Assessing a New *In Vitro* Wound Model as a Pre-Clinical Test for Evaluating the Adherent Properties of Wound Dressings

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INTRODUCTION

During wound healing, the formation of a fibrin clot is a key early step in wound repair, playing a critical role not only in hemostasis but also in providing structural scaffolding for new tissue growth and serving as a barrier against microbes.[1,2,3] Premature disruption of the fibrin clot within a wound can cause the patient significant pain and negatively impact the wound healing process leading to delayed/stalled healing, excessive scar tissue formation, and potential infection.[4] Therefore, it is important that the dressings selected to treat wounds should be designed to resist adhesion to the wound bed.

Currently, pre-clinical assessments of wound dressings with non-adherent/non-stick technologies are conducted using *in vivo*, partial-thickness dermal wound models. In these studies, multiple superficial skin excisions are made on test animals, and the test articles are applied to the wound sites for a predetermined period of time (typically 24 hours) to assess adhesion. The maximum peel force (MPF) required to remove a test article from a wound bed is then measured. A greater MPF indicates stronger adhesion to the wound bed or fibrin clot. Not only are such studies expensive and time consuming, but there has been a recent push by regulatory agencies for the development and adoption of comparable *in vitro* assays due to the ethical issues associated with animal testing.

Driven by the desire to identify alternatives to *in vivo* studies, several different *in vitro* models have been explored within academia, but few have been standardized. In this study, we compared the performance of different non-adherent/non-stick pads using both a new standardized *in vitro* fibrin clot adhesion test and a conventional *in vivo* excisional wound model to assess the ability of this *in vitro* test to replace, or reduce the use of, the *in vivo* model.

MATERIALS & METHODS

Test Articles

The test articles examined in this study consisted of two different types of non-stick pads (positive controls) and a gauze sponge (negative control). Both samples provided were sterile. The only modifications made to the test articles were trimming them to the appropriate size for the respective tests and those samples used in the *in vivo* model were fitted with a suture 5mm from the dorsal side of the dressing to create a small loop for connecting to a digital force device (see Figure 2a).

In Vitro Fibrin Clot Model

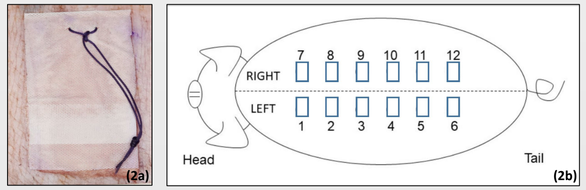
In this model, test articles were evaluated for their ability to resist adhesion to a wound bed, simulated by a laboratory-synthesized fibrin clot, using YY/T 1477.4-2017-Part 4.[5,6] In short, fibrin clots were prepared by mixing fibrinogen with thrombin in PBS containing BSA at room temperature. This mixture was incubated at 37°C for 1h and subsequently cooled to room temperature for 1h to allow the fibrin clot to form and solidify. The clot was carefully removed, positioned between two pieces of the test specimen (5cm x 10cm), a 100g weight was applied on top, and the whole assembly was incubated for 24h at 37°C and 85% relative humidity. Afterward, the portion of the test specimen where the fibrin clot was located was supported, by hand, in a 90° orientation, while the free ends were mounted to a tensile tester via clamps (see Figure 1). The tensile tester measured the maximum peel force exhibited by each test specimen.

Figure 1. Photograph illustrating how the test samples were supported while the tensile tester measured the peel force required to separate the test article from the synthesized fibrin clot.

In Vivo Excisional Wound Model

The non-adherent properties of the test articles were also assessed using a partial-thickness excisional wound model conducted with Yucatan miniature swine. In this animal model, 12x partial-thickness wounds (6 sites/side) were created on each animal in two paraspinal columns between the crest of the shoulders and the ilium (see Figure 2b). A dermatome was used to create the partial-thickness excisional wounds (5mm depth and 2.5cm x 4.0cm in size) and the wounds were appropriately cleaned prior to placing the pre-sutured test articles on the designated wound sites. A standard barrier dressing was applied over each test article and the entire wound area was covered with a foam pad and tear-resistant mesh to prevent dislodgement of the test articles. After a 24-hour exposure period, the animals were anesthetized, barrier dressings removed, and a digital force meter was used to measure the maximum peel force required to remove each dressing.

Figure 2. Photograph of a test sample fitted with a suture for connecting to a digital force meter for measuring the MPF for each dressing (2a) and an illustration of 12 wound site locations made on each test animal used in the *in vivo* excisional wound model study.



RESULTS

Figure 3. Comparison of the average adhesive strength measured between the test articles and laboratory synthesized fibrin clot during testing that utilized the *in vitro* fibrin clot model.

