 ± 0.88	16.53 ± 0.74	14.89 ± 0.92
Aquacel Ag	30.51 ± 1.25	25.40 ± 1.34	23.91 ± 0.58
Acticoat AA	9.80 ± 0.8	15.24 ± 0.6	16.32 ± 1.1
Optimised Psyllginate Ag 0.05% $AgCO_3$	21.72 ± 0.98	28.94 ± 1.12	26.31 ± 2.50

2.1.4. Silver release

1 g of each fibre was suspended in nutrient broth saline solution (a mixture of standard nutrient broth and saline solution 1:1 at a ratio of 1:100 (w/v). The samples were kept at room conditions for three days. During this period, aliquots were removed at timed intervals of 1, 4, 24, 48 h and the liquid was topped up to maintain a constant volume. Samples were filtered, diluted appropriately and analysed using atomic absorption spectrometry

2.1.5. Antimicrobial efficacy testing

The AATCC 147-2004 (Antibacterial Activity Assessment of Textile Materials: agar diffusion plate test) and zone of inhibition tests were used to determine the antimicrobial efficacy of test fibres using three selected species of bacterial cells.

3. Results and discussion

Linear density and tensile properties of all the fibres are given in Table 1. To eliminate unnecessary variables, all fibres were drawn at the same draw ratio i.e. 1.25. As seen from the table, inclusion of Psyllium increases linear density of the fibres by as much nearly twice the control fibre. However, the tenacities or fibre strengths remain virtually identical for all the fibres. Psyllginate Ag showed the most reduction in extension compared to the control and the Psyllginate fibre.

3.1. Liquid absorption properties of optimised Psyllginate Ag and commercial fibre dressings

The prime intention of including Psyllium is to improve absorption characteristics of alginate fibres. It is therefore most important to illustrate this achievement and how it would ultimately compare to commercial wound dressings of similar calibre. Table 2 clearly illustrates these findings. Aquacel Ag shows the highest g/g water absorption compared to Acticoat AA and the optimised Psyllginate Ag fibre. However, water is not the best example of bodily fluid, it lacks the appropriate salt contents. Therefore, saline and solution A better represent the exudate and are commonly used as pseudo exudate solutions. When considering absorption of such solutions, Psyllginate Ag clearly outperforms both the Acticoat AA and Aquacel Ag, known as the gold standard within wound care arena, by at least 10%. Based on statistical *t*-test analysis made between each set of results, the difference between calcium alginate i.e. the control and Acticoat AA as compared to the optimised Psyllginate fibre are significant. But the difference between Aquacel Ag and optimised Psyllginate is statically not significant. However, other attributes of Psylliginate fibre, as will be shown later, justifies Psyllginate's superiority to Aquacel Ag.

3.2. Silver content in the fibres

Silver contents of Psyllginate Ag and the two commercially available silver dressings; Aquacel Ag, and Acticoat AA were measured and have been illustrated in Table 3. The results show that Psyllginate Ag has very low silver content compared to Aquacel Ag and Acticoat. Acticoat AA has the largest quantity of silver per gram of fibre.

The fact that Psyllginate Ag fibre contains up to 4.5 times less silver than Aquacel Ag and as much as 9 times less silver than Acticoat AA in itself would be of considerable commercial benefit, particularly if it is proven to be effective against different bacteria at such low silver content.

3.3. Silver release analysis

Dispensation or release of silver from the fibre dressing is another important consideration; fast silver release rate could instantly kill most if not all bacteria but fail to maintain antibacterial environment with time or too slow release may not provide sufficient antibacterial potency to kill some if not most bacteria. Hence, greater knowledge of release mechanism and assessment of its potency is crucial. Fig. 1 shows silver release from Aquacel Ag and Acticoat AA both these dressing fibres show very fast and large quantities of silver release in the first 4 h and then slow down for the next 48 h. Acticoat AA shows further rise in silver release rate after 48 h. Psyllginate Ag, on the other hand, follows a steady and slow rate of release up to 72 h. Psyllginate silver release mechanism is clearly very different to the other two silver fibre dressings. Judging by these results it appears unlikely that Psyllginate Ag would be able to compete with the commercial fibre dressings in killing and containment of bacteria, unless proved otherwise.

