Effect of *Solanum tuberosum* on Muslin-Gauze Composite for Healthcare Applications

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Abstract

The research work is aimed to develop an alternative technique for immediate blood coagulation using natural starch extract (*Solanum tuberosum*) and to promote haemostasis in medical application. The natural starch exhibits significant anti-bacterial activity and blood coagulation ability by subjecting it to standard test methods. From the results, it was found that the natural starch coated on the muslin-gauze cloth coagulates blood and stops bleeding at a faster rate than the product made out of base human (or) cow plasma and kaolin-coated gauze cloth. Hence, this research work shows a commercial viability and potentiality of the developed product for healthcare applications.

**Introduction**

Haemostatic agents have been gaining popularity for use in emergency traumatic life threatening bleeding control [1,2]. Anti-haemorrhagic agents used in medicine have various mechanisms of action. 1) Systemic drugs work by promoting coagulation 2) Locally acting haemostatic agents work by promoting platelet aggregation. They are available in two forms, as a granular powder poured on wounds, or embedded in a dressing [3]. Micro Fibrillar Collagen Haemostat (MCH) attracts platelets and allows for the formation of a blood clot when it comes into contact with blood. Chitosan haemostat (chitosan and its salts) bonds with platelets and red blood cells to form a gel like clot which seals a bleeding vessel [4,5].

The haemostatic products are available to meet variety of medical applications at defense, accidents and surgical procedure and it is mainly used to improve clotting times and provide infection-resistance without side effects [6]. Though these products assists in achieving homeostasis, they have complicated preparation methods, after application they may turn exothermic on contact to the skin, causing side effects [7,8]. The inert minerals used in certain hemostat powders are quite expensive though they are effective. The potato tuber is mainly used to improve clotting times and provide infection-resistance without side effects [6]. Though these products assists in achieving homeostasis, they have complicated preparation methods, after application they may turn exothermic on contact to the skin, causing side effects [7,8].

During the formation of the tubers, starch granules were formed in the tuber cells. Several researches have used plasma from the human and animals for haemostasis and proved the possibilities of allergic reactions. Considering all these demerits, the research work is aimed to encapsulate natural starch granules in non-woven gauze cloth enabling to act as non-chemical, non-allergic, less expensive haemostatic product [9,10].

**Materials and Methods**

**Materials**

- Cotton gauze & muslin-cloth sourced from M/s Ramraju surgical cotton mills.
- Starch-extracted from *Solanum tuberosum* (potato starch)
- Triclosan-an anti-bacterial agent.

The muslin-cotton gauze composite is prepared as shown in figure 1. The starch extracted from *Solanum tuberosum* is applied on the muslin-gauze cloth using pad-dry-cure method. The starch along with triclosan (anti-bacterial agent) was applied to the muslin-gauze cloth and the cloth was soaked containing 2% *Solanum tuberosum* and 3% triclosan agent at 50°C for 15 minutes with MLR 1:20 dried at 90°C for 5 minutes and cured at 120°C for 3 minutes.

**Methods**

1. Development of muslin-gauze fabric
2. Extraction of starch from *Solanum tuberosum*
3. Application of starch over fabric
   - Sample preparation
   - Testing of samples
   - Clinical trials

**Synthesis of starch**

*Extraction of potato starch*: The starch from *Solanum tuberosum* was extracted through “horizontal spiral centrifugal machine” using the following procedure as explained in figure 2.

During the formation of the tubers, starch granules were formed in the tuber cells. These starch granules are sized between 10 µm and 100 µm. Powdered materials generally have very higher surface area and hence they can absorb more fluids as shown in figure 3.

The resizing is done, so that these granules can be applied on the...
fabric evenly without any uneven distribution. This is to ensure that all the area of the potato starch coated fabric provides an equal effect without smudging as shown in figure 4.

Characterisation of starch: Potato starch was analyzed by FTIR spectroscopy method as shown in figure 5. The results infer the presence of hydroxyl group in its molecular structure. The rate of absorbency on to the fabric specimen was also determined using absorbency test.

Testing

SEM analysis

The treated and untreated samples of muslin-gauze fabric were examined under scanning electron microscope and the images were compared. From the above SEM images, the extent of deposition of the starch extracted from *Solanum tuberosum* (potato) on the muslin-gauze cloth was clearly visualised. The images were captured for every 100 µm, 50 µm, 10 µm and 5 µm respectively. The difference between the treated and untreated gauze-muslin cloth has been clearly seen.

