To: technicalreports@afosr.af.mil
Subject: Annual progress report to Dr. Hugh DeLong

Title: Silica Nanofiber Combat Hemostat (SiNCH)
Award #: FA9550-07-1-0563

Reporting Period: 7/14/07 to 7/14/08

Annual Accomplishments:
We screened a panel of nanomaterials and identified high aspect ratio silica nanofibers (>100) as the best promoter of coagulation in vitro. These materials require combination with a carrier in order to provide adequate handling characteristics. We evaluated a panel of carrier material candidates for their ability to improve handling, act as a bulking agent, and improve hemostatic performance. Preliminary tests identified a combination of silica nanofibers and glass microspheres (SiNCH1) as our best performing material in vitro. SiNCH1 produced faster clotting than zeolites (as measured on a thromboelastograph) with no measurable exothermic reaction. Initial small animal wound models to evaluate SiNCH1 further demonstrated its hemostatic capabilities, showing clotting rates equivalent to those for quikclot, but without the exothermic reaction. The nanofibers were designed to dissolve within 2 weeks both in vitro and in vivo, however the non-nanofiber carrier portion of SiNCH1 was not rapidly resorbed and further work will be required to identify or modify carriers to allow rapid resorption.

Archival Publications: None

Change in research objectives: None

Change in AFOSR program manager: None

Extensions granted or milestones missed: No-cost 6 month extension granted 1/15/08

New discoveries, inventions or patent disclosures during this period: None

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6. AUTHOR(S)
Robert Hugh Daniels Ph.D

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14. ABSTRACT

We screened a panel of nanomaterials and identified high aspect ratio silica nanofibers (>100) as the best promoter of coagulation in vitro. These materials require combination with a carrier in order to provide adequate handling characteristics. We evaluated a panel of carrier material candidates for their ability to improve handling, act as a bulking agent, and improve hemostatic performance. Preliminary tests identified a combination of silica nanofibers and glass microspheres (SiNCH1) as our best performing material in vitro. SiNCH1 produced faster clotting than zeolites (as measured on a thromboelastograph) with no measurable exothermic reaction. Initial small animal wound models to evaluate SiNCH1 further demonstrated its hemostatic capabilities, showing clotting rates equivalent to those for quikclot, but without the exothermic reaction. The nanofibers were designed to dissolve within 2 weeks both in vitro and in vivo, however the non-nanofiber carrier portion of SiNCH1 was not rapidly resorbed and further work will be required to identify or modify carriers to allow rapid resorption.

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silica nanofibers, hemostat, resorbable, biocompatible, non-exothermic

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Final Report

Prepared for AFOSR by: R Hugh Daniels PhD[1], Esther Li[1], Oscar Abilez MD[2], Chengpei Xu MD PhD[2] and Christopher Zarins MD[2]
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Our overall goal for this phase of the program was to answer all the key technical questions that will validate this technology as a combat hemostat. These goals included:

1) Characterizing the impact of nanofiber aspect ratio as well as concentration within the blood on clotting rate both *in-vitro* and *in-vivo.*
2) Characterizing the impact of nanofiber/carrier hemostat combination on clotting and heat generation.
3) Characterizing the immune response to the nanofiber hemostat *in-vivo*.
4) Characterizing the resorption of the nanofiber hemostat.

We have successfully carried out both in vivo and in vitro evaluation of our nanofiber hemostat. We have developed an effective nanofiber/carrier formulation and compared its efficacy in vitro and in vivo against other formulations and existing hemostatic materials. In addition to this we have demonstrated that the clotting activity of our material is non-exothermic and that the nanofiber portion of our hemostat is completely resorbable and does not lead to an increased inflammatory response in vivo.
To describe these results effectively the report has been broken down into 2 major sections: the first describes the in vitro evaluation of our material set in terms of clotting, resorption, and heat generation, and the second section describes our in vivo data.

1. In vitro characterization, formulation and selection of nanofiber based hemostats

1.1 Determination of optimal material type and aspect ratio of nanofibers.

The first step for this program was to identify the optimal nanomaterial to use as the basis for our nanofiber hemostat. To effectively evaluate a panel of materials it was necessary to implement a robust, information rich in vitro test system to determine the rate of hemostasis and the nature of the formed clot. The best device available to carry out these tests is the thromboelastograph (TEG). This system is used as a routine clinical tool to determine the clotting rate of whole blood and it provides information on the time of initiation of clot formation, the rate of fibrin build up and the overall strength of the formed clot. Figure 1 shows a typical trace from a TEG and the important information that can be gathered from the trace.

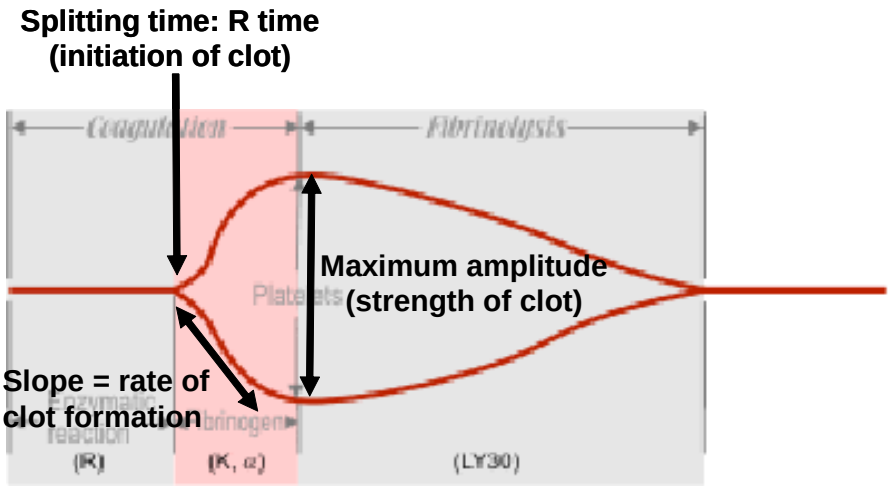


Figure 1: Typical TEG trace and the information that can be gathered from each trace. Typically we recorded the initiation, rate and strength of the clot formed.

We also secured a supply of citrated human blood from the Stanford blood center and evaluated the clotting rate of blood stored under various conditions for a week. We found that citrated blood could be stored at 4C for up to a week with little variation in the subsequent activated clotting rate of the blood. Therefore, once we had the system in house and working we used refrigerated whole, citrated human blood as the in vitro test bed for our panel of materials. We focused on silica nanofibers of various aspect ratios but also evaluated other nanomaterials such as zinc oxide, zirconium oxide, titanium oxide and aluminum oxide. Table 1 summarizes some of these data:

Table 1: Evaluation of in vitro clotting time of various inorganic nanomaterials.

Material	Optimized Amount	Correspondent SA (m2)	MA (mm)	Splitting (R)Time (sec)
100nm low aspect ratio silica (100nm x 10-100nm)	0.1mg	0.064	62.8	325
10nm medium aspect ratio silica	5mg	3.2	49	310
3um medium aspect ratio Silica fibers (3um x 30-40nm)	1.5mg	0.6	65	205
High aspect ratio silica fibers (30um x 60nm)	9mg	0.63	58.9	140
Kaolin (TEG control)	0.2mg	n/a	59.8	155
TiO2 high aspect ratio	7mg	0.6	61	190
ZrO medium aspect ratio	2mg	0.1	59.2	185
Al2O3 medium aspect ratio	5mg	2.625	59.8	275

As Table 1 shows the high aspect ratio silica nanofibers were the fastest inorganic clotting agent analyzed using the TEG assay when the material was added at its optimal concentration. We therefore selected high aspect ratio silica nanofibers as the base nanofiber hemostat for the remainder of this study.

1.2 In vitro analysis of silica nanofiber dissolution.

One important requirement for an effective combat hemostat is that it can be safely resorbed by the body thus removing the need for extensive surgical intervention to clean the hemostat from the wound. We evaluated the rate of dissolution of our high aspect ratio silica nanofibers in vitro. Nanofibers grown on solid substrates were immersed in a solution of phosphate buffered saline and were incubated at 37C or 60C (to accelerate natural dissolution) for various times. The solution was changed every 3 days to mimic turnover of bodily fluid in vivo. At various time points the substrates were removed and the nanowires on the substrate were evaluated by electron microscopy. As figures 2 and 3 show the nanowires steadily broke down over time and were almost completely dissolved by 1 week at 37C and completely dissolved by 1 week at 60C. These data suggest that a silica nanofiber hemostat left in tissue would breakdown completely in about two weeks. As a control we also monitored breakdown of titania nanofibers and, as would be expected, we saw no significant dissolution over the time course of the experiment. We further evaluated this by evaluating nanofiber hemostats in vivo as will be shown in section 2.4 below.

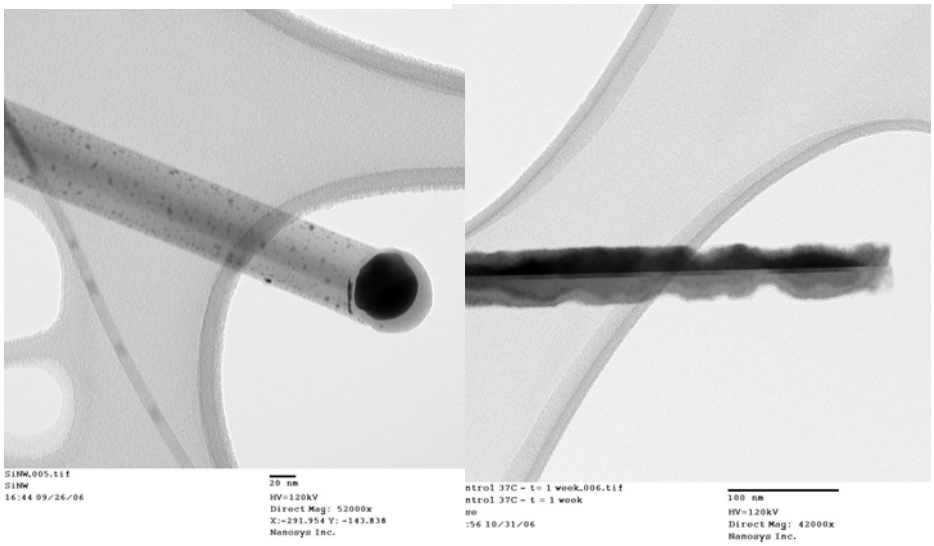


Fig 2. TEM analysis of silica nanofibers monitored after 0 and 1 week in PBS at 37^{0}C. Note the significant dissolution of the fiber over this time frame. No silica nanofibers were found at later time points.