3.4. Antimicrobial efficiency

3.4.1. Bacterial suspension method

Bacterial suspension method is normally used to determine the minimum amount of antimicrobial agent necessary to kill a certain population of test microorganism which is known as minimum inhibitory concentration (MIC) of antimicrobial. This is a quantitative procedure for evaluation of the degree of antibacterial activity. Test samples are inoculated with bacterial suspension in bacterial growth media and incubated at required growth temperature (37 °C) for the test bacteria. After a specified time intervals (i.e. 1 h, 4 h, 24 h, and 48 h used in this work) 0.1 ml of inoculate is separated and spread over the agar plate. The plates are incubated overnight at 37 °C and numbers of colonies are counted as number of bacteria per millilitre using the following formula:

$$\text{Colony forming units per millilitre (CFU/ml)} = n \times 10 \times 10^5$$

where n – number of colonies counts in 0.1 ml; 10 – conversion number of colony forming units per millilitre; and 10^5 – dilution of culture.

Antimicrobial activity of the optimised Psyllginate Ag was also compared with the two commercial dressings Aquacel Ag and Acticoat AA. Gram positive *Staphylococcus aureus*, methicillin resistant *S. aureus* type 16 (MRSA 16) and Gram negative *E. coli* were used. 10^6

Table 3
Total silver contents of Psyllginate Ag and the commercial dressing fibres.

Sample type	Silver contents (mg/l)	Average silver content (mg/l)
Psyllginate Ag (0.05% $AgCO_3$)	5.72–8.42	7.39
Acticoat AA	38.83–92.53	65.83
Aquacel Ag	18.75–42.45	30.60

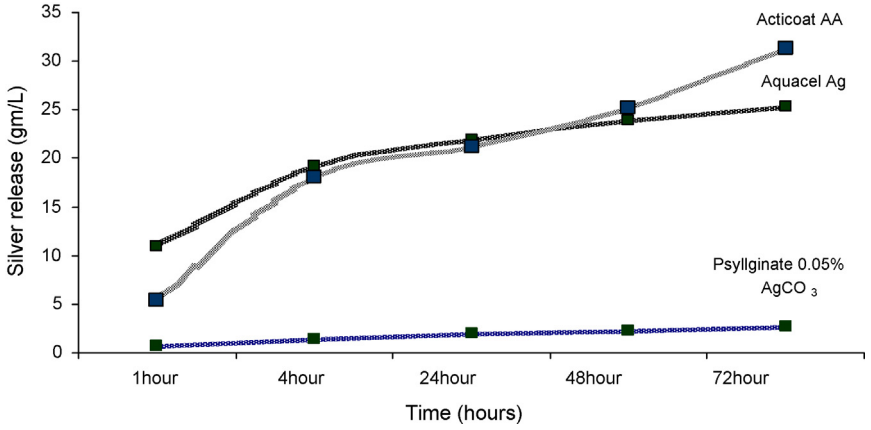


Fig. 1. Silver release as a function of time for the three test fibres.

Table 4
Antimicrobial activity results of optimised Psyllginate Ag, Aquacel Ag and Acticoat AA against *S. aureus*.

Type of fibre	0 (h) LN (CFU/ml)	1 (h) LN (CFU/ml)	4 (h) LN (CFU/ml)	24 (h) LN (CFU/ml)	48 (h) LN (CFU/ml)
Control	21.717	23.477	28.931	37.696	49.495
Optimised Psyllginate 0.05% AgCO3	21.717	21.344	20.395	0	0
Acticoat AA	21.717	20.880	18.603	0	0
Aquacel Ag	21.717	20.069	0	0	0

Table 5
Antimicrobial activity results of optimised Psyllginate Ag, Aquacel Ag and Acticoat AA against *E. coli*.

