Final Report for ScarFree Foundation Grant:

Novel Haemostatic Medical Devices for Acute Conflict Wound Treatment

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EXECUTIVE SUMMARY

In the current proposal, we aimed to advance a novel haemostatic material technology to work towards commercialization of it. Our novel haemostatic material strongly repels blood and do not soak in it, thus preventing blood loss when pressed on the wound. Yet, it causes fast clotting to seal the wound, and after clotting, it detaches with extreme ease without re-opening the wound, enabling safe dressing removal. We had three key advancements to enhance our ability to move forward on commercialization. Firstly, we discovered the mechanism by which our novel nanofibrous material could cause fast clotting. We found that this was through the activation of factor XII of the intrinsic pathway, which is similar to how the well-known haemostat, kaolin, achieves fast clotting. This information is important for eventual regulatory approval for our technology. Secondly, we showed that our material had low toxicity in human skin cell cultures and on human skin tissues, again enhancing our ability to achieve regulatory approval. We showed that a large concentration of the nanofibers did not reduce viability of the cells, and did not bring about cell deaths or DNA damage. Thirdly, we established methods to enhance our material by coating additives to them to enhance clotting performance. We achieved this by spray coating kaolin and chitosan particles and a binding polymer, in a manner that did not change its core properties, such as its hydrophobic repellency of blood. Overall, this Scarfree foundation grant enabled us to bring our technology closer to regulatory approval and investor funding.

WORK UNDERTAKEN

We performed work in 3 areas: (A) investigation of the clotting mechanism of our carbon nanofibers, (B) investigation of the toxicity of our carbon nanofibers to keratinocytes, and (C) established techniques for coating additives to the material to enhance clotting performance.

A. INVESTIGATION OF CARBON NANOFIBERS CLOTTING MECAHNISM

The understanding of the exact mechanism by which our nanofibers could elicit fast clotting responses is important because this will be needed for applications for regulatory approval. To achieve this understanding, we tested carbon nanofibers with human plasma, to determine the mechanism by which fast clotting was achieved. By comparing platelet rich plasma (PRP) to platelet poor plasma (PPP), we determined that platelet activation did not play a crucial role in the fast clotting. Next, by adding corn trypsin inhibitors (CTI) to plasma to inhibit the intrinsic pathway, we determined that the intrinsic pathway was the primary mechanism responsible for the clotting. Finally, we tested specifically for factor XII activation by the nanofibers, and determined that this was the mechanism for the fast clotting of our nanofibers.

A.1 Methodology

A.1.1 Superhydrophobic carbon nanofiber coating

To coat cotton woven gauze, CNFs (719811-25G, Sigma Aldrich) and polydimethylsiloxane (PDMS, SYLGARD® 184) were separately dispersed in dichloromethane with QSONICA Q500 Ultrasonicator for 25 min and 10 min. They were mixed and sonicated for 5 min, then sonicated for further 1 min in presence of curing agent. The composite dispersion was spray-coated onto cotton woven gauze (Smith & Nephrew Ptc Ltd). The coated gauze was baked at 80°C for 1 hr. To coat 96-well plate (CytoOne plate flat bottom, Starlabs, UK), CNFs were dispersed in 100% isopropanol with ultrasonicator for 25 min, then added to wells with multichannel for 200 µl per well. The plate was incubated at room temperature in fume hood with lid open for 24 hr. To remove any loosely attached CNFs, the coated gauze and plate were exposed to compressed air from a spray gun for 1 min.

A.1.2. Contact angle and surface morphology

Contact angle (CA) was measured using a custom-built device which consists of adjustable frame, LED backlights and the SWIFTcam microscope camera (SC503-K, SWIFToptical, Texas, US) with an adjustable focus lens. 5 µl of water was pipetted onto a flat sample of CNF-coated gauze for taking images which were then analysed in ImageJ. CA was calculated by averaging triplicate measurements each site (n=3). Surface morphology of the coated materials were characterised by Scanning Electron Microscopy (SEM, Zeiss Auriga CrossBeam). Images were captured at 5kV and 4000× magnification.

A.1.3. Calibrated Automated Thrombogram (CAT) assay

The CAT assay was carried out in 96-well plate which was partially coated by CNF. Both PRP and PPP were prepared using fresh human blood from blood donors and used for assay no longer than 2 hr after blood sampling. Phospholipids (PE: PC: PS= 20 : 60 : 20 in weight) were added to all PPP wells to final concentration of 4.8µM. Tissue factor (TF, final conc. 1 pM) and corn trypsin inhibitor (CTI, Enzyme Research Laboratories, UK, final conc. 65 µg/ml) were selectively added either to activate the extrinsic pathway or to block the intrinsic pathway. The thrombin generation (TG) was induced by adding of $CaCl_2$ (final conc. 16.7 mM) to all wells simultaneously and monitored using a plate reader fluorometer (Fluoroskan Ascent, ThermoLabsystems, Helsinki, Finland) and corresponding software (Thrombogram-Thrombinoscope™ assay; Synapse b.v., Maastricht, The Netherlands) for 90-120 mins. For analysis, lag time and time to peak were shown as 90 min or 120 min if no TG was recorded at the end of the assay.

