

Development of an *in vitro* model to evaluate the potential for adherence of wound healing dressings

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Introduction

Adherence of wound dressing material to the wound bed has previously been reported, *in-vivo*, with fibrous dressings (Bell & Hart, 2007). Fibres may remain in the wound bed that could negatively affect the inflammatory response, as demonstrated *in-vivo* by giant cell reactions. Adherence of wound dressings can occur in clinical practice, possibly as a consequence of changing wound conditions' as a wound infection is resolved or healing progresses.

Methods

A unique quantitative *in-vitro* model has been developed, based upon the adherence of a wound dressing to a fibrin clot. The clot provides an adherent, biological matrix simulating the wound bed surface and is applied between two pieces of dressing. The force required to remove the dressing material from a fibrin clot is measured.

A design of experiments approach was employed to investigate all interactions between variables. A number of fibrous (mainly alginate) wound dressings were assessed in conjunction with non-adherent wound dressings, including a non-adherent antimicrobial dressing.

Results

The model was optimized for clot size, fibrin and protein concentration and adhesion conditions. The maximum force measured, gf, to separate the dressing material from the clots for the non-adherent wound dressings was 160gf. The force required to separate the fibrous dressings from the clots was significantly higher, ranging between 384 - 910gf.

A linear relationship existed between the adherence value for the dressing and the percent open area, dependent on the diameter of the hole in the non-adherent layer.

Conclusion

Decreasing the potential adherence of dressings and limiting the number of fibres that remain at the wound surface, may positively influence the wound healing process. This model distinguishes between non-adherent wound dressings and dressings that have a potential to adhere to a wound bed.

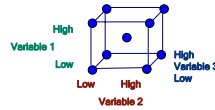
This *in-vitro* model has the potential to be used as a replacement to current *in-vitro* models.

Test Method Optimization

Design of Experiment (DoE) was used to optimize the variables of the method.

DoE principles

An approach for effectively and efficiently exploring the cause-and-effect relationship between numerous variables (Xs) and the output or process performance variable (Y)



Test variables: Fibrin Clot size, Sample size, Sample repetition, Thrombin concentration, Drying duration.

Method

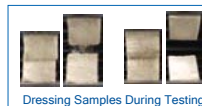
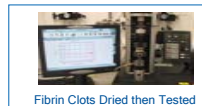
Fibrin clots were formed in circular polystyrene pots, combining fibrinogen (3mg/ml) and Thrombin (in excess) in Phosphate Buffered Saline (PBS) containing 10mg/ml Bovine Serum Albumin (BSA) at room temperature.

The reaction mixture was incubated for 1 hour at 37°C and then cooled for 1 hour allowing time for the fibrin clot to form and set.

A clot was positioned between the dressing materials, weight applied and further incubated at 37°C.



The maximum force to remove the dressing material from the clot was measured with an Instron Tensile Tester.



Test method validation

The method was validated using different dressing types and different operators, demonstrating the method to be both reproducible (between operator) and repeatable (within operator). Three dressing materials were evaluated, a dressing with a non-adherent wound contact layer and two fibrous type wound dressings. Five samples per dressing material were evaluated on three separate occasions by both operators. In total, three different batches of each dressing material were assessed.

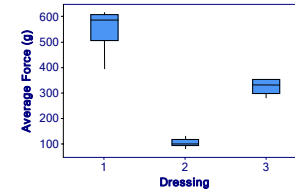
Summary of statistical analysis

Sample	p=
Operator	0.003
Sample and operator interaction	0.128
	0.709

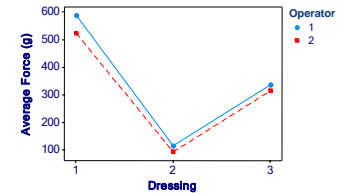
Two-way ANOVA results summary

Repeatability and Reproducibility assessed in total Gage R&R analysis.	
Total Gage R&R	22.68%
Acceptance criteria	<30%

Method Distinguishes Between Dressing Materials



Operator to Operator Reproducibility



Objective

To develop an *in-vitro* test model, and validate its use for evaluation and comparison of the potential adherence of fibrous based (mainly alginate) wound dressings.

Method Development

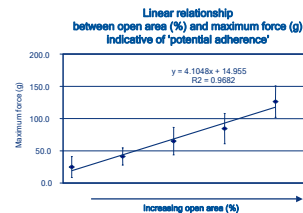
The principle of this method is based on the production of fibrin, a building block for the haemostatic plug, the end product of the coagulation cascade. Fibrin is produced by the cleavage of fibrinogen by thrombin. The fibrin polymers; in combination with albumin, form a fibrin clot providing a biological matrix.



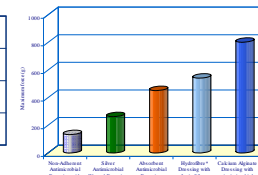
This matrix provides the necessary medium for cells and other matrix proteins to aggregate and provide a haemostatic plug for blood vessel repair and scab formation on the surface of the wound.

Effect of open area on potential adherence

The addition of perforated film to a fibrous (alginate/CMC) material provides the dressing with a non-adherent wound contact layer. The number and size of perforations were assessed covering a range of open areas (%). A linear relationship was observed between open area and maximum force to separate the dressings from the clot. A reduction in the maximum force is seen by adding the film layer compared with other fibrous (mainly alginate) wound dressings.



Comparison of a non-adherent antimicrobial dressing with commercially available wound dressings



The data presented is the average force required to separate the dressing material from a fibrin clot. For each dressing tested, shown from left to right on the graph, the average result was 132g, 263g, 452g, 542g and 802g, respectively.

Conclusion

The fibrin clot *in vitro* model was developed, optimised and validated for use in the assessment of the potential adherence of wound dressing.

The test demonstrated both repeatability and reproducibility. It also demonstrated the ability to distinguish between different dressing materials.

This potential adherence was reduced by the addition of a perforated film as a wound contact layer on the Non-Adherent Antimicrobial Dressing with Silver.

The number and size of holes and the open area of the perforated film layer can affect potential adherence.