Superior performance of cellulose nanofibers as a topical haemostat

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Introduction:

- Topical haemostatic agents play a significant role in managing bleeding in the first hours post-injury and in reducing the associated mortality in surgical and trauma settings.
- Oxidized regenerated cellulose (Surgicel®) is one of the most commonly-used absorbable cellulose-based haemostats, and functions by decreasing the pH of its surrounds. This results in red cell lysis and haemoglobin oxidation. The acidic nature also damages nearby cells, increases inflammation in the surrounding tissues and delays wound healing [1].
- We previously synthesized non-oxidized cellulose nanofibers using a ball-milling method [2], demonstrating a direct relationship between the morphology of nanofibers and their pro-coagulant properties. The morphologically-optimized non-oxidized cellulose nanofibers induced rapid and robust clotting through activation of plasma coagulation and platelet entrapment [3].
- **In this project, we aimed to test the haemostatic efficacy, biocompatibility and degradability of these non-oxidized cellulose nanofibers (CNFs) *in vitro* and *in vivo*.**

Materials and Methods:

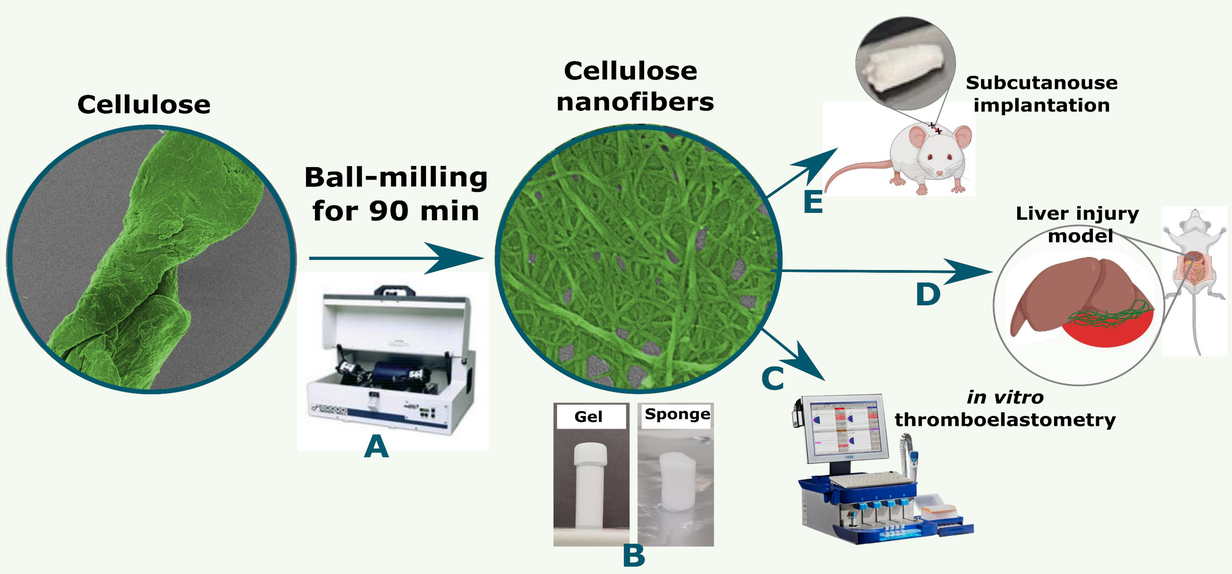

Figure 1. Schematic image demonstrating **(A)** CNFs synthesis through ball-milling and **(B)** CNFs preparation in two forms: sponge and gel, and assessment of their haemostatic efficacy and biocompatibility using **(C)** *in vitro* non-activated rotational thromboelastometry, **(D)** *in vivo* liver injury model, and **(E)** *in vivo* subcutaneous implantation model. Toxicity studies of CNFs and Surgicel® against red, endothelial, fibroblast cells were also performed.

Results and Discussion:

1. Haemostatic efficacy in *in vitro* thromboelastometry studies using healthy donors' blood:

- CNFs outperformed Surgicel® by inducing rapid and robust clotting in healthy donors' blood in both forms, gel and dry sponge.
- Using scanning electron microscopy (SEM) of the clots, CNFs were observed to form a mesh-like structure intermingling with blood components and increasing fibrin formation.
- In comparison, deformed red blood cells and a distinct absence of fibrin fibers were observed in the Surgicel® treated samples.