A.1.4. Chromogenic substrate assay for FaXIIa activity

FaXII activation by CNF was investigated by incubating 120 µl per well of 200 nM FaXII (Enzyme Research Laoboratories, HFX1212, diluted in TBS) in both CNF-coated and uncoated wells in 96-well plate for 0 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 150 min and 180 min at 37 °C. After incubation, 90µl of each samples were mixed with 10µl chromogenic substrate H-D-Pro-Phe-Arg-pNA (Chromogenix, S-2302™, 0.5mM) in an uncoated 96-well plate and immediately measured for absorbance at 405 nm in BioTek ELx808 plate reader at 37 °C for 20 min (1 scan/min). FaXIIa activity was quantified by fitting the initial (linear) slopes of substrate conversion for 20 min to a standard curve of FaXIIa (0-125 nM, Enzyme Research Laboratories, HFX1212a, diluted in TBS)

A.3. Results

A.3.1. Intrinsic Pathway was the Main Mechanism for CNF Fast Clotting

Results of incubating the CAT assay of CNF in human plasma are shown in figure 1 and 2, for platelet poor plasma (PPP) and platelet rich plasma (PRP), respectively. In Figure 1A, we observed that incubation with CNF elicited thrombin formation while incubating with PDMS coating or uncoated wells did not elicit any thrombin generation. The wells with 1pM tissue factor were used as positive control. Compared to this positive control, CNF had an activation lag time that was about 50% longer and a slightly longer time to peak thrombin, but the peak concentration of thrombin generated was about three times higher, and the endogenous thrombin potential (ETP, or the area under the thrombin versus time plot) was about twice as high. This suggested that CNF had a potency to induce fast clotting.

In Figure 1B, corn trypsin inhibitor (CTI) was added to the same experimental setup with PPP, to inhibit factor XII of the intrinsic pathway, to check with intrinsic pathway was the cause of the fast-clotting mechanism. Results show that CTI removed thrombin generation response of PPP in the CNF coated well, to the same level as that of the negative control (uncoated well) and PDMS coated well. This confirms that CNF causes clotting via the intrinsic pathway.

In Figure 2, we repeated the same study with PRP. Here, we had observations as the above. CNF elicited thrombin generation but the PDMS coated wells did not, and negative control wells had a very late thrombin generation response and low thrombin peak. With PRP, CNF had a similar delayed thrombin generation time and thrombin peak time, and an elevated thrombin peak concentration and

ETP, but the peak concentration and ETP were not as highly elevated above the positive control as in the experiments with PPP. With CTI inhibition of the intrinsic pathway, we observed a similar decimation of thrombin generation responses in all wells except for the positive control (tissue factor) wells. Collectively, this again demonstrated that CNF had a good potency to induce fast clotting, and that this was primarily via the intrinsic pathway.

Further, comparing PPP to PRP results, we had very similar observations. This thus suggested that the presence of platelets in the plasma did not play a major role in CNF-induced clotting. However, this does not rule out the possibility that CNF may induce platelet, which is part of our current investigation.

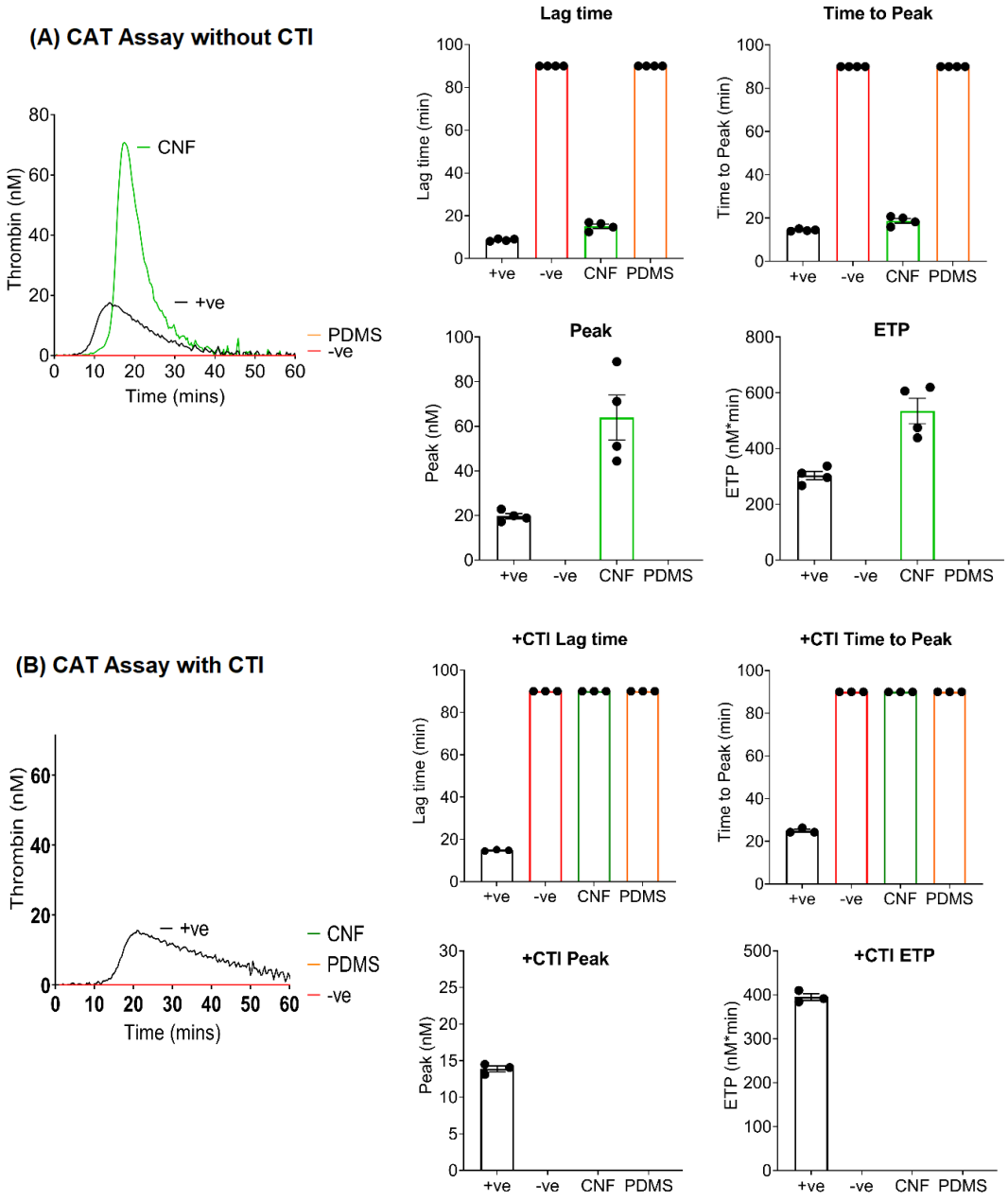


Figure 1. *(A) CAT assay on PPP results, (A) without CTI inhibition factor XII, and (B) with CTI inhibition. +ve: positive control, which was the addition of 1pM of tissue factor; -ve: negative control, which were an uncoated well; PDMS: wells spray-coated with PDMS, which served as a second negative control; CNF: well spray-coated with carbon nanofibers. Results show that CNF activated thrombin generation, but could be inhibited by CTI, demonstrating that intrinsic pathway was the primary*

mechanism.