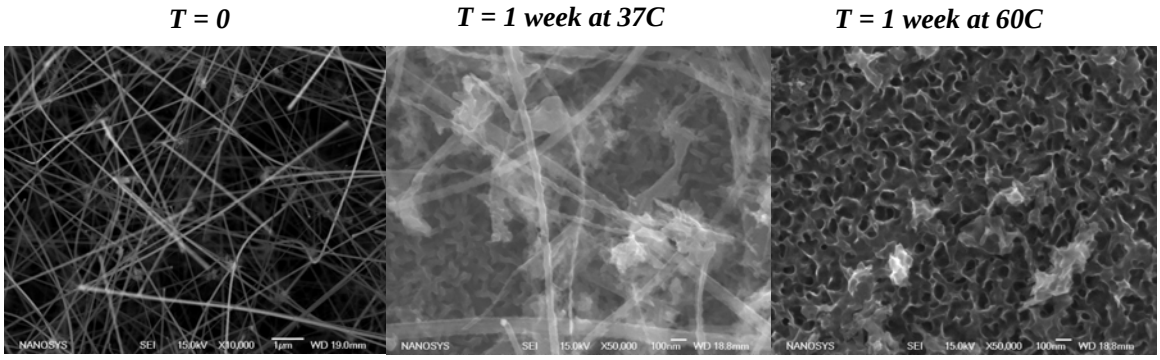


Fig 3. SEM analysis of silica nanofibers monitored after 0 or 1 week in PBS at 37^{o}C or 60^{o}C. This confirms that within a week at physiological temperatures there is significant dissolution of the silica nanofibers. There is no detectable dissolution of titania nanofibers over this time frame.

1.3 Formulation and evaluation of nanofibers in a usable format.

High aspect ratio silica nanofibers have a high surface area to volume ratio and thus the material is difficult to handle in an uncontrolled environment. It is easily dispersed and is not easy to apply to a specific area. As a result it was important to design a formulation of nanofibers that could be easily handled and yet retained (or increased) the beneficial procoagulative characteristics of the base material. We evaluated several types of carrier agents with the aim of retaining or improving the hemostatic capabilities. The best performing material we evaluated we term SiNCH1 which was a combination of silica nanofibers with a 10X excess of 3-10um glass microspheres (Fig 4). This material was easy to handle in a powdered form and analysis of the hemostatic potential showed that in the TEG it induced clotting even faster than the zeolite material quikclot. We routinely achieved R-times of less than 100seconds on the TEG using SiNCH1 – the only comparable material was quikclot (Fig 5).

Figure 4: The left hand panel shows vials of prepared SiNCH1 material. The right hand panel shows an SEM image of the same material.

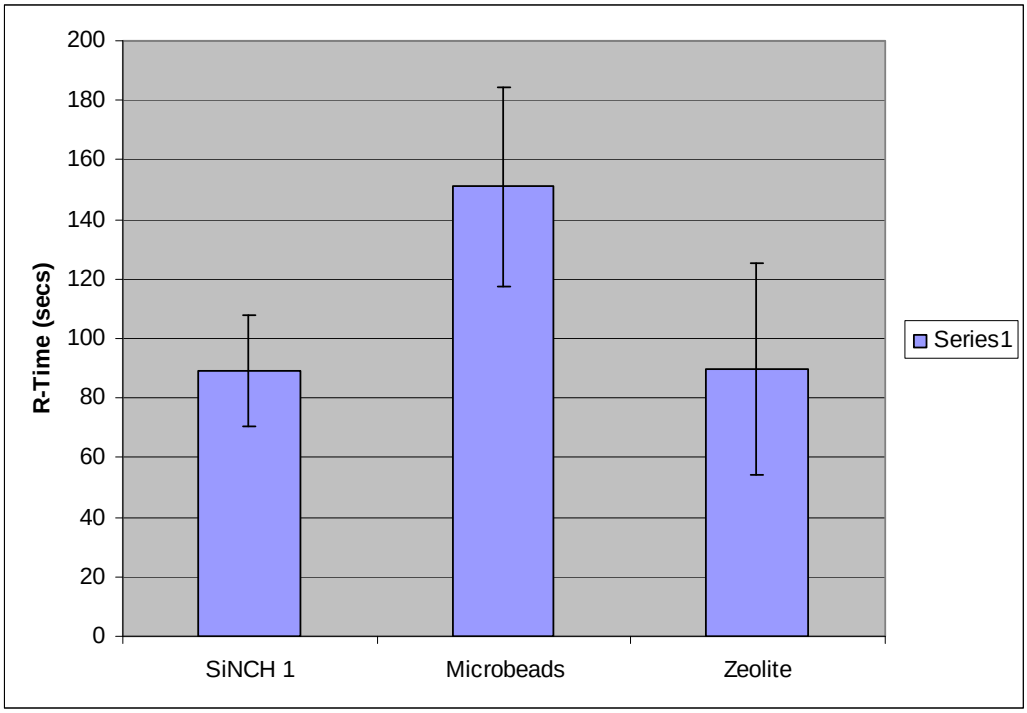


Figure 5. In vitro clotting rate of SiNCH1 is equivalent to Zeolite (crushed) and significantly better than microbeads alone – n=6 (P<0.05 that SiNCH is faster than 3um microspheres alone).

One important issue remaining with SiNCH1 is that although the nanofiber portion of the material will dissolve in a reasonable time frame the silica microspheres will not dissolve for many months in vivo. As a result we also investigated materials that would resorb as carrier agents. One promising candidate was cellulose. This material will dissolve, is currently used as a hemostatic agent and has this activity enhanced in combination with nanofibers (Fig 6). Even though the rate of hemostasis was not as fast as our SiNCH1 material the data were promising enough for us to evaluate this in vivo as a completely resorbable material. As a result we have also designed a construct termed SiNCH2 which is a composite of silica nanofibers and woven oxidized cellulose. This was also evaluated in our in vivo studies below.

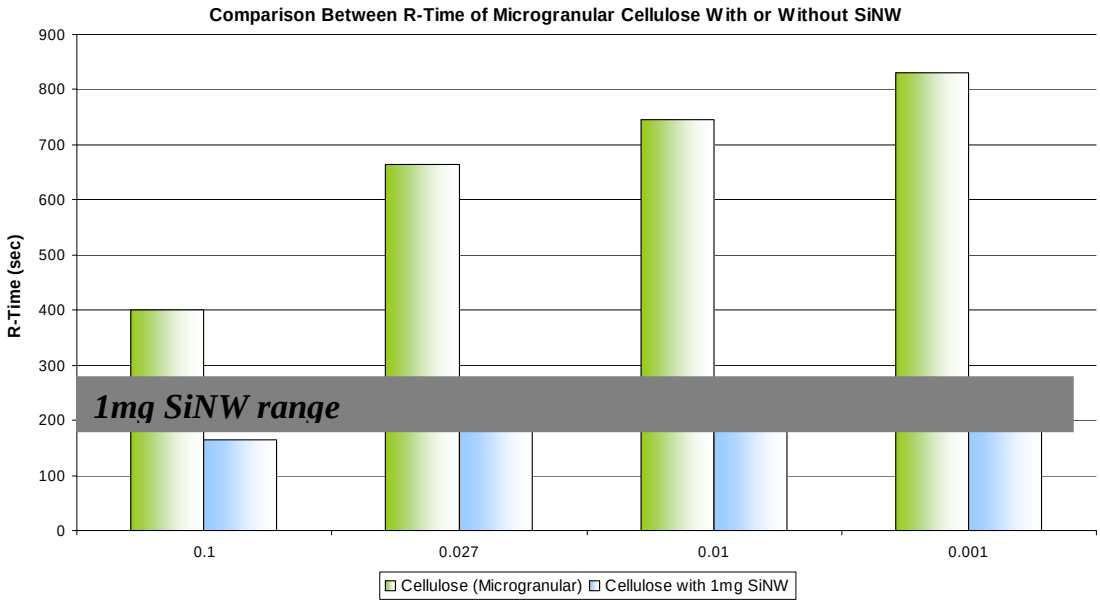


Figure 6. Combination of microgranular cellulose with silica nanofibers. The graph shows the r-time (y-axis) and amount of cellulose added (x-axis). For each concentration of cellulose the clotting time is shown in the absence (left bars) and in the presence of 1mg of silica nanofibers.

1.4 Evaluation of heat generation in vitro.

We developed a test method using a small thermistor (2mm long 1mm wide) that can be inserted into a mound of hemostat and left there while blood or saline is added. We tested the measured temperature change when 100mg of hemostat (either SiNCH1 or Zeolite: Quikclot) was exposed to 1ml of saline pre-warmed to 37C.

Test material	Average increase in temperature
SiNCH1	no detectable change
Quikclot	14^{o}C

This change in temperature induced by quikclot would represent an apparent skin temperature of 51C during hemostat application and this number falls within the range of reported values.

We did not detect any exothermic reaction when liquids interacted with SiNCH1.

2. In vivo evaluation of SiNCH formats

Aims: The experiments were designed to demonstrate the life-saving potential for SiNCH as a clotting accelerant in cases of acute trauma, proof-of-concept demonstrations were planned to show that clotting is accelerated and the materials are safe to use. To validate the in-vitro TEG analysis in an in-vivo living system, and demonstrate its relevance to an actual wound, we performed haemostasis testing using rat acute bleeding models. Work was performed at the Zarins lab under an approved animal protocol.

2.1 Materials, Methods and Protocols used for in vivo study

Materials and Methods: We used various control and experimental materials and material combinations in a rat model in the Clark Center surgical laboratory setting on the Stanford campus. Different tissue types and different injuries were created to exhibit different bleeding modes and were evaluated for relevance, repeatability, and efficacy. The haemostatic materials tested include the following: cotton gauze, various SiNCH formats, zeolite (QuikClot), forms of oxidized cellulose (Surgicel), microfibrillar collagen haemostat (Avitene). Bleeding without haemostatic material was used as control. Nanosys provided the experimental SiNCH formats, the zeolite, and the oxidized cellulose test materials.