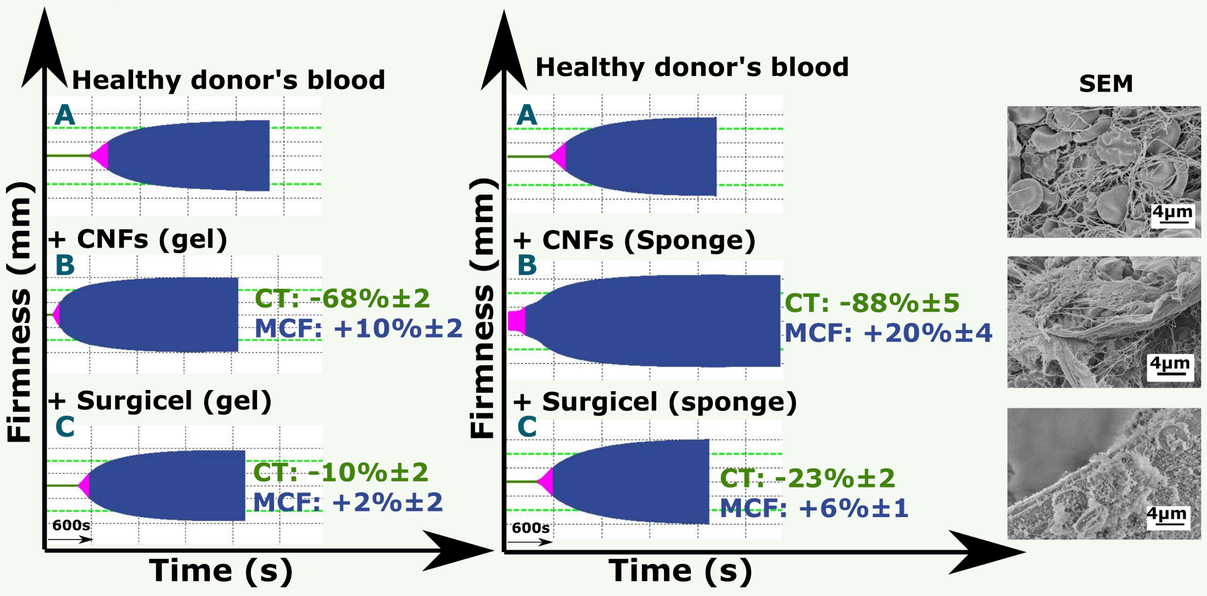

Figure 2. Non-activated rotational thromboelastometry clotting profiles obtained from healthy donors' blood **(A)** without and **(B)** with addition of CNFs and **(C)** Surgicel®. Samples were compared in gel (1wt.% in PBS: left panel) and dry sponge form (1.5 mg: right panel).

2. Haemostatic efficacy in *in vitro* thromboelastometry studies using thrombocytopenic patients' and heparinised blood:

- Rapid and robust clotting was also obtained with the addition of CNFs to thrombocytopenic patient's blood and to heparinized blood.