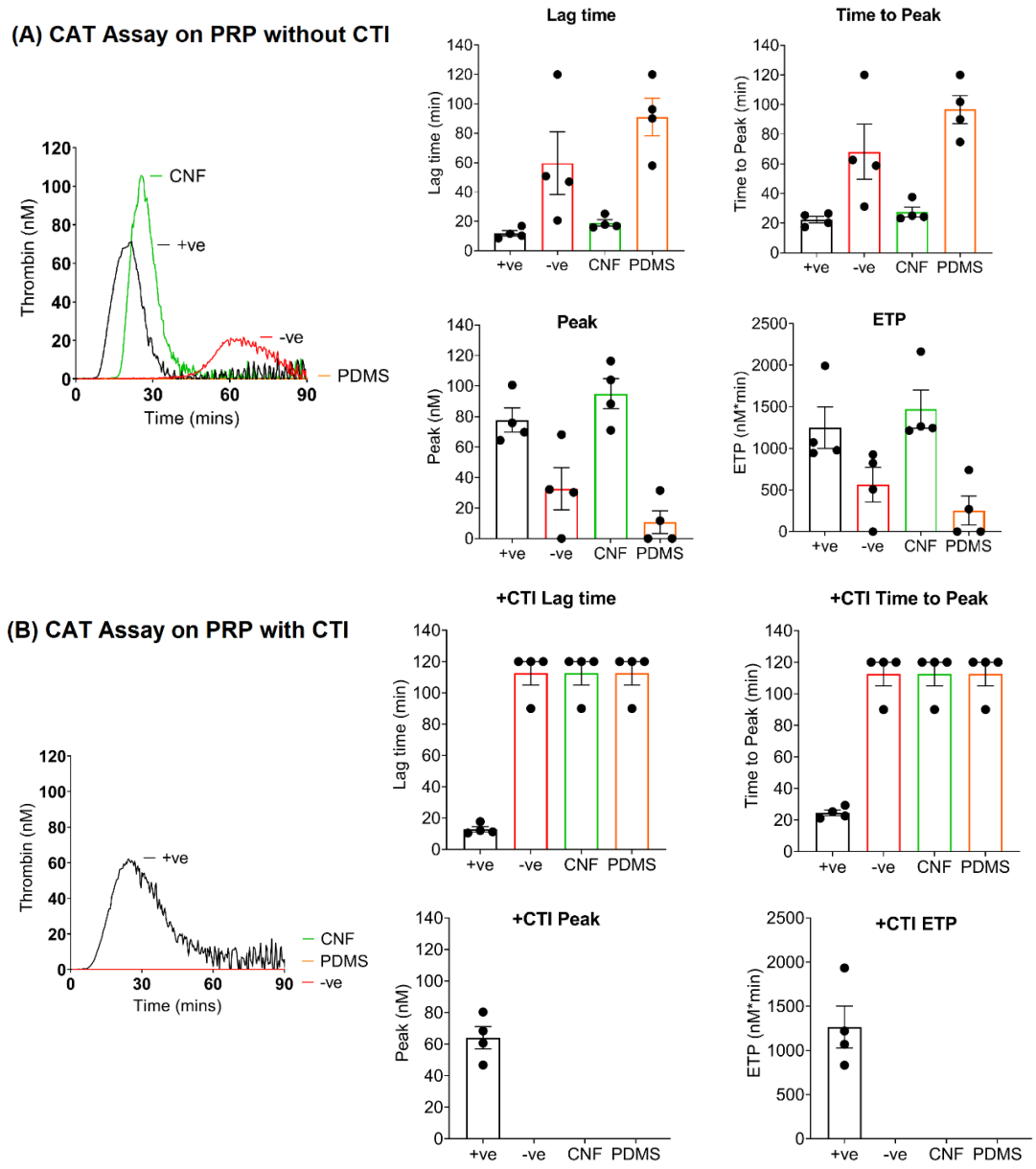


Figure 2. *(A) CAT assay on PRP results, (A) without CTI inhibition factor XII, and (B) with CTI inhibition. +ve: positive control, which was the addition of 1pM of tissue factor; -ve: negative control, which were an uncoated well; PDMS: wells spray-coated with PDMS, which served as a second negative control; CNF: well spray-coated with carbon nanofibers. Results were similar as in Figure 1, showing that CNF activated thrombin generation, but could be inhibited by CTI, demonstrating that intrinsic pathway was the primary mechanism.*

A.3.2. Factor XII activation was the Primary Mechanism for CNF Fast Clotting

Next, we attempted to narrow down which part of the intrinsic pathway was CNF relying on for the fast-clotting mechanism. We tested CNF for its ability to activate factor XII. Results in Figure 3 showed that CNF activated factor XII, much more significantly than the control wells, and wells with CNF but where incubation was stopped very quickly after commencement. We further validated this results with Western blotting (Fig 3C) This confirmed that CNF activated factor XII as the clotting mechanism. Since factor XII was at the top of the intrinsic pathway, there was no further need to investigate other factors in the pathway.