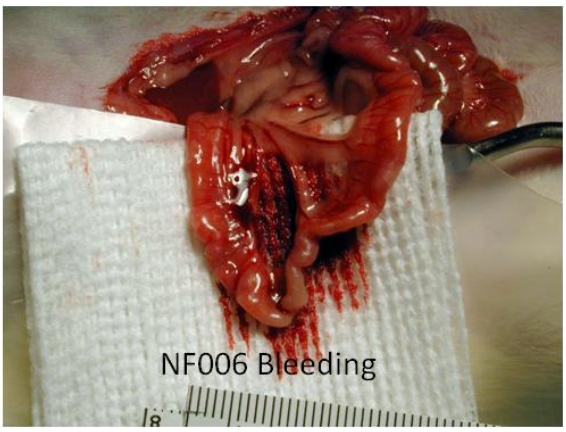
Procedures: Two bleeding models were created surgically under an approved protocol. The mesenteric artery at the proximal loop of the small intestine to the duodenum was cut completely to induce bleeding. Three bleeding sites were made for each animal. The liver bleeding model was created by trimming off 1 cm-piece from the anterior cordial lobe of the liver. Care was taken to make sure the trimmed pieces were equal in size. After injury, the haemostatic agent was applied to the injury site and bleeding was allowed to proceed for 3 minutes and 30 seconds, which was found in a pilot study to be average clotting time. The gauzes used to collect blood from the wound were weighed for calculation of blood loss. The animals were then euthanized. For survival experiments, after haemostasis was induced, and blood loss was documented, the surgical field was closed and the animals were housed and monitored for four weeks. Wound site histology was then carried out to evaluate residual effects of the haemostat and overall tissue health.

Data analysis: The blood loss was recorded in excel database and ANOVA/Fisher's PLSD (Protected Least Significant Difference) were performed as statistical analysis using StatView V5.5. Statistical significance was defined as $p<0.05$.

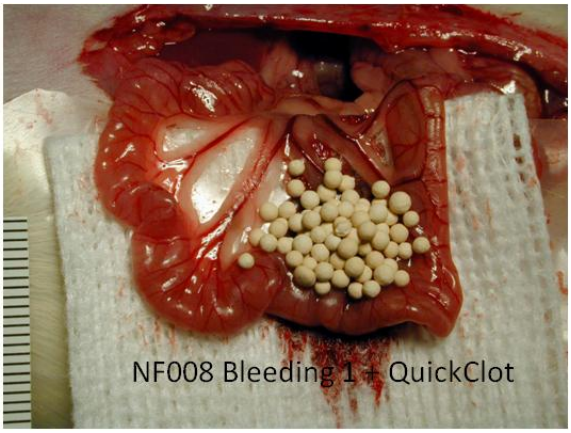
Results:
Acute mesenteric artery bleeding model:

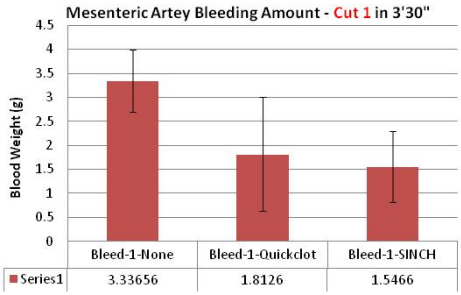
- Acute mesenteric bleeding (NF02-16, 10/11/07)
- 3 bleeding sites, (first bleed the most)
- n=5, SiNCH, Quicklot, none, amount applied varied, 66-100mg for SiNCH, 406-1600mg for QuickClot

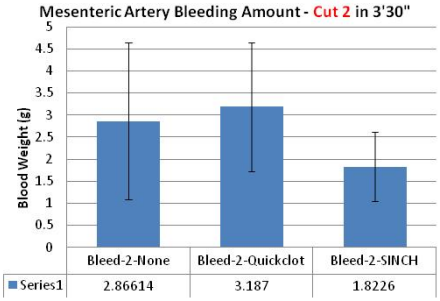

NF006 Ready for bleeding

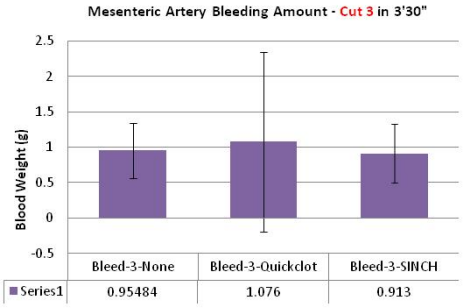

NF006 Bleeding

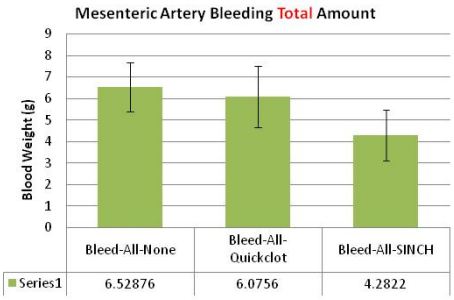

NF007 Bleeding 1 + SiNCH


NF008 Bleeding 1 + QuickClot







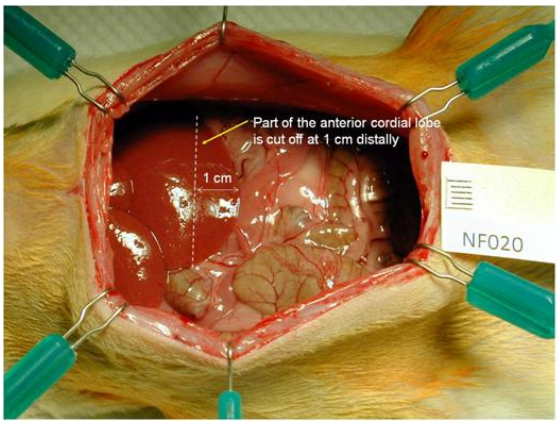


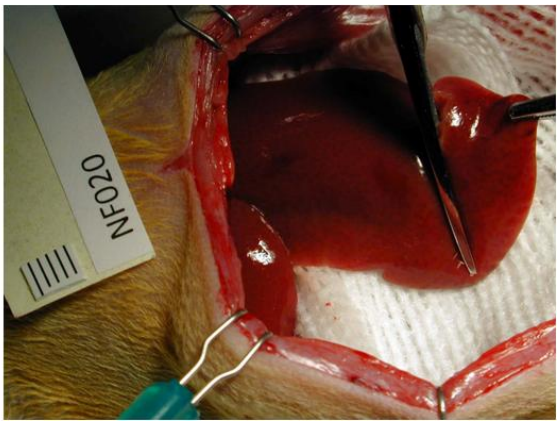
SiNCH appear to have more effect, but the amounts varied

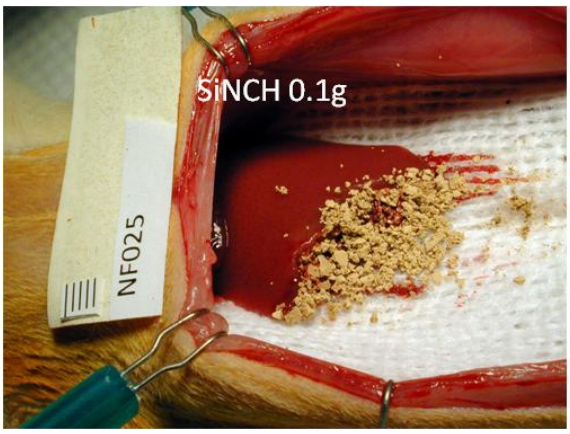
	Mean Diff.	Crit. Diff.	P-Value	
Bleed-All-None, Bleed-All-Quickclot	.453	1.421	.5244	
Bleed-All-None, Bleed-All-SINCH	2.247	1.421	.0026	S ←
Bleed-All-Quickclot, Bleed-All-SINCH	1.793	1.421	.0145	S ←

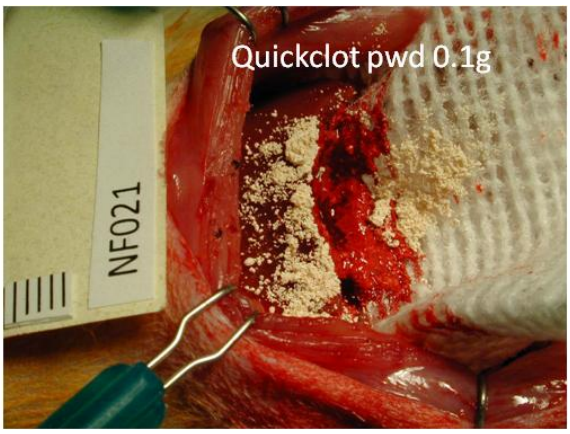
Acute liver bleeding (NF20-34, 10/25/2007) (survived for histology)

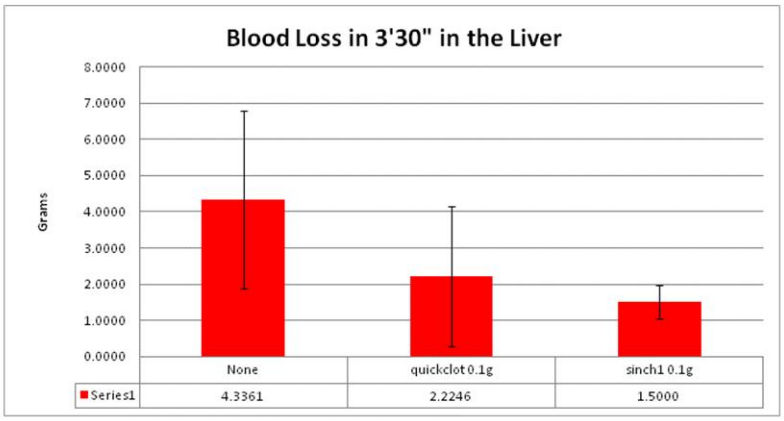
- trimming off 1 cm-piece from the anterior cordial (AC) lobe of the liver
- n=5, SiNCH1, QuickClot, 100mg each, none