Type of fibre	0 (h) LN (CFU/ml)	1 (h) LN (CFU/ml)	4 (h) LN (CFU/ml)	24 (h) LN (CFU/ml)	48 (h) LN (CFU/ml)
Control	21.271	23.689	28.446	39.569	53.281
Optimised Psyllginate 0.05% AgCO3	21.271	20.913	0	0	0
Acticoat AA	21.271	20.743	0	0	0
Aquacel Ag	21.271	20.549	0	0	0

Table 6
Antimicrobial activity results of optimised Psyllginate Ag, Aquacel Ag and Acticoat AA against MRSA 16.

Type of fibre	0 (h) LN (CFU/ml)	1 (h) LN (CFU/ml)	4 (h) LN (CFU/ml)	24 (h) LN (CFU/ml)	48 (h) LN (CFU/ml)
Control	22.443	26.094	30.736	42.504	53.872
Optimised Psyllginate 0.05% AgCO3	22.443	22.066	21.709	0	0
Acticoat AA	22.443	22.089	21.218	0	0
Aquacel Ag	22.443	21.663	20.595	0	0

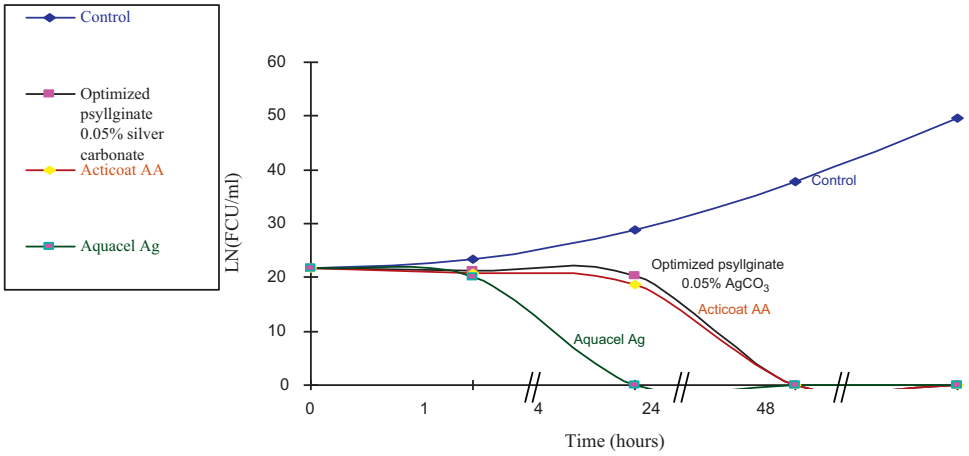


Fig. 2. Antibacterial activities of test dressing fibres against *S. aureus* (values are in log number CFU/ml).

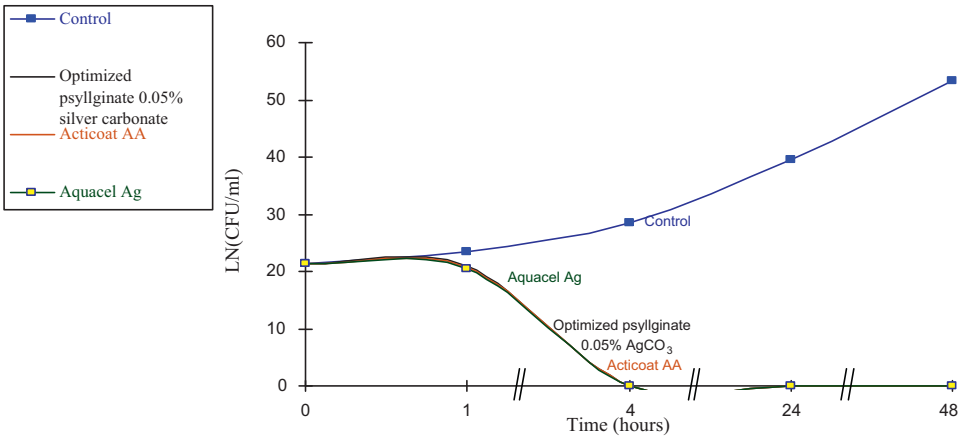


Fig. 3. Antimicrobial activity of test dressing fibres against *E. coli* (values are log number of CFU/ml).