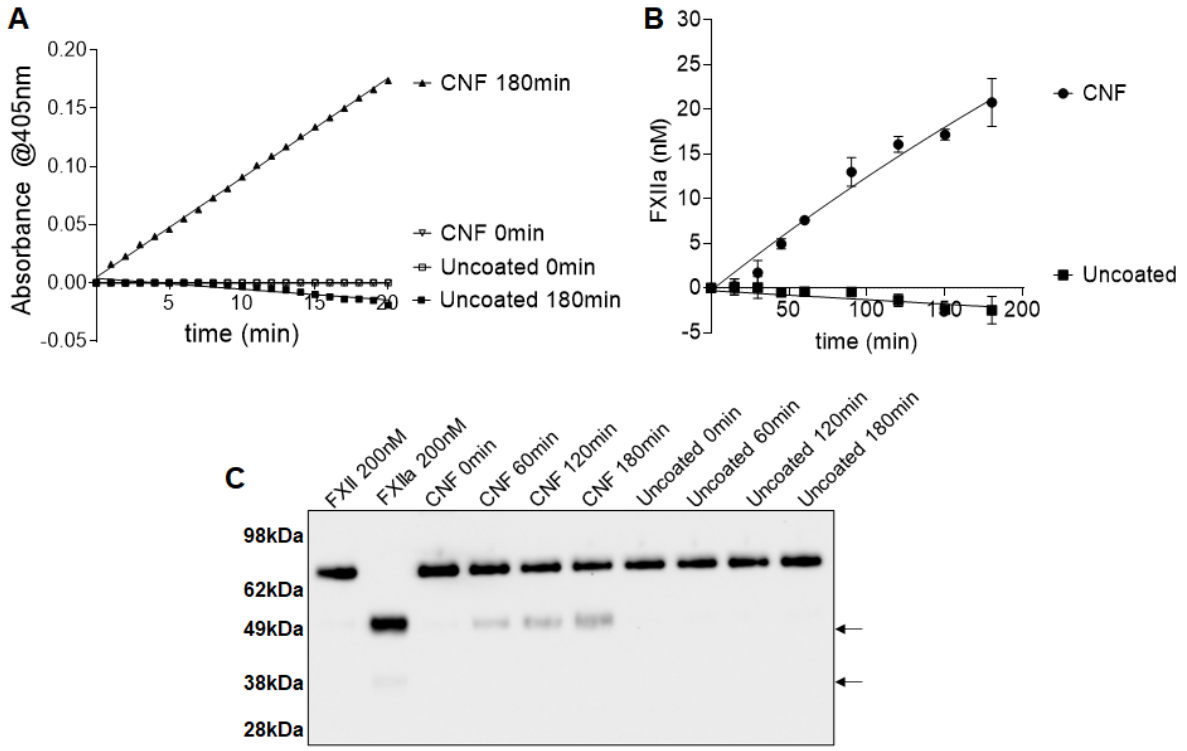


Figure 3. (A) *Chromogenic substrate assay for FaXIIa activity in CNF-coated and uncoated wells in 96-well plate.* ***(B)*** *Quantification of FaXIIa concentration in CNF-coated and uncoated wells. Substrate conversion (linear) was monitored in the assays and fitted to a standard curve of FaXIIa concentration.* ***(C)*** *Western blotting of FaXII at different time points of incubation in CNF-coated and uncoated wells under reducing conditions. The arrows indicates FaXIIa heavy & light chains.*

A.4. Conclusions

Through our work, we determined that our CNF haemostatic material generated thrombin via the intrinsic pathway, by activating factor XII. This was a similar mechanism as how the well-known haemostatic compound, kaolin, causes fast clotting. We determined that platelet activation was not needed for this effect of CNF, but cannot yet rule out that CNF may be activating platelet, although this is likely to be minimal. This result suggests that a strategy to improving our material's clotting responses could be to enhance our haemostatic material so that it can activate platelet as well, so that platelet activation can augment the intrinsic pathway clotting mechanism to produce even faster clotting. We are in the process of developing a means to achieve this strategy.

The current results are important because an understanding of the mechanism for fast clotting is necessary for regulatory approval applications.

B. INVESTIGATION OF TOXICITY ON OUR NANOFIBER HAEMOSTATIC MATERIAL

The demonstration of low toxicity of our haemostatic nanofiber material is important as it will be needed for regulatory approval. To investigate this, we tested our nanofibers in two ways. Firstly, we incubated it with human keratinocyte cells in a culture, and secondly, we incubated a nanofibrous coated cotton gauze on human skin tissue samples. We show that in both experiments, minimal toxicity responses were observed.

B.1. Methodology

B.1.1. Cell viability assay

Keratinocytes were seeded into 96-well plate for 5×10^3 cells per well on a monolayer of 3T3 fibroblasts, previously treated with mitomycin C (4 µg/mL—Sigma Aldrich), at a density of 2.2 to

$3.2x10^4$ cells/well in 100 µl FAD high calcium media with 10% FCS, glutamine, cocktail and insulin. The cells were incubated at 37 °C for 3 days to 60-80% confluence. The cell medium was changed to DMEM Phenol free (Gibco, 31053028) with 1% FCS and glutamine. The cells were incubated at 37 °C for 3 hr. CNFs were dispersed in the same medium using a water bath sonicator for 15min at maximum power, and then added to the wells to achieve a concentration gradient of CNF ranging from 0.01-5,000 µg/ml. Triton X-100 was added to 0.1% v/v in cell media of other wells as positive control. The negative control was cells in normal media. The wells with normal media alone were set as blank. All of them were done in triplicates. After 24hr incubation at 37 °C, all media were replaced with new media containing 10 % v/v Alamar blue (Invitrogen, DAL1100). The plate was covered with foil and incubated at 37 °C for another 3 hr. The plate was read by Fluorometer SpectraMax® iD3 with excitation wavelength at 540nm and emission wavelength at 590nm. The cell viability (%) was calculated as: (Average test or positive control - average blank) / (Average negative control - average blank) × 100%. The assay was carried out for 3 times. The average cell viability and SEM for each group were calculated and presented by GraphPad Prism.