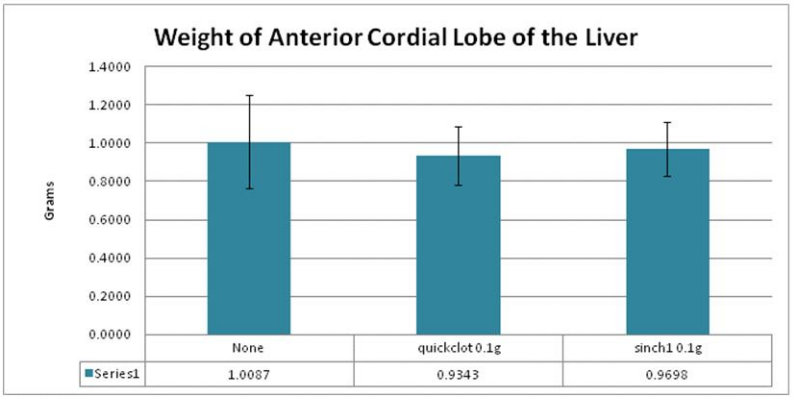










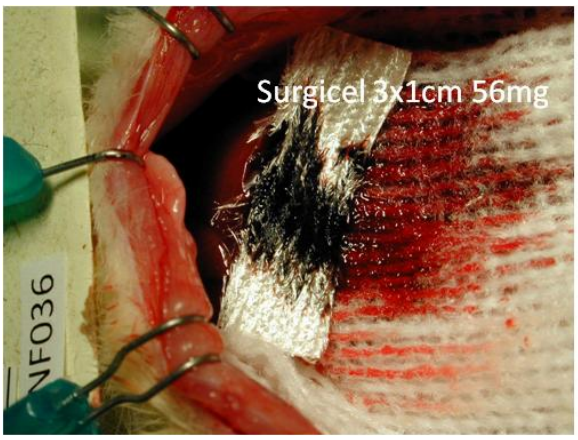


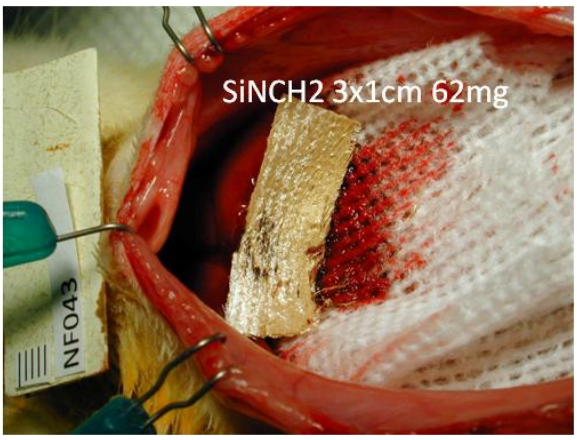
NS, since p=.0751 >0.05, and mostly NS for PLSD

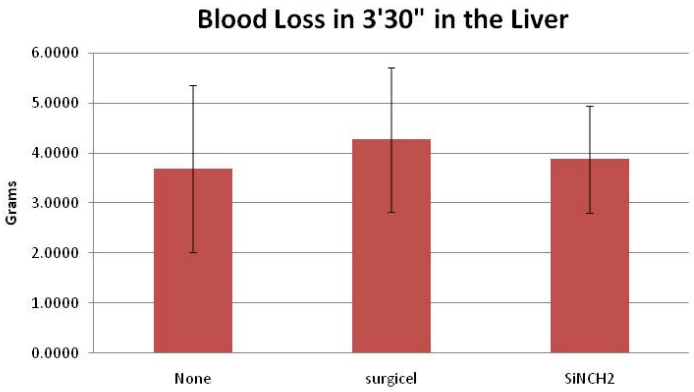
AC was trimmed equally

Acute liver bleeding (NF35-49, 11/1/2007)

- trimming off 1 cm-piece from the anterior cordial (AC) lobe of the liver
- n=5, SiNCH2 57-62mg, surgicel 3x1cm 56-62mg, none







ANOVA Table for Blood Loss (g)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Group	2	.905	.453	.227	.8005	.453	.077
Residual	12	23.956	1.996				

ANOVA Table for AC Lobe (g)

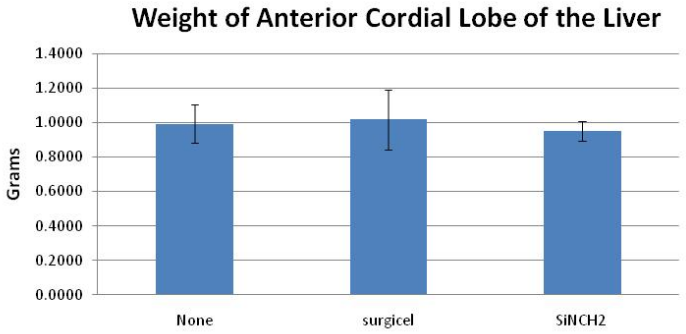
	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Group	2	.011	.005	.363	.7031	.726	.094
Residual	12	.180	.015				

Fisher's PLSD for Blood Loss (g)
Effect: Group
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value
None, surgicel	-.598	1.947	.5218
None, SiNCH2	-.191	1.947	.8347
surgicel, SiNCH2	.399	1.947	.6932

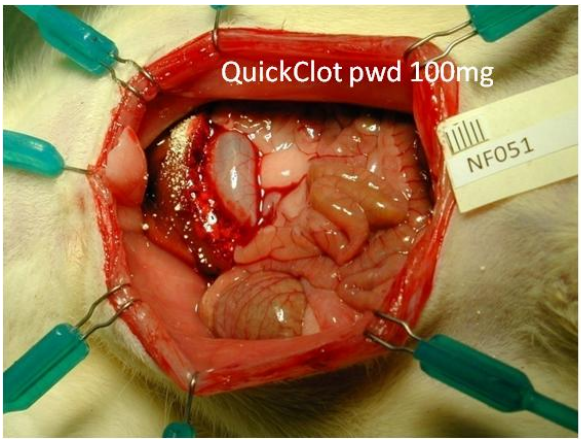
Fisher's PLSD for AC Lobe (g)
Effect: Group
Significance Level: 5 %

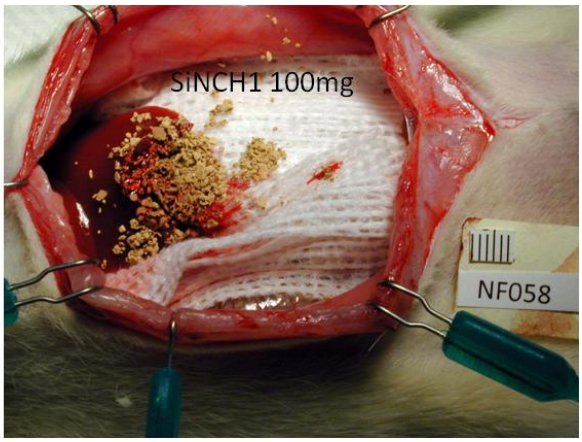
	Mean Diff.	Crit. Diff.	P-Value
None, surgicel	-.024	.169	.7699
None, SiNCH2	.041	.169	.6059
surgicel, SiNCH2	.065	.169	.4168

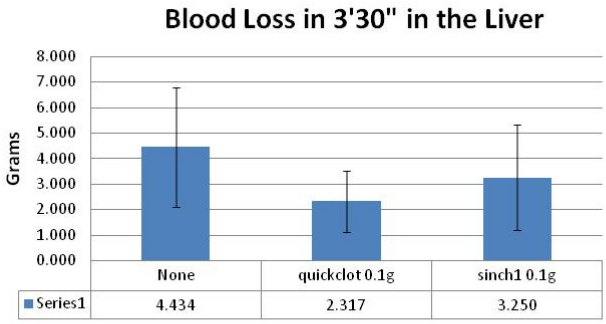


Acute liver bleeding (NF50-68, 12/6/2007)

- trimming off 1 cm-piece from the anterior cordial (AC) lobe of the liver
- n=5, SiNCH1 100mg, QuickClot pwd 100mg, none







ANOVA Table for Blood Loss (g)

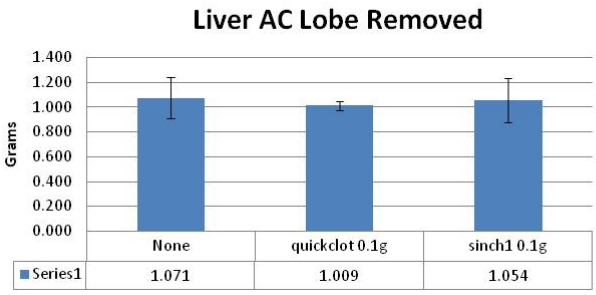
	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Group	2	11.248	5.624	1.519	.2582	3.038	.254
Residual	12	44.433	3.703				

ANOVA Table for AC Lobe (g)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Group	2	.010	.005	.250	.7827	.500	.080
Residual	12	.240	.020				

Fisher's PLSD for Blood Loss (g)
Effect: Group
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value
None, quickclot	2.116	2.652	.1076
None, sinch1	1.184	2.652	.3499
quickclot, sinch1	-.932	2.652	.4584



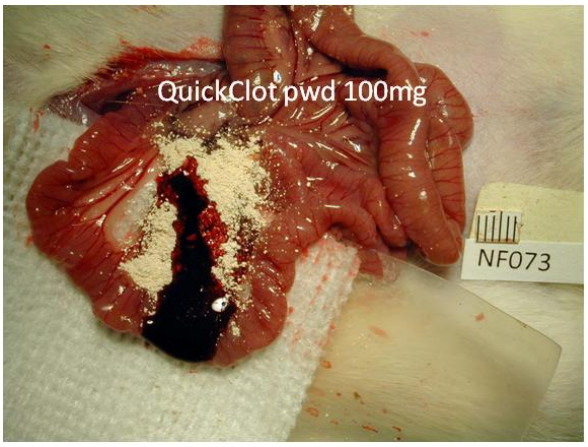
Fisher's PLSD for AC Lobe (g)
Effect: Group
Significance Level: 5 %