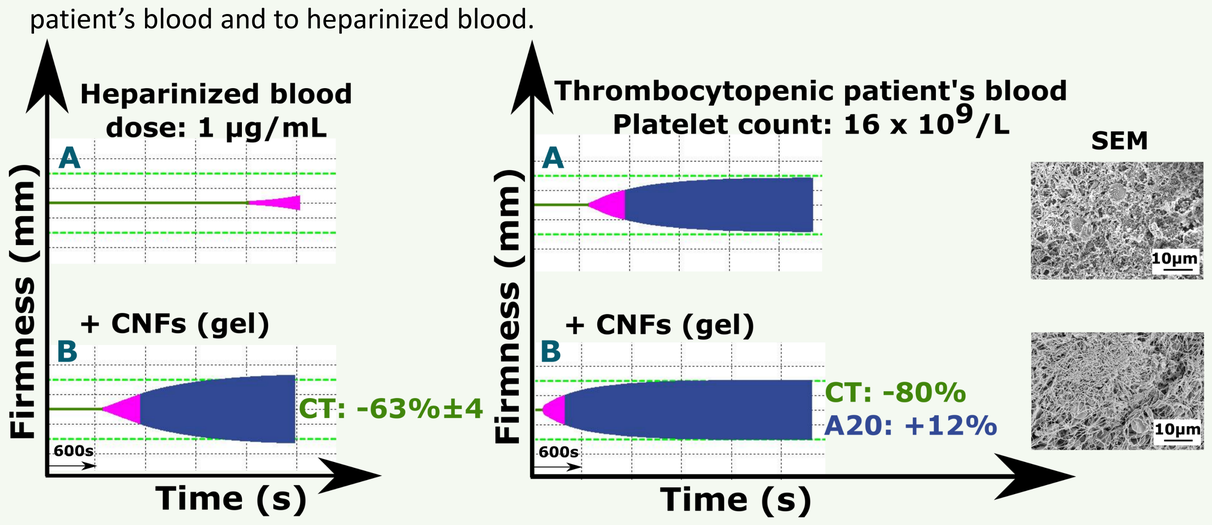

Figure 3. Non-activated rotational thromboelastometry clotting profiles of the blood collected from heparinized blood (left panel) and thrombocytopenic patient (right panel) **(A)** without and **(B)** with addition of CNFs (1wt.% in PBS).

3. Haemostatic efficacy of CNFs in an *in vivo* liver injury model:

- CNFs outperformed Surgicel® reducing blood loss by 38% versus 15%, respectively **(A)**.
- CNFs increased fibrin formation within the injury site compared to control **(B)**.
- Surgicel® dehydrated and blackened the liver tissue (insert), caused red cell lysis and inhibited fibrin formation **(B)**.

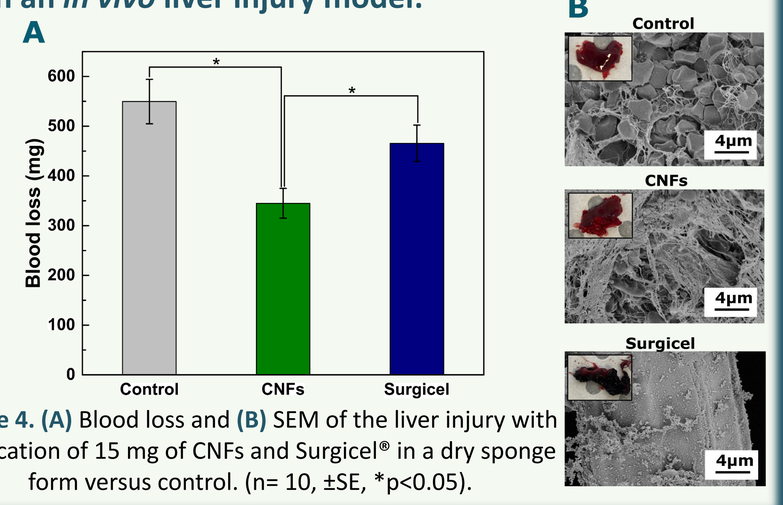

Figure 4. (A) Blood loss and **(B)** SEM of the liver injury with application of 15 mg of CNFs and Surgicel® in a dry sponge form versus control. (n= 10, ±SE, *p<0.05).

4. Biocompatibility of CNFs in *in vitro* toxicity studies on red, endothelial and fibroblast cells:

- CNFs caused no damage to red cells (**A-B**) and had no significant effect on the proliferation of fibroblast and endothelial cells (**C-D**).
- Surgicel® caused up to 5% (±SE 1%) lysis of red cells (**A-B**) and exhibited significant toxicity towards endothelial and fibroblast cells (**C-D**).