B.1.2. Immunofluorescence staining

One 13mm cover glass (631-0150, VWR) was put into each well in 24-well plate. $1×10^4$ Keratinocytes were seeded into each well on a monolayer of 3T3 fibroblasts ($2-3×10^4$ cells per well). Cells were incubated a humidified incubator at 37 °C with 5% CO2 for 3 days. Staurosporine (positive control for apoptosis, final conc. 0.5µM), Triton X-100 (positive control for necrosis, final conc. 0.1% v/v), CNF (final conc. 200µg/ml and 20µg/ml, dispersed in cell media using a water bath sonicator for 15min at maximum power), DNA double-strand break (DSB) inducer (positive control for DSB, 1:150) were separately added to cell media, leaving wells with nothing added as negative control. All cells were incubated for a further 24 hr or 1 hr (DSB inducer only). Cover glasses with cells attached were washed, fixed and stained according to standard protocols of Annexin V-FITC apoptosis staining/detection kit (ab14085, Abcam) and Gamma H2A.X staining kit (ab242296, Abcam). All cover glasses were also stained by DAPI (1:3000, 62248, Thermo Scientific) for nuclear staining, then mounted on glass slides in mowiol (Calbiochem). Images were acquired randomly with an Olympus Provis BX51 microscope (magnification 60x), a SPOT RT monochrome camera and SimplePCI software (Hamamatsu), and analysed using Image J.

B.1.3. Human ex vivo skin model

Ex vivo human skin tissues (NSA11, NativeSkin access®) with silicone ring and 11-mm diameter were purchased from Genoskin (Toulouse, France). The biopsies were taken from abdominal region of a healthy 47-year-old female donor via surgical procedure. According to the manufacturer, full ethical approval for the study protocol had been obtained from the French ethical research committee, and authorization had been provided by the French Ministry of Research.

The skin tissues were cultured in 12-well plate, with the media provided by manufacturer in a humidified incubator at 37 °C with 5% CO2 upon arrival. After overnight incubation for 18 hr, all skin tissues were punched by a 1.25 inch needle (BD Microlance) at the centre to create wounds. The top surfaces of skin tissues were separately covered with normal cotton gauze (NCG), CNF-coated cotton gauze (CCG) and nothing (Untreated). Sterilised plastic weights were put on top of the gauzes to maintain firm gauze-skin contact. All treatments were made in triplicates. The skin tissues were incubated for 24 hr before being harvested for skin histology, hematoxylin and eosin (H&E) staining and immunohistochemistry.

B.1.4. Skin histology and epidermal skin thickness measurement

All skin tissues were collected and fixed in 4% paraformaldehyde (Sigma-Aldrich) at 4 °C overnight. 5µm-thick paraffin sections of each skin tissue were mounted on glass microslides and stained with H&E according to a standard protocol. Images were taken using a ZOE Fluorescent Cell Imager (Bio-Rad) with Brightfield channel. Three images from each section were randomly photographed for

analysis. Epidermal thickness for each image was measured from the stratum corneum to the beginning of the dermal layer at six different points using Image J. The average epidermal thickness (μm) and SEM for each group were calculated and presented by GraphPad Prism.

B.1.5 Immunohistochemistry (IHC)

Sections of each skin tissue on glass microslides were deparaffinised and rehydrated in a Leica ST5020 Multistainer Bath Array. Antigen retrieval was carried out in citric acid buffer at pH6.0 in a Microwave at high power for 10min. Tissue sections were then blocked in 5% goat normal serum in PBS at room temperature for 1 hr, then separately incubated with 5μg/ml rabbit monoclonal anti-gamma H2A.X (ab81299; Abcam), rabbit IgG isotype and PBS at 4 °C overnight. The tissue sections were incubated with secondary antibody Goat anti-Rabbit HRP (1:250 dilution, P0448, DAKO) at room temperature for 1 hr. Methyl green was used for nuclear counterstain. Three images were randomly photographed using EVOS XL Core Imaging System (Invitrogen) for each tissue section and used for signal quantification in ImageJ. The percentage of dermal gamma H2A.X staining area was calculated by dividing anti-gamma H2A.X antibody stained nuclear area in dermis with the total area of dermis on each image. The average percentage and SEM of each group were calculated and presented by GraphPad Prism.

B.2. Results

Cell viability results are shown in figure 3. Alamar Blue assay showed that up to 5,000 μg/ml of CNF, keratinocytes remained largely viable even after a 24 hours of incubation. 24 hours is much longer than the intended use of our haemostatic device, which is up to 2 hours. Figure 4 shows that representative fluorescent microscopy images, showing that up to 200 μg/ml of CNF did not stain positively for gamma H2A.X, which suggest minimal DNA double-strain break, or low genotoxicity. Figure 4B shows that up to 200 μg/ml of CNF did not elicit apoptosis, as gauged via Annexin-V stains, and did not elicit necrosis, as gauged via propidium iodide stains.

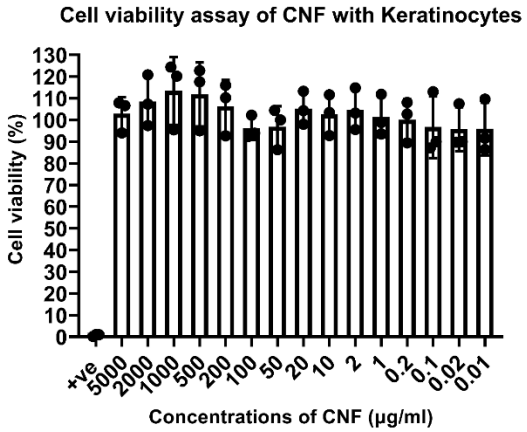


Figure 3. *Cell viability assay of Keratinocytes incubated with different concentrations of CNF in media for 24hrs using alamar blue. The positive control (+ve) contained 0.1% Triton X-100 in media instead of CNF. The negative control was Keratinocytes in normal media. The wells with normal media alone were read as blank. Cell viability was calculated as: (Test or positive control - blank)/(Negative control - blank) × 100%.*