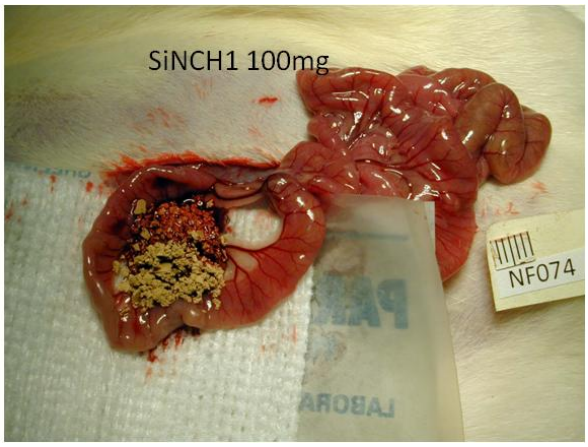
	Mean Diff.	Crit. Diff.	P-Value
None, quickclot	.061	.195	.5067
None, sinch1	.017	.195	.8541
quickclot, sinch1	-.044	.195	.6285

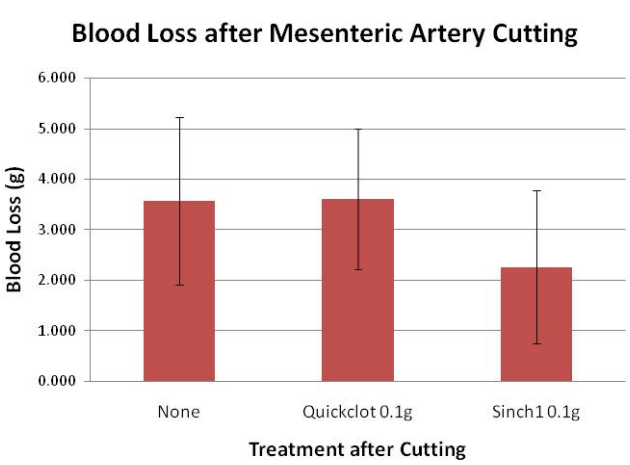
P=.2582, NS

Acute mesenteric bleeding (NF69-84, 12/19/07)

- 1 bleeding site, (first bleed the most)
- n=5, SiNCH1 100mg, Quicklot pwd 100mg, none







ANOVA Table for Blood Loss (g)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Groups	2	5.903	2.951	1.271	.3158	2.541	.218
Residual	12	27.871	2.323				

Means Table for Blood Loss (g)
Effect: Groups

	Count	Mean	Std. Dev.	Std. Err.
Bleed-1-None	5	3.565	1.659	.742
Bleed-1-Quickclot	5	3.603	1.388	.621
Bleed-1-SiNCH	5	2.254	1.513	.677

Fisher's PLSD for Blood Loss (g)
Effect: Groups
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value
Bleed-1-None, Bleed-1-Quickclot	-.038	2.100	.9694
Bleed-1-None, Bleed-1-SiNCH	1.311	2.100	.1987
Bleed-1-Quickclot, Bleed-1-SiNCH	1.349	2.100	.1869

P=.3158, NS

2.2 Summary of In Vivo Analysis

To demonstrate the potential for SiNCH as a clotting accelerant we performed haemostasis testing using rat acute bleeding models. The results showed that SiNCH had some effects on the bleeding; however, more experiments are needed to reveal statistical significance. In general our material performed at least as well as the gold standard "quikclot" in this preliminary evaluation and thus we are encouraged that with further development and analysis in the appropriate bleeding models we will be able to improve this further. In addition to assessing blood loss with SiNCH1 we also measured heat generated during application of the material. This was achieved by placing a small (2mm) thermister against the wound site as the hemostat was applied. We were unable to detect any change in temperature upon application of the hemostat in five different tests.

2.3 Preliminary histological evaluation

All treated with bleeding liver AC lobe, 1cm; recover for 4 weeks
Two histology sections were taken from the cutting edge

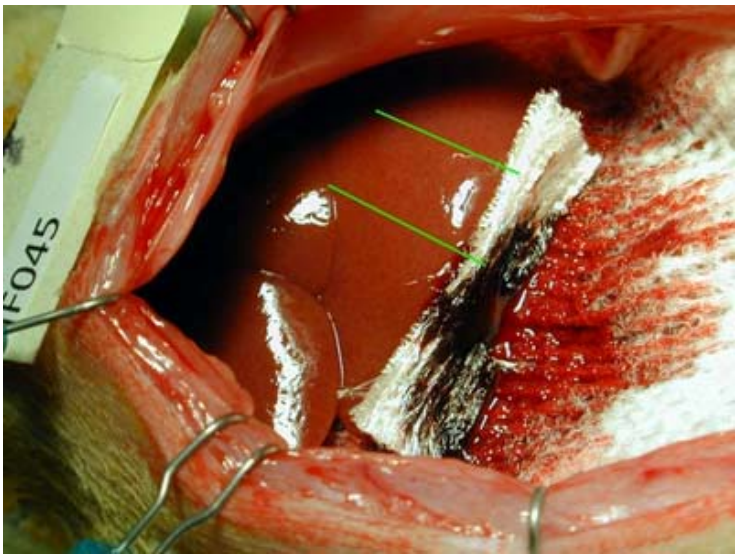
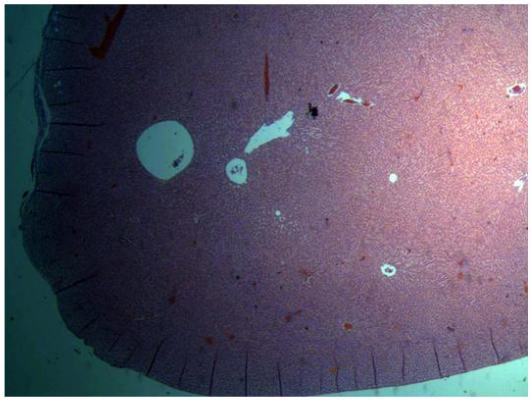
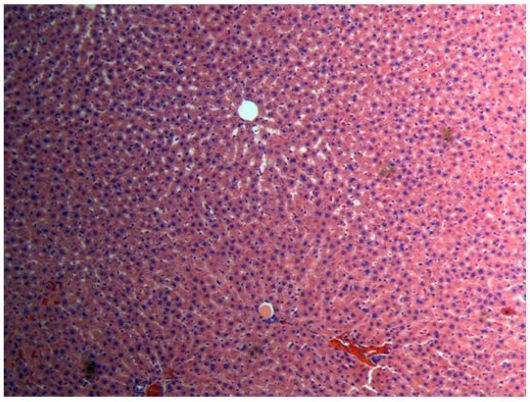


Table 2: Panel of material evaluated histologically.

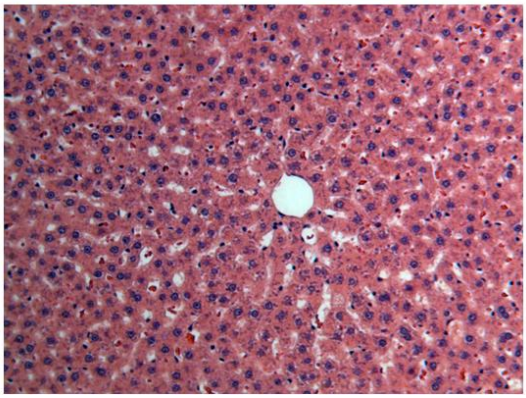
	Note on		AClobe			Note on		AClobe
ID	Haemostat Materials	Blood Loss (g)	wt. (g)		ID	Haemostat Materials	Blood Loss (g)	wt. (g)
NF020	none	0.6704	0.615		NF035	none	3.898	0.863
NF023	none	5.806	1.2569		NF038	none	2.256	0.92
NF026	none	5.288	1.002		NF041	none	3.984	1.146
NF029	none	6.839	1.03		NF044	none	2.072	0.98
NF032	none	3.077	1.1396		NF047	none	6.203	1.05
NF021	quickdot pwd 0.1g	1.209	0.7378		NF036	surgicel 3x1cm 56mg	2.368	0.825
NF024	quickdot pwd 0.1g	1.474	0.962		NF039	surgicel 3x1cm 58mg	5.393	1.25
NF027	quickdot pwd 0.1g	5.619	1.01		NF042	surgicel 3x1cm 61mg	5.991	0.915
NF030	quickdot pwd 0.1g	1.957	1.13		NF045	surgicel 3x1cm 55mg	3.881	0.96
NF033	quickdot pwd 0.1g	0.8641	0.8318		NF048	surgicel 3x1cm 57mg	3.728	1.13
NF022	sinch10.1g	1.359	0.819		NF037	SINCH2 3x1cm 56mg	4.993	0.968
NF025	sinch10.1g	1.179	0.9175		NF040	SINCH2 3x1cm 58mg	3.335	0.898
NF028	sinch10.1g	0.999	1.03		NF043	SINCH2 3x1cm 62mg	2.373	0.907
NF031	sinch10.1g	2.022	1.18		NF046	SINCH2 3x1cm 59mg	4.742	0.941
NF034	sinch10.1g	1.9409	0.9023		NF049	SINCH2 3x1cm 58mg	3.923	1.04

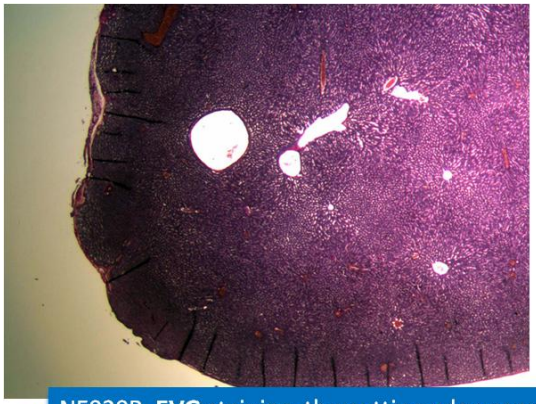
Observation Remarks (all H&E staining unless indicated)