Anti-microbial test

The anti-microbial activity of the samples was evaluated quantitatively. The shake flask method, a standard test method, was used to measure the reduction rate in number of bacterial colonies formed and provided our quantitative data. *Staphylococcus aureus*, AATCC 6358, a gram positive bacterium, was the testing bacterium. In this procedure, +0.1 g sample was dipped into a test tube containing *Staphylococcus aureus* culture solution in which the bacteria concentration was 1.5-3.0*10^4/ml. The test tube was shaken at 35°C for 1 hour on a rotary shaker at 100 rpm, and 1:100 dilutions of the test solution were made. One millimeter of the dilute test solution was poured onto TGE agar broth, and when this had been incubated at 35°C for 24 hours, the number of bacterial colonies in the agar broth was counted. The reduction rate in the number of bacterial colonies was calculated using the following equation counted.
Reduction rate in number of colonies (%) = (A-B)/A * 100

Where A=number of bacterial colonies before shaking and B= number of bacterial colonies after 1 hour shaking.

Absorbency test

AATCC MM TS-04: Absorbent Capacity–Absorbency Testing System (ATS) (standard absorbency test) measures the water absorbency, the rate at which the water was absorbed, and the direction of absorption was determined with an infrared sensor for analysing the maximum absorbent capacity of the fabric.

Clotting time test

The test was also referred to as haemostatic test. Testing using human blood samples is a difficult criteria. Blood from animals can be used for testing the clotting time. In order for blood to clot, the enzyme thrombin must be generated from the plasma precursor prothrombin. Thrombin then converts soluble fibrinogen into insoluble fibrin. Generation of thrombin involves the sequential activation of a number of other plasma clotting factor, this process is also being assisted by Ca++ and by factors released by platelets and damaged tissues. The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), clotting time will be prolonged. The expected range for clotting time is 4-10 minutes.

- Blood sample of the domestic animal species for which coagulation values have been reported, the sheep most closely resembles those of humans.
- Test sample: Blood sample from Ovis Aries
- Sample details: Fresh blood sample drawn from domestic sheep. Three sheep's were involved in the experiment.
- Method used: Lee and white method

Under aseptic precaution venipuncture was done and one ml. of blood is collected in each 3 small clean test tubes. Time was noted with a stopwatch as soon as the blood was taken. The test tubes are kept in a water bath maintained at 37°C. After two minutes, the test tubes were examined every 15 seconds, by gently tilting them. Clotting time was noted when the tube can be completely inverted without spilling the blood. Average value of the results in the 3 test tubes gives the clotting time.

Normal CT value: 2 to 7 minutes.

Results and Discussion

The anti-bacterial, absorbency and blood clotting properties of potato starch coated muslin-gauze fabric were superior to that of unfinished cotton gauze used for medical purposes. The samples were tested accordingly and the results were discussed. The detailed results and discussions are as follows:

Effect of anti-bacterial activity on starch coated samples

The mean ratings for anti-bacterial activity for each untreated (control) & treated sample stored for different time periods about 2, 4, 6 & 8 hrs respectively were compared. Differences in these ratings were apparent and strongly influenced by the concentration of potato starch. The anti-bacterial properties of the potato starch coated samples were analyzed as per the standards and it was found to be significantly good. The samples showed 100% bacterial reduction compared to the untreated one which showed only 75% bacterial reduction as shown in figure 6.

Effect of absorbency test on treated and untreated potato starch coated samples

Absorbency test was conducted for untreated and treated samples to find out the efficiency of the sample towards water absorbency rate. There was 1.972% (approx 2%) increase in absorbent capacity of the processed sample (muslin-gauze treated with potato starch) compared to the control sample.

Effect of blood clotting time on starch coated samples

Effect of blood clotting time on the natural starch coated fabric:

From the table 1, the standard clotting time for blood in kaolin-coated gauze cloth was approximately 170-190 seconds. The normal clotting time for blood is found to be around approximately 150-180 seconds without coating any material on the cloth. But in a processed sample (a sample that is coated with potato starch), the clotting time was observed to be 90-120 seconds. This rate of clotting was very quick than other normal samples used for clotting blood. Thus, from the standard test method, it was proved that the potato starch coated fabric clots blood much faster than the product made out of base human (or) cow plasma and kaolin-coated gauze cloth.

Effect of SEM on treated& untreated fabric

The effect of treated and untreated SEM photographs was represented in figure 7 and 8 respectively. It provides the clear evidence of surface etching caused by the Solanum tuberosum.

Conclusion

From the research work, it was inferred that the starch extracted from Solanum tuberosum (potatoes) provide better haemostasis than any
other natural and synthetic agents used for clotting blood. The project was aimed in the development of a natural, quicker and non-allergic agent that can be coated on muslin-gauze fabric used for clotting blood and reduces the rate of blood transfusions. The sample’s anti-microbial activity and the rate of absorbency of the soaked muslin-gauze cloth were found to be extremely good due to the treatment with triclosan. The sample was tested for its ability to clot blood using the clotting time test method. Thus, a novel procoagulant dressing was developed for coagulation of blood showed good results than the normal agents used in medical field.

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