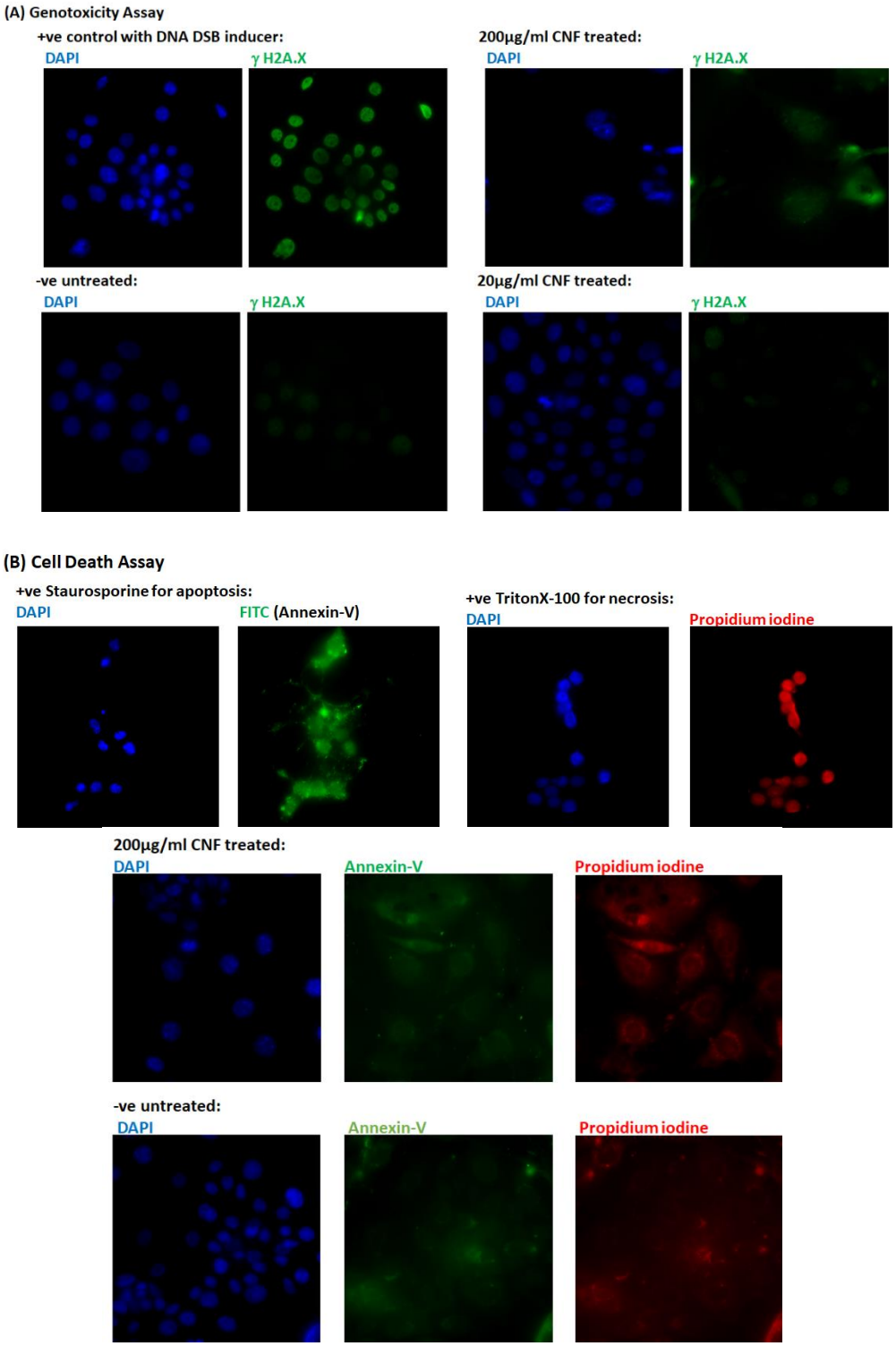


Figure 4. *Cell Toxicity Assay Results. (A) Representative images of keratinocytes treated with DNA double-strand break (DSB) inducer (+ve control) in media, 200µg/ml and 20µg/ml CNF in media and untreated (-ve control) for 24hrs, showing Gamma H2A.X (green) and DAPI nuclear staining (blue). (B) Representative images of keratinocytes treated with 0.5µM Staurosporine (+ve control for apoptosis) in media, 0.1% Triton X-100 (+ve control for necrosis), 200µg/ml CNF in media and untreated (-ve control) for 24hrs, showing Annexin-V (green), Propidium iodine (red) and DAPI nuclear staining (blue). The magnification is 60×.*

We further tested our CNF material on human skin tissue constructs. Skin tissues were punched at the centre and topically treated for 24 hrs with CNF-coated cotton gauze, normal cotton gauze and with no treatment. Results are shown in figure 5. CNF enhances epidermal thickness and maintains dermal stability in commercially purchased human ex vivo skin tissue samples. Immunostaining of skin tissue samples further showed that genotoxicity signals measured from samples covered by CNF-coated and plain gauzes, were not stronger than that from the control untreated samples.

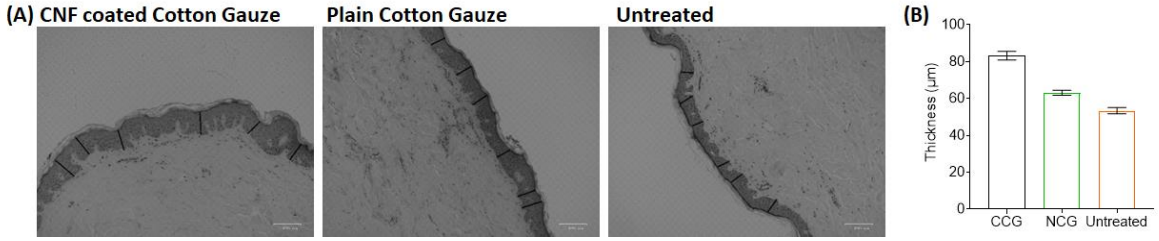


Figure 5. *(A) Representative images of Hematoxylin and Eosin (H&E) stained skin tissue sections with black bars measuring epidermal thickness. White scale bar is 100µm. (B) Quantification of epidermal thickness from three groups of skin tissue sections: CNF coated Cotton Gauze (CCG), Plain Cotton Gauze (NCG) and Untreated. Results are based on triplicate measurements of each group. Horizontal and error bars represent means and SEM.*