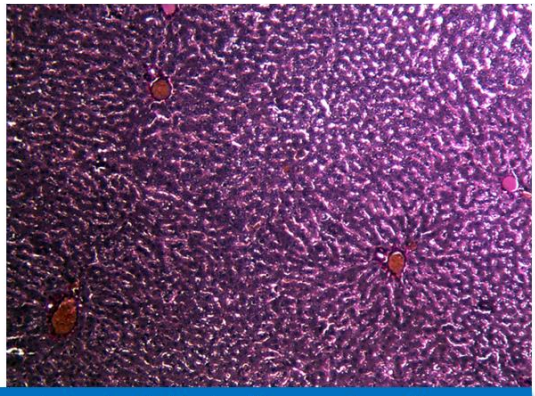




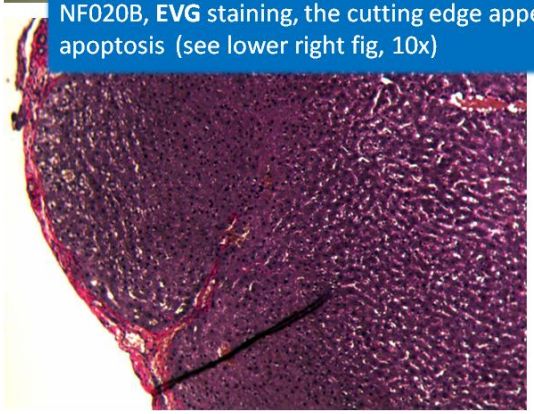
NF020B, no haemostat materials applied. the cutting edge appears to be healed but with necrosis and/or apoptosis (fig above 2.5x); nearby liver shows normal structure with lobular structure which has a central vein (fig upper right 10x, and right 20x)

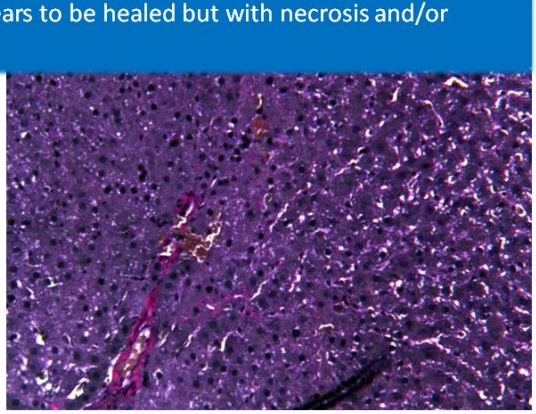


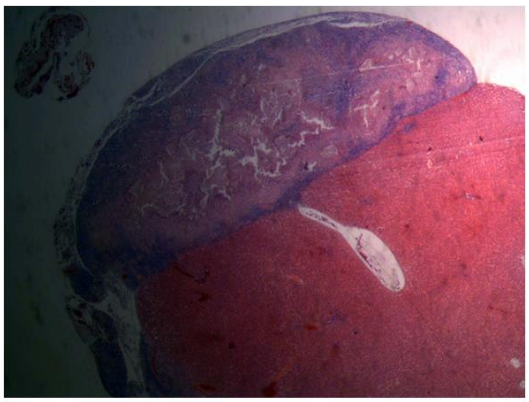


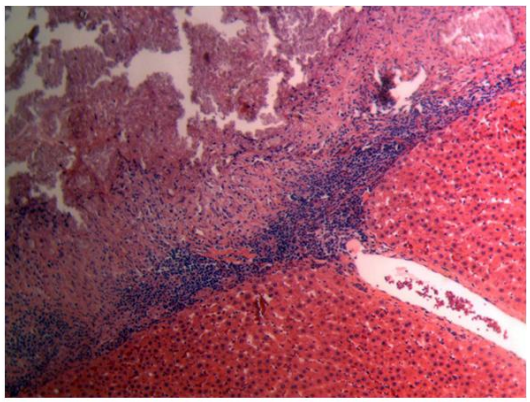


NF020B, **EVG** staining, the cutting edge appears to be healed but with necrosis and/or apoptosis (see lower right fig, 10x)

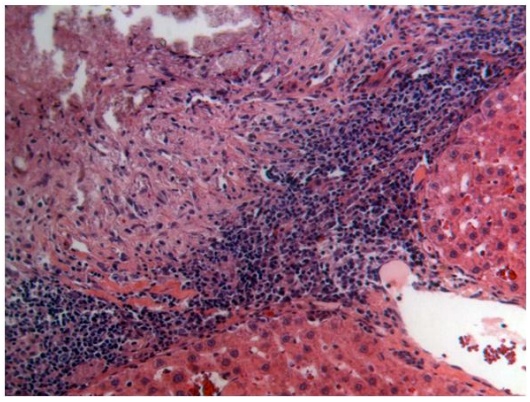


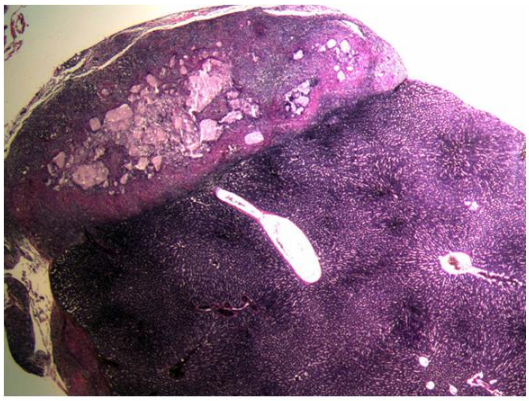


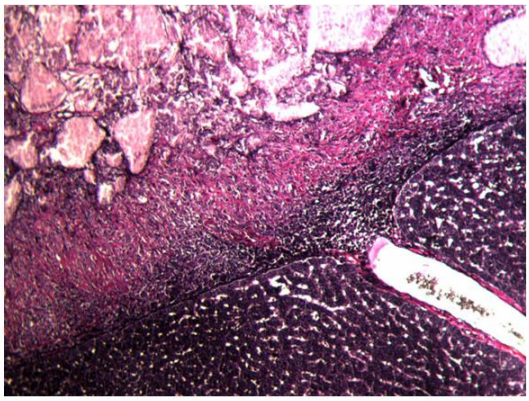




NF021A, **quickclot** pwd 0.1g.
The materials well adhered to the cutting edge and well capsulated with large number of inflammatory cells, mainly lymphocytes, and fibroblasts. (fig above 2.5x); nearby liver shows mild inflammatory reaction (fig upper right 10x, and right 20x)

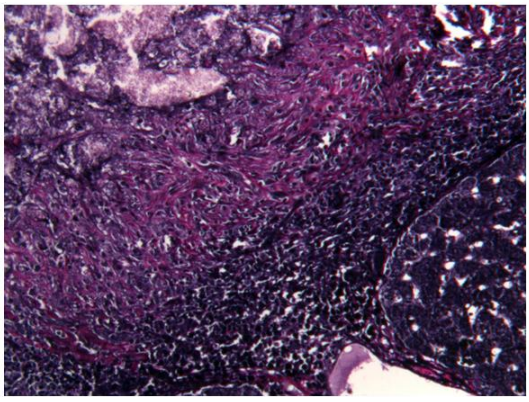


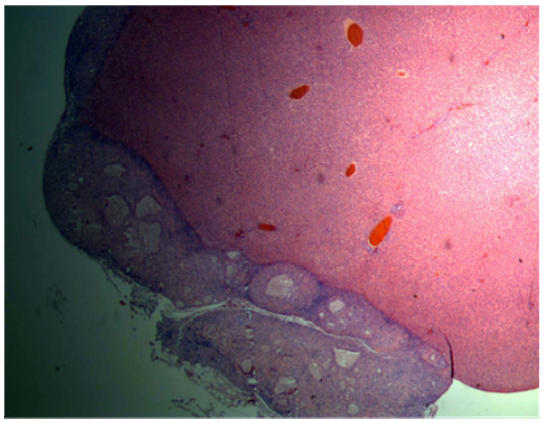


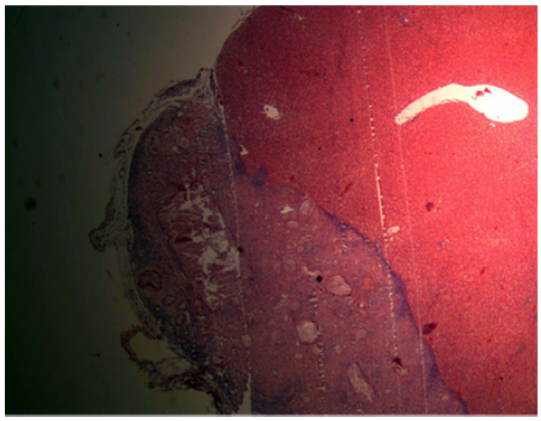


NF021A, **EVG** staining, collagen appears red, elastin dark purple.

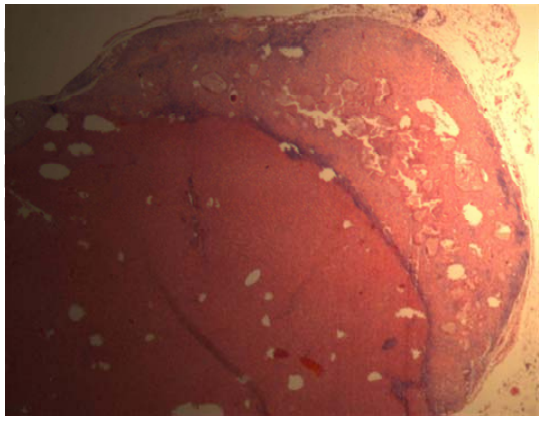
(fig above 2.5x, fig upper right 10x, and right 20x)