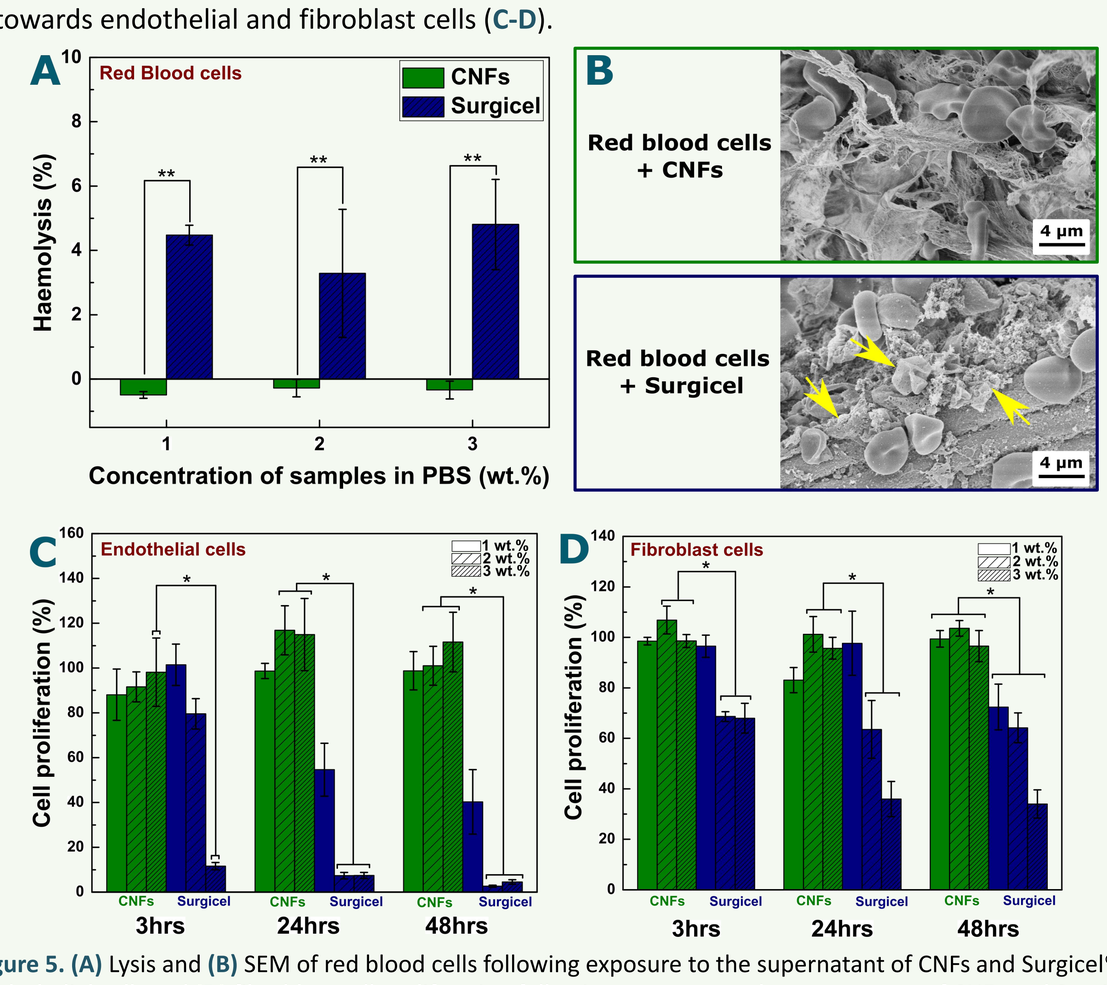

Figure 5. (A) Lysis and **(B)** SEM of red blood cells following exposure to the supernatant of CNFs and Surgicel®, **(C)** endothelial cell- and **(D)** fibroblast cell proliferation following exposure to the supernatant of CNFs and Surgicel®. (n= 9, ±SE, *p<0.05 and **p<0.01).

5. Biocompatibility and degradation of CNFs in an *in vivo* subcutaneous implantation model:

- Macroscopically, CNFs were detectable for up to 4 wks, while Surgicel® degraded within 1 wk.
- Histologically, more foreign material and foreign body reaction were observed in the CNFs implantation site for up to 2 wks. At 4 wks post-implantation, this had significantly resolved with slow degradation of CNFs evident.
- At 4 wks, dermal scar tissue was more prominent in the Surgicel® treated groups than the CNFs or control groups where the dermal tissue had largely returned to normal.

Conclusion:

- CNFs (in gel and sponge forms) outperformed the commercial benchmark, Surgicel®, by inducing rapid and robust clotting (*in vitro*) and reducing blood loss (*in vivo*) through increased fibrin formation.
- CNFs also proved effective in blood from thrombocytopenic and heparinized patients.
- In contrast to Surgicel®, pH-neutral CNFs did not damage isolated red blood cells nor impede proliferation of cultured fibroblast or endothelial cells.
- CNFs degraded slowly in the body with minimal scar formation.

References:

[1] E. Mohamed *et al.,* Materials Today Nano, 2021.
[2] T. Tsuzuki *et al.*, Cellulose, 2015.
[3] E. Mohamed *et al.*, Carbohydrate polymers, 2021.

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