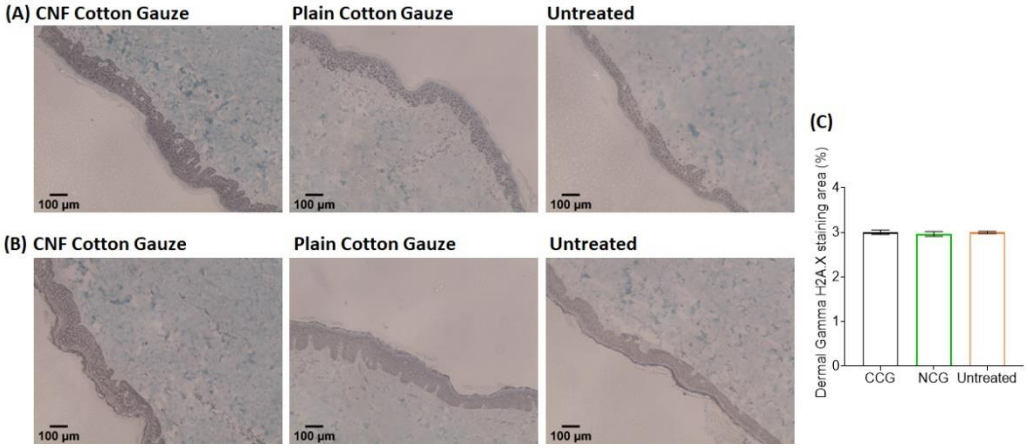


Figure 6. Representative images of skin tissue sections stained with (A) Rabbit anti-Gamma H2A.X (brown), (B) Rabbit IgG isotype (brown), and methyl green (all sections, green). (C) Percentage of Gamma H2A.X stained area in dermis of tissue sections treated by CNF Cotton Gauze (CCG), Plain Cotton Gauze (NCG) and Untreated. Results are based on triplicate measurements.

B.3. Conclusions

Our work here showed that CNF, after 24 hours of incubation with keratinocytes skin cells, did not elicit any significant genotoxicity or cell toxicity responses. This is thus an important result for continued commercialization of our CNF haemostatic material.

C. ESTABLISHING WAYS TO ENHANCE CLOTTING MECHANISM

Our data suggested that there was room to increase the clotting speed of our nanofibrous haemostatic material. We thus investigated ways to adding additional haemostatic compounds to our material. We established a spray coating technique to add Kaolin and Chitosan to our nanofibrous haemostatic gauze, using a binding polymer to hold the compounds to the surface

C.1. Methodology

C.1.1 Fabrication of nanofibrous gauze with Chitosan or Kaolin Additives

A double spraying method was employed to bind these additives, therefore for the initial spraying the

method of fabrication of the original CNF only gauze is used. For the second spraying Instead of CNF, kaolin and chitosan were used. The methodology did not change as the solvent DCM does not disrupt kaolin and chitosan structure. 1mg, 5mg, 10mg, 20mg, 40mg, 80mg, 200mg and 400mg of Sigma Aldrich Chitosan and Kaolin were weighed into a falcon tube on an analytical balance (resolution 0.1mg). 10ml of Dichloromethane was added to the falcon tube and ultrasonicated at an amplitude of 30 for 25 minutes. This was done to dissolve the kaolin or chitosan and to increase its surface area. After ultrasonication, the falcon tube is placed in a cold-water bath to prevent premature cross-linking. Then the PDMS/DCM solution was made as before, then solutions were combined in a similar manner to just the CNF and subsequently airbrushed in a similar manner.

C.1.2 Scanning Electron Microscopy (SEM) Characterization

To explore and compare the surface topography of the gauzes, scanning electron micrographs were taken of the uncoated plain cotton gauze, CNF gauze and gauzes with different concentration of the additives. 1x1cm sections of the gauzes were cut and then all but the 2 uppermost layers were removed and the 2 final layers were stuck to double sided carbon tape. These were then mounted to SEM stubs and spluttered with gold to ensure the additives became conductive. This allowed primary electrons to be released from SEM to provide the electrons of the coatings (CNF only and CNF with additives) on the gauze with sufficient energy to be released as secondary electrons. These secondary electrons are collected and form an image allowing the coating to be seen in the micrographs. Therefore the detector used was an SE2 detector. The micrographs were captured at an electron high tension voltage of 5kV at varying magnifications and working distances using a Zeiss Leo 1525 SEM.

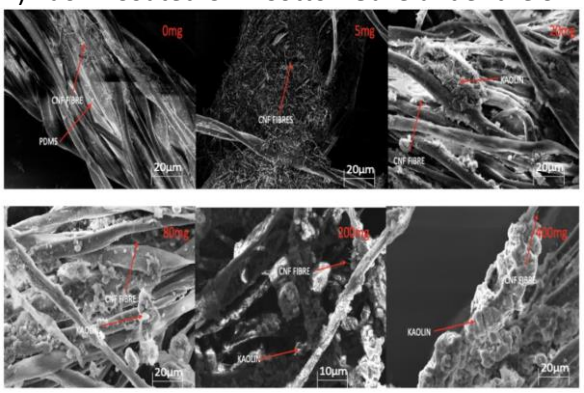
C.1.4. Contact Angle Measurements

To investigate the additives effect of hydrophobicity, contact angle measurements were used to identify wetting angle. The gauze was cut into 1x3cm rectangles , half the layers were removed and it was taped to a microscope slide. Then 10µl of water was pipetted onto this flattened piece of gauze taped to the microscope slide. A lab constructed goniometer with a SWIFT optical SWIFTcam SC503-K microscope camera, adaptable frame, adjustable lenses, and light-emitting diode backlights was used to capture an image of the water droplet. Then the complimentary Swift SC SwiftCam Series Camera Software 3.0 was used to calculate the contact angle. A horizontal line was drawn at the bottom of the droplet and the angle was taken from one end of the horizontal line to the opposite bottom edge of the water droplet, figure 2.2. The contact angles measurements were repeated 3 times on different sites of the same gauze and the averages were calculated using Microsoft Excel.