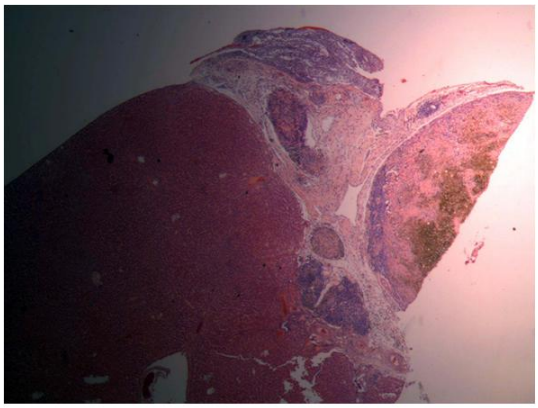


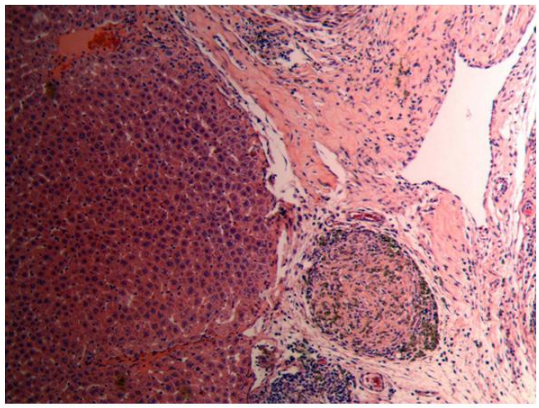




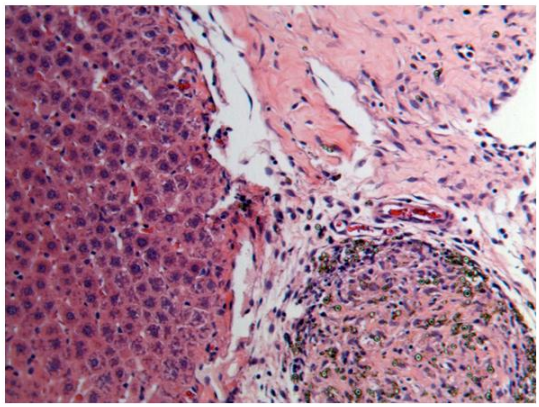
NF024B, NF030B, NF033B, all quickclot pwd 0.1g.
Histology is similar to NF021 in previous slide. (fig above 24B, 2.5x; fig upper right 30B, 2.5x, and fig right 33B 2.5x; higher power images are available.)

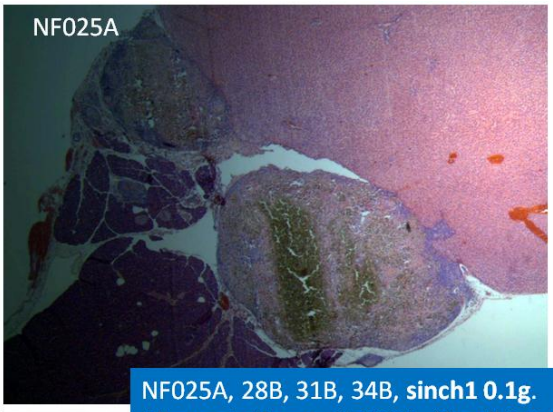


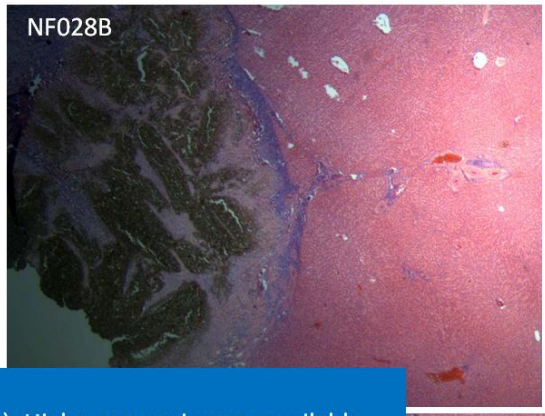




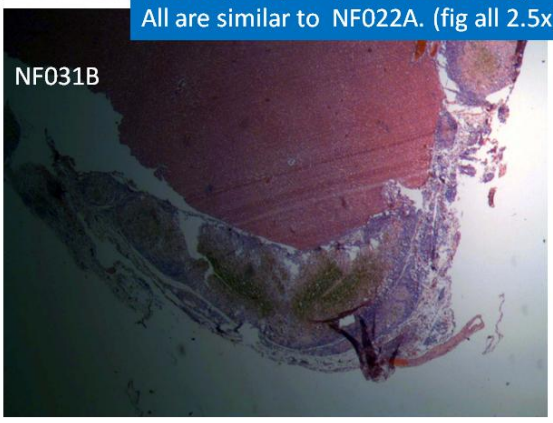
NF022A, **sinch1 0.1g**.
The materials appears brown on the sections, which is well well capsulated with large number of inflammatory cells, mainly lymphocytes, and fibroblasts. Large amount of connective tissues make the adhesion between the material and the cutting edge (fig above 2.5x); nearby liver shows mild inflammatory reaction (fig upper right 10x, and right 20x)

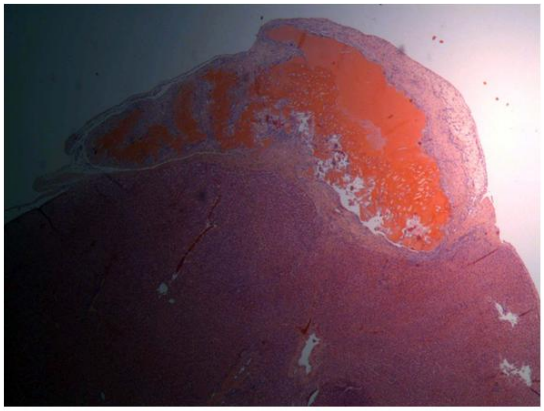


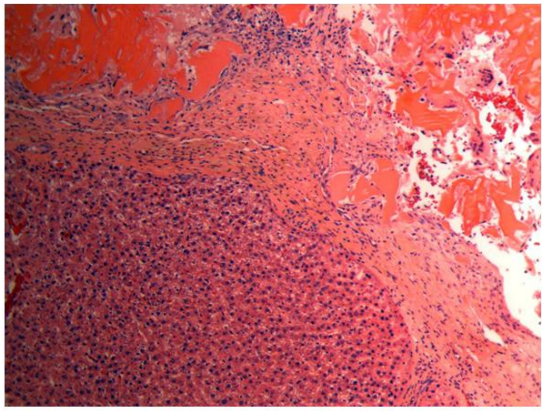




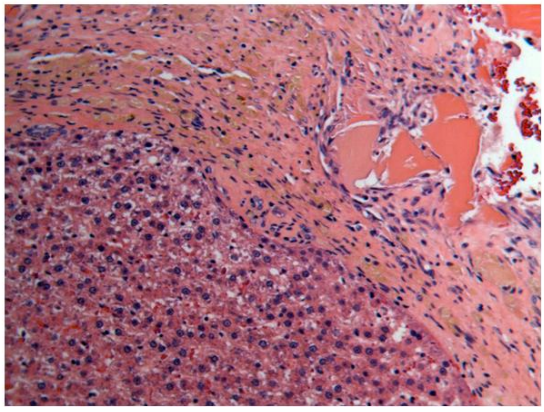
NF025A, 28B, 31B, 34B, **sinch1 0.1g**.
All are similar to NF022A. (fig all 2.5x). Higher power images available.

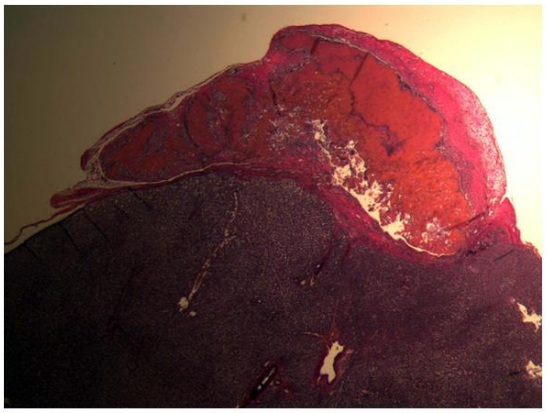


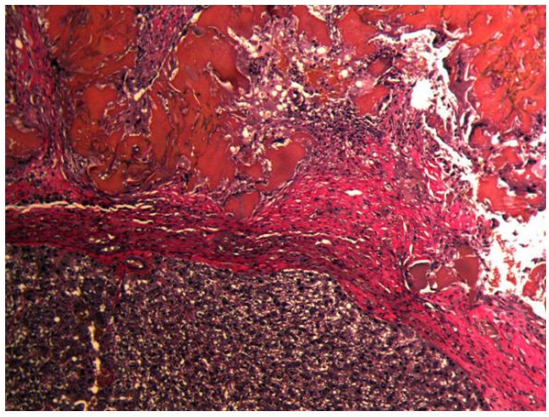




NF036B, **surgicel 3x1cm 56mg**.
The materials appears red on the sections, which is well capsulated with large number of inflammatory cells, mainly lymphocytes and fibroblasts. Large amount of connective tissues make the adhesion between the material and the cutting edge (fig above 2.5x); nearby liver shows mild inflammatory reaction (fig upper right 10x, and right 20x)

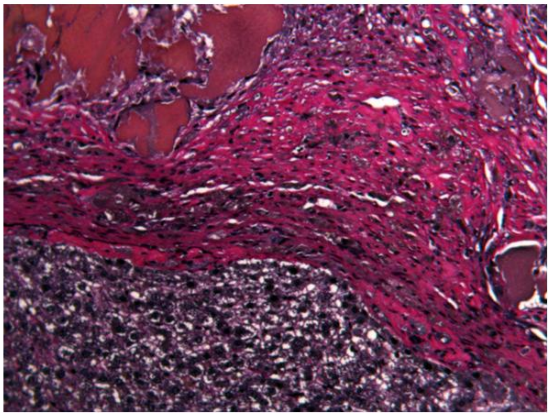


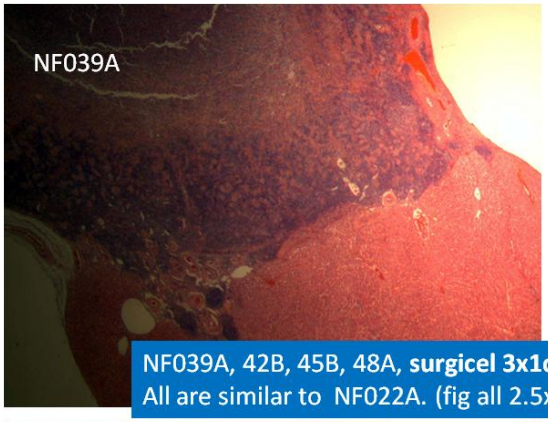


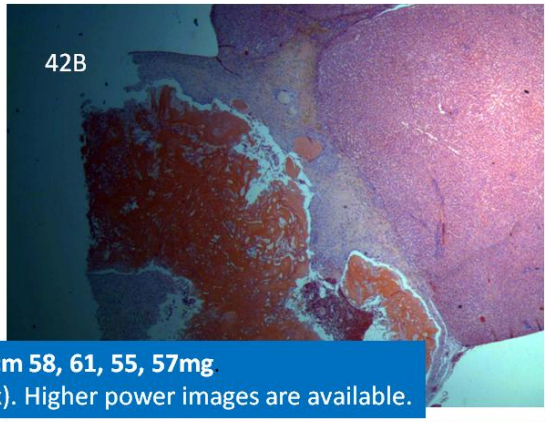


NF036B, **EVG** staining, collagen appears red, elastin dark purple.