dilution of overnight grown cultures were used and bacterial activity of test fibres were recorded by reduction of CFU/ml over time. A control sample with no fibres was also used to compare antibacterial activities. Plate counts were performed at 1 h, 4 h, 24 h and 48 h of inoculation and incubation at 37 °C. Results are presented in Tables 4–6 and graphical representations are shown in Figs. 2–4.

For Gram positive bacterium; *S. aureus*, MRSA 16 and Gram negative bacterium *E. coli*, test dressing fibres showed no significant difference in antibacterial activity over 24 h and 48 h of inoculation. Complete reduction in CFU/ml of these bacterial strains was achieved with optimised Psyllginate Ag, Aquacel Ag and Acticoat AA after 24 h and 48 h of inoculation. The bacterial suspension study results signify that optimised Psyllginate Ag is as effective as Aquacel Ag and Acticoat against Gram positive bacterium *S. aureus*, MRSA and *E. coli*, even though, optimised Psyllginate has very low quantities of silver in comparison to the commercially available silver loaded dressing fibres.

Small and sustained release of silver ions from fibre composition containing silver into the wound environment is very important for consistent antimicrobial activity. Release of excessive silver into wound environment normally results in the formation of silver chloride. Precipitation of silver chloride into wounds environment is thought to be undesirable as it may lead to irritation, cyto-toxicity and possible skin staining. Poon & Burd demonstrated that silver has toxic effect on keratinocytes and fibroblasts and can adversely affect wound healing (Margaret, Lui, Poon, Lung, & Burd, 2006; Poon & Burd, 2004). Acticoat AA contains the highest amount of silver and releases relatively high quantities of the silver particles. Smith & Nephew claim that their Acticoat AA has stable silver release characteristics for up to 48 h, clearly this not has been found in this study (Dunn & Edwards-Jones, 2004). Aquacel Ag (Convatec) contains lower quantities of silver than Acticoat AA but it is also claimed to deliver and maintain a steady delivery of silver (Bowler et al., 2004).

Silver contents and silver release analysis in this work have shown that both commercial dressings generate high concentration of silver in liquid media very quickly. This in turn would lead to generation of silver chloride and cause possible skin rash. The optimised Psyllginate Ag, on the other hand, has shown to contain very low amount of silver with a very low and steady release of silver particles whilst being as effective against all the tested bacteria.

3.4.2. Zone of inhibition

Zone of inhibition is qualitative method used to determine the diffusion of antimicrobial agent from its carrier into the appropriate growth environment required for the test microorganisms. However the diffusion rate of the antimicrobial agent is directly related to its chemical structure, composition and concentration. The area formed without microbial growth around the test sample is generated by the antimicrobial substance diffused from the sample which is called the zone of inhibition wherein effect of the antimicrobial agent ceases the bacterial growth.

Test fibres were matted and placed on agar plates that were inoculated with *S. aureus*, *E. coli* and, MRSA16. Bacterial growths were determined visually after 24 h of incubation at 37 °C. Antimicrobial activity was demonstrated by zone of inhibition on and around the matted test fibres. The test results are shown in Fig. 5 (*S. aureus*), Fig. 6 (*E. coli*), and Fig. 7 (MRSA 16).