C.2. Results

We successfully fabricated the gauze with Chitosan and Kaolin additives. In Figure 7 shows that under the SEM, we could observe the CNF as fibrous-like structures, and additional blobs of material with increasing concentrations of kaolin and chitosan. We further tested these additions for their hydrophobicity, since kaolin and chitosan are hydrophilic compounds. We found that at these amounts of additives, the contact angle of the gauze is not significantly altered (Figure 8). We thus have a strategy of introducing additive to modulate clotting performance without compromising the core nature of our hydrophobic haemostatic material.

(A) Kaolin Coated CNF-Cotton Guze under the SEM



(B) Chitosan Coated CNF-Cotton Guze under the SEM

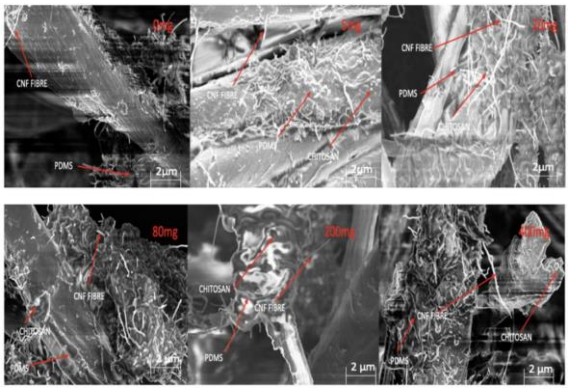


Figure 7. *SEM microscopy images of CNF-cotton gauzes with a second spraying to introduce haemostatic additives: (A) kaolin, and (B) chitosan. With increasing amounts of these additives added, additional blob like surface microstructures were observed to be attached on the surface, which are likely the additives.*

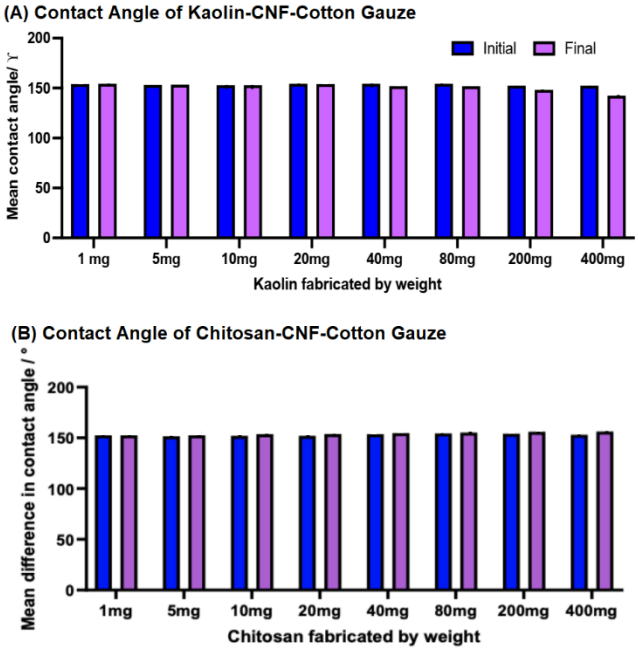


Figure 8. *The contact angle of the CNF-cotton gauze before and after the second spray-coating to add haemostatic additives to the gauze, for (A) kaolin additives, and (B) chitosan additives.*

C.3. Conclusions

We devised a method for adding haemostatic additives to our CNF-cotton gauze surface, to

enhance clotting mechanism. Haemostatic additives are hydrophilic, but we find that if coated in moderate amounts, the hydrophobicity of the gauze is not significantly altered. This should allow us to retain the core properties and performance of our CNF haemostatic gauze, but provide us with a strategy to modulate clotting performance. We have not yet tested these constructs for clotting performance, and are in the process of doing so.

D. CENTRE OUTPUTS

D.1. List of publications and presentations planned or produced:

With the data gathered from the current grant, we are planning to write a manuscript for publication, on the factor XII mechanism of CNF-induced fast clotting, and on the cell and tissue toxicity data.

D.2. Intellectual property arising

No intellectual property arose from the grant work.

D.3. Use of results to other funding bodies or for collaborations

We are currently in the process of applying for further funding, and the data collected from this grant work are key data we use in these further funding applications. We have attempted a Rosetrees Grant, and will be attempting an EPSRC grant next.

D.4. Year by year list of funding leveraged due to Scar Free Foundation Funding

We have not yet been able to secure further funding for this research

D.5. Impact

We have achieved excellent data to demonstrate the mechanism by which our CNF nanofibrous haemostatic material causes fast clotting. Further, we have shown that the material used are not toxic to skin cells and tissue, adding to our current data of preliminary animal studies showing no adverse skin reactions after 24 hours of patching. These data are very valuable, as they will be essential inputs that are needed for regulatory approvals for our haemostatic device. We believe that we are much closer to being able to commercialization with these data gathered. We have also been able to establish a new strategy of enhancing our material, by placing additives on it that can speed up clotting. Overall, our impact is to push our new haemostatic technology closer towards commercialization, and to de-risk it to make investor funding more likely.

D.6. List of Centre academic staff

The research work in this grant were conducted by
(1) Postdoctoral Research Associate, Dr. Yaoxian Xu,
(2) Senior Lecturer, Dr. Choon Hwai Yap,
(3) Lecturer, Dr. Josefin Ahnstrom,
(4) Reader, Dr. Vania Braga,
(5) PhD student, Leticia de Souza, and
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