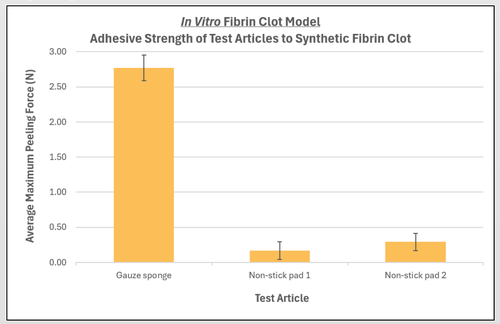


Figure 4. Comparison of the average adhesive strength measured between the test articles and wound sites during testing that utilized the *in vivo* excisional wound model.

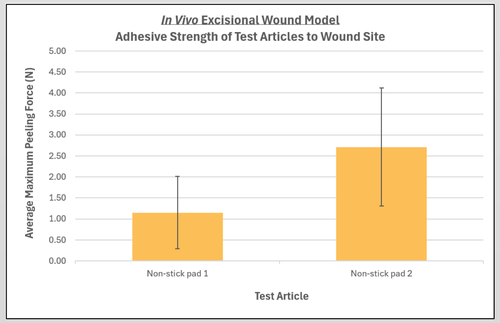


Table 1. Summary of statistical analyses assessing differences in adhesive strength measurements for each test article in both the *in vitro* fibrin clot model and the *in vivo* excisional wound model.

Wound Model	Data Set 1	Data Set 2	Statistically Significant Difference
In Vitro Fibrin Clot Model	Gauze sponge [M = 2.77N; SD = 0.18N]	Non-Stick Pad 1 [M = 0.17N; SD = 0.13N]	Yes [$t(16) = 37.20$, $p < 0.001$]
	Gauze sponge [M = 2.77N; SD = 0.18N]	Non-Stick Pad 2 [M = 0.29N; SD = 0.12N)	Yes [$t(16) = 35.74$, $p < 0.001$]
	Non-Stick Pad 1 [M = 0.17N; SD = 0.13N]	Non-Stick Pad 2 [M = 0.29N; SD = 0.12N]	Yes [$t(18) = -2.26$, $p = 0.036$]
In Vivo Excisional Wound Model	Non-Stick Pad 1 [M = 1.15N; SD = 0.86N)	Non-Stick Pad 2 [M = 2.71N; SD = 1.40N)	Yes [$t(38) = -4.61$, $p < 0.001$]

DISCUSSION

During the *in vitro* fibrin clot tests, a much greater maximum peel force was associated with the gauze sponge (2.77N). This was anticipated because there is nothing to prevent a fibrin clot from becoming imbedded within the gauze sponge during the exposure period resulting in the formation of a clot-gauze composite. The gauze sponge exhibited an average MPF that was 16.3-fold and 9.5-fold greater than was observed from non-stick pad 1 (0.17N) or non-stick pad 2 (0.29N), respectively, which utilize a perforated, non-adherent layer that contacts the wound. The difference in average MPF between the gauze sponge and non-stick pads was shown to be statistically significant (Table 1). Additionally, the *in vitro* fibrin clot model was able to distinguish a significant difference in the clot adhesion properties between the non-stick pads, with non-stick pad 2 exhibiting an average MPF that was 1.7-fold higher than non-stick pad 1.

The *in vivo* excisional wound model also revealed a statistically significant difference in MPF between the non-stick pads, with non-stick pad 2 (2.71N) exhibiting a MPF 2.4-fold greater than non-stick pad 1 (1.15N). Furthermore, the average MPF values for both non-stick pads were approximately 8.1-fold higher in the *in vivo* model when compared to the *in vitro* model. This difference is not unexpected, as the *in vivo* wounds are complex and dynamic environments with a number of different parameters that could impact the adhesion of a material to a wound bed. In contrast, *in vitro* models are designed to focus on the variables most vital to clot-material interactions and improving test-to-test reproducibility. What is notable is that the *in vivo* data was consistently 8.1-fold greater than the data obtained from *in vitro* data, indicating that the *in vitro* fibrin clot model effectively predicts test article performance in an *in vivo* partial-thickness dermal wound model.

CONCLUSIONS

- The *in vitro* fibrin clot wound model effectively discerned and quantified the difference in clot adhesion properties between a standard gauze sponge and non-stick dressings.
- Furthermore, this *in vitro* model was able to recognize the difference in the clot adhesion properties of two different non-stick dressings, a difference which was reaffirmed using a conventional *in vivo* partial-thickness excisional wound model.
- This study demonstrates that the *in vitro* fibrin clot wound model could be an extremely useful surrogate that can be used instead of, or in combination with, the conventional *in vivo* wound model in developing and evaluating different “non-stick” dressing technologies.
- Utilizing this *in vitro* wound model could reduce, or potentially eliminate, problems commonly associated with conducting *in vivo* studies (i.e., high costs, long timeframes, and ethical concerns regarding the use of animals).

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