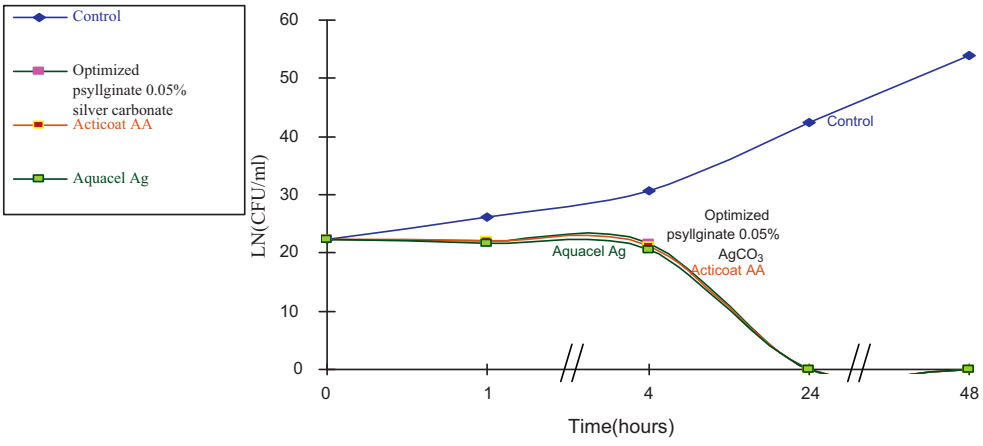


Fig. 4. Bacterial activities of test dressing fibres against MRSA16 (values are log numbers of CFU/ml).

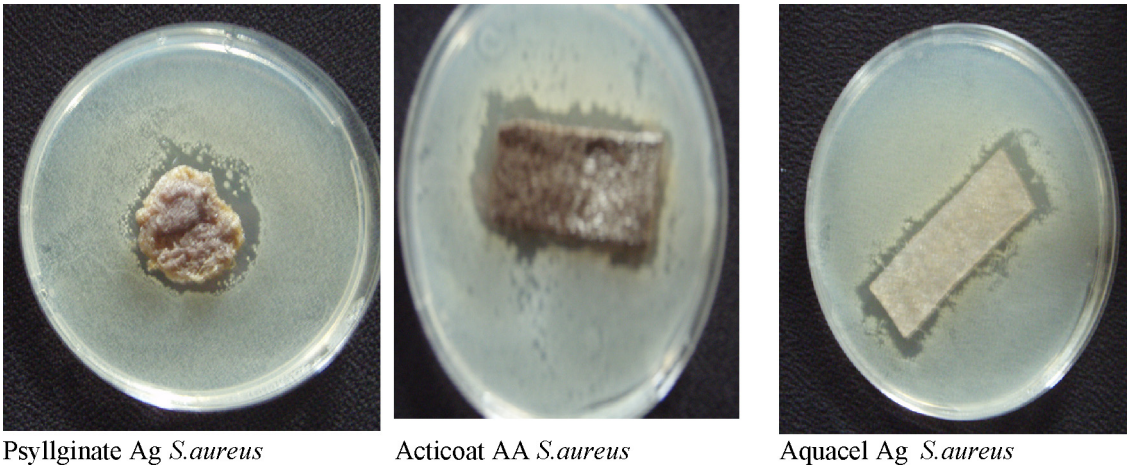


Fig. 5. Appearances of inhibitory zone with *S. aureus*.