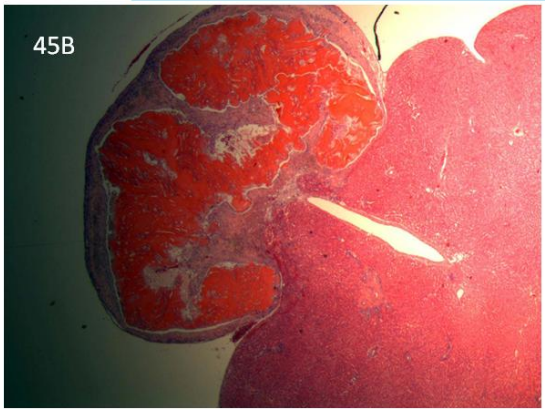
(fig above 2.5x, fig upper right 10x, and right 20x)

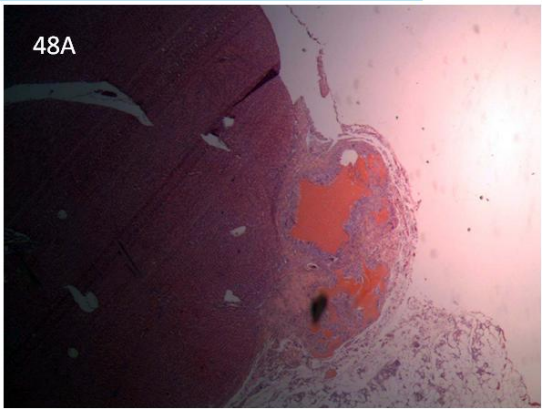


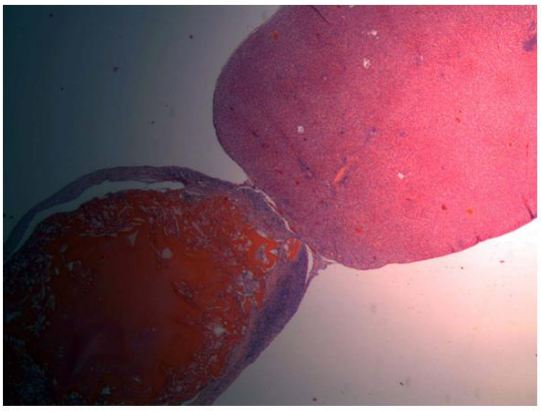


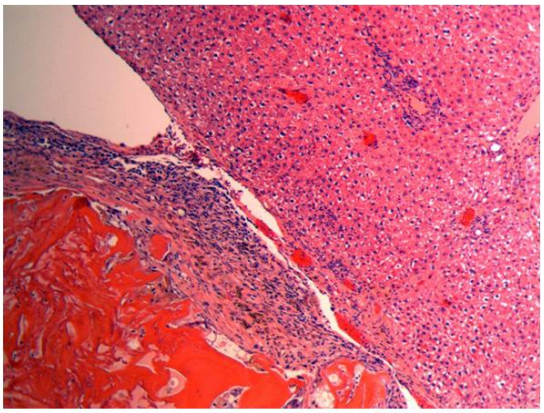


NF039A, 42B, 45B, 48A, **surgicel 3x1cm 58, 61, 55, 57mg**.
All are similar to NF022A. (fig all 2.5x). Higher power images are available.

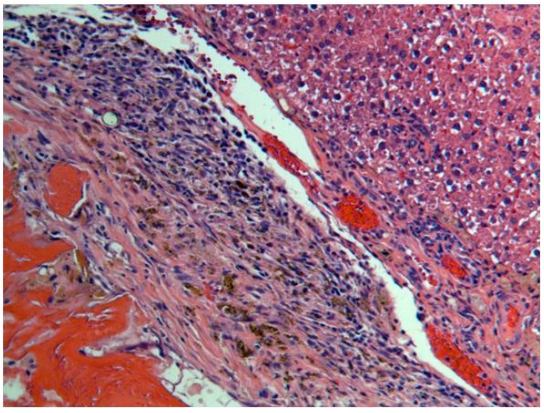


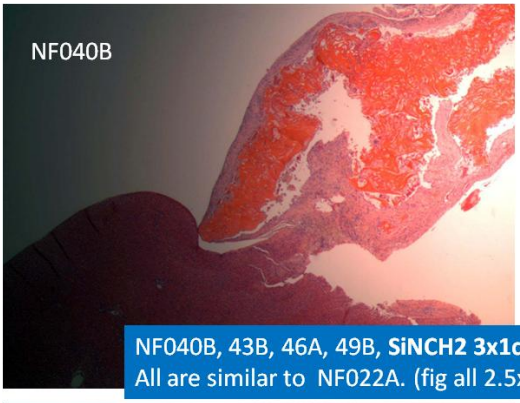


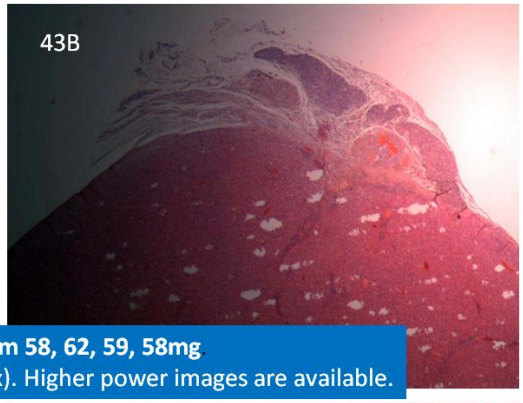




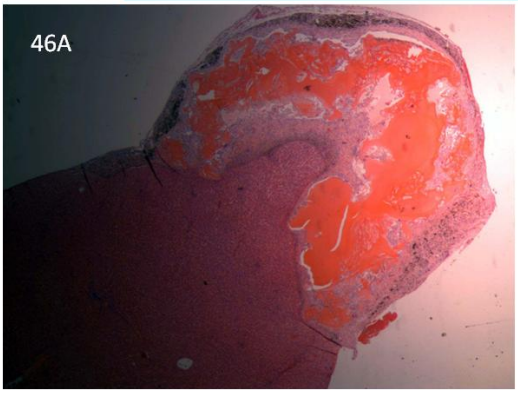
NF037A, **SiNCH2 3x1cm 56mg**.
The materials appears also red on the sections, which is well capsulated with large number of inflammatory cells, mainly lymphocytes and fibroblasts. Large amount of connective tissues make the adhesion between the material and the cutting edge (fig above 2.5x); nearby liver shows mild inflammatory reaction (fig upper right 10x, and right 20x)

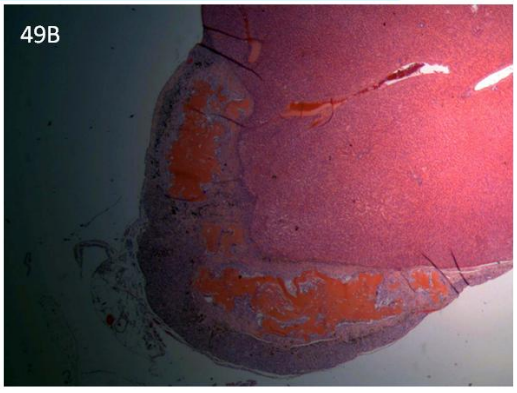






NF040B, 43B, 46A, 49B, **SiNCH2 3x1cm 58, 62, 59, 58mg**
All are similar to NF022A. (fig all 2.5x). Higher power images are available.





2.4 Summary of histological evaluation

- All evaluated wounds show sub-acute to chronic inflammatory reactions
- Haemostat materials are mostly capsulated by inflammatory connective tissues
- Liver tissue at cutting edge appears to be healed well with mild necrosis and/or apoptosis
- Haemostat materials do not seem to cause any additional deleterious effect on the liver.

These data were supported by additional in vitro studies on nanofibers implanted into the soft tissue of rabbits. In that study the response of the soft tissue at the site of implantation at 1, 2 and 12 weeks post-implantation was indistinguishable from a benign control implant (USP polyethylene negative control). In total these data suggest that the nanofibers do not induce any significant increase in inflammation when introduced into animal tissue.

In addition to their benign nature we were unable to detect the presence of the nanofibers on implanted substrates 2 weeks after implantation supporting the contention that nanowires will dissolve in vivo and be expelled from the body.

3. Summary and Conclusions

In this pilot study we have demonstrated the following:

1. High aspect ratio silica nanofibers are an effective hemostatic material *in vitro*.
2. SiNCH1 (silica nanofibers + glass microsphere carrier) were as effective a hemostat as quikclot *in vitro* and *in vivo* with no detectable heat generation.
3. SiNCH1 does not induce any significant inflammatory response 4 weeks after use.
4. The nanofiber portion of SiNCH1 is completely resorbable but the carrier is not resorbed within 1 month.

These data suggest that a silica nanofibers are a very promising candidate for development into an effective battlefield hemostat. There are several major efforts that would be required to fully develop a silica nanofiber based hemostat.

1. Development of new bulking agents that give the hemostatic performance of SiNCH1 but are fully absorbable.
2. Development alternate form factors for the delivery of SiNCH. These could be in the form of a powder like SiNCH1 or a more conformable solid scaffold that would allow application to various battlefield injuries.
3. Further demonstration the hemostatic activity of SiNCH formats in a large animal wound model with a sufficient number of experiments to produce statistically significant data.
4. Scale up of nanofiber production in a cost effective process to supply sufficient quantities of nanofibers for this application.
5. Development of appropriate packaging and sterilization schemes appropriate for SiNCH formats.