Nano hemostat solution: immediate hemostasis at the nanoscale

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Abstract

Hemostasis is a major problem in surgical procedures and after major trauma. There are few effective methods to stop bleeding without causing secondary damage. We used a self-assembling peptide that establishes a nanofiber barrier to achieve complete hemostasis in less than 15 seconds when applied directly to a wound in the brain, spinal cord, femoral artery, liver, or skin of mammals. This novel therapy stops bleeding without the use of pressure, cauterization, vasoconstriction, coagulation, or cross-linked adhesives. The self-assembling solution is nontoxic and nonimmunogenic, and the breakdown products are amino acids, which are tissue building blocks that can be used to repair the site of injury. Here we report the first use of nanotechnology to achieve complete hemostasis in less than 15 seconds, which could fundamentally change how much blood is needed during surgery of the future.

Key words: Hemostasis; Surgery; Trauma; Nanotechnology; Self-assembling peptide


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The authors declare a competing financial interest: S.Z. is a co-founder and board member of 3D Matrix, the licensor of one of the materials used.


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calcium to produce fibrin glue, which acts as an artificial clot [1,2,5,6,8,10,14,19].

There are five major issues related to the limitations and applicability of many of these hemostatic agents. First, some of the materials are solid, such as powder formulations, and are not able to flow into the area of injury to bring about their hemostatic effects [1,10,14]; second, some liquid agents, such as cyanoacrylates, require a dry environment to be effective [8]; third, some materials can create an immune response resulting in the death of adjacent cells, placing additional stress on the body that can prolong or prevent healing [8,10,14,15,20]; fourth, some agents have a short shelf-life and very specific handling requirements [6,10,14,16,17]; and finally, many currently used hemostats are difficult to use in uncontrolled environments [1,7,8,10,14]. Moreover, if a therapy uses swelling as part of its hemostatic action, then extra care must be taken to ensure that the local blood supply is not reduced or stopped, which could cause additional tissue damage or even death. This is particularly crucial when using expanding foams [19]. Many hemostatic agents must be prepared just before use because of their short shelf-life.

Surgical instruments, such as cautery devices, clamps and clips, must be used by a skilled individual in a controlled environment [2,5,8-10,16,20].

Our discovery, observed during a neurosurgical procedure, introduces a new way to stop bleeding using a self-assembling peptide that establishes a nanofiber barrier and incorporates it into the surrounding tissue to form an extracellular matrix (ECM). Surmising that nanotechnology might be useful in our central nervous system regeneration studies, we injected the material into wound sites in the brain of hamsters to determine whether it would facilitate neuronal regeneration [21]. To our surprise, it also stopped bleeding.

We then wanted to know if the rapid hemostasis that we had observed in our nerve regeneration experiments was tissue specific or would also work in other tissues. The seven experiments we designed and performed demonstrate that in less than 15 seconds complete hemostasis can be achieved after (1) a transection of a blood vessel leading to the superior sagittal sinus in both hamsters and rats, (2) a spinal cord cut, (3) a femoral artery cut, (4) a sagittal transection of the left lateral liver lobe, (5) a transverse transection of the left lateral liver lobe including a cut in a primary branch of the portal vein, (6) a 4-mm liver punch biopsy, and (7) multiple 4-mm skin punch biopsies on nude mice.

Materials and methods

Adult Syrian hamsters were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), and adult rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg). The experimental procedures adhered strictly to the protocol approved by the Department of Health and endorsed by the Committee on the Use of Laboratory Animals for Teaching and Research of the University of Hong Kong and the Massachusetts Institute of Technology Committee on Animal Care.

Cortical vessel cut experiment

The animals were fitted in a head holder. The left lateral part of the cortex was exposed, and each animal received a transection of a blood vessel leading to the superior sagittal sinus (Figure 1, A). With the aid of a sterile glass micropipette, 20 μL of 1% NHS-1 solution (see below under “Preparation of the self-assembling solutions”) was applied to the site of injury or iced saline in the control cases. The animals were allowed to survive for as long as 6 months.

Spinal cord injury experiment

Under an operating microscope, the second thoracic spinal cord segment