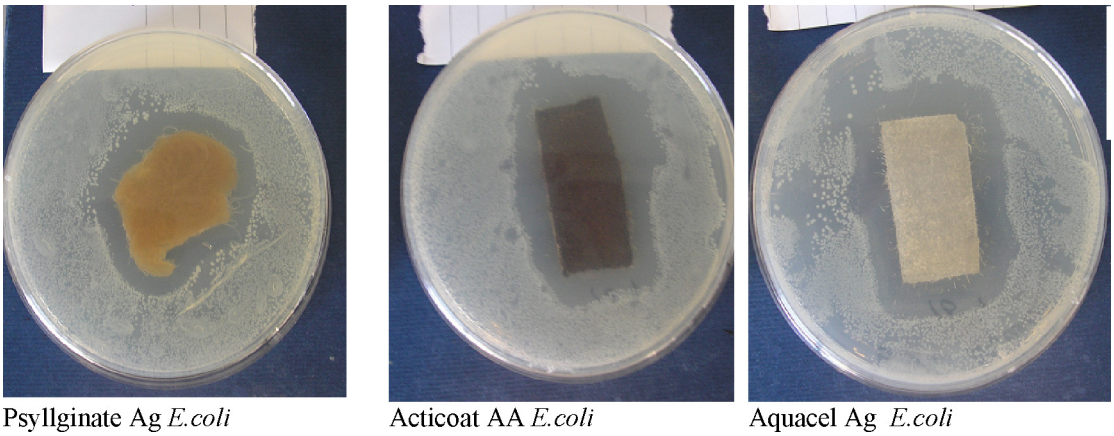


Fig. 6. Appearances of inhibitory zone with *E. coli*.

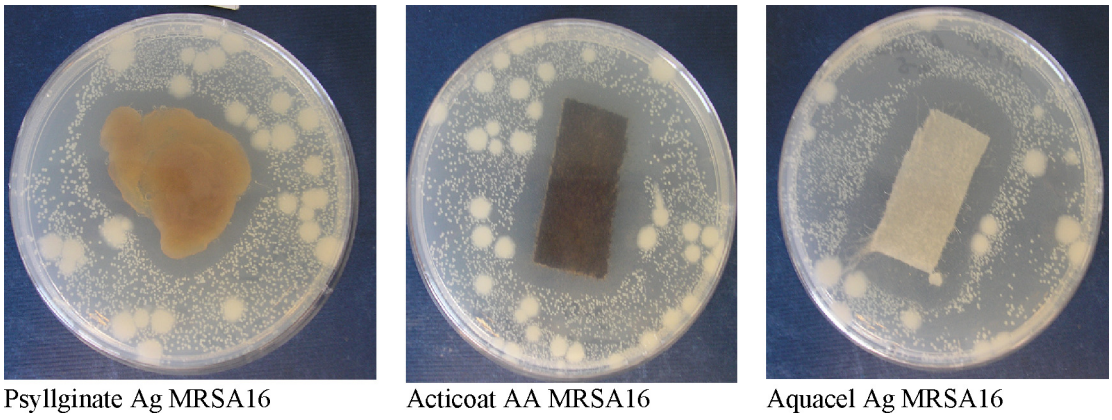


Fig. 7. Appearances of inhibitory zone with MRSA 16.

The results of antibacterial sensitivity in Figs. 5–7 show that Aquacel Ag, Acticoat AA and the optimised Psyllginate Ag have all clear zones of inhibition against Gram negative *E. coli*, *S. aureus* and MRSA16. The clear zone of inhibition occurred as a result of silver release from the test fibres/dressings. These are clear visual and qualitative proofs that Psyllginate Ag is certainly a fibre with serious and unavoidable advantages that must be considered as a serious contender in the wound dressing arena.

4. Conclusion

In this study, a new hybrid fibre has been developed from natural polymers with inbuilt ability to gel and absorb large quantities of pseudo exudates. Exudate absorption capacity of the developed biomaterial fibre is at least 10% more than carboxymethyl cellulose (Aquacel), known as the gold standard in the wound dressing world. It also contains up to six times less silver than the

conventional silver dressings i.e. 7.39 mg/l as opposed to 30.6 mg/l and 65.68 mg/l for Aquacel Ag and Acticoat AA respectively. Furthermore, the developed fibre also displays very slow rate of silver release whilst maintaining full potency over time against known micro-organisms including MRSA. Silver is a premium material and therefore has a high cost value. Excessive use of silver not only increases manufacturing and hence retailing costs but can also initiate unnecessary complications in the wound healing process. The Psyllginate Ag developed in this work is much cheaper to produce and is highly effective against Gram positive and Gram negative bacteria with unlikely danger of causing discoloration, damage, allergic reaction or staining based on its very low silver content. The study concludes that the developed hybrid fibre has long lasting antimicrobial and gelling properties that are comparable, if not better, than Acticoat AA and Aquacel Ag, two commercially available silver dressings and therefore a serious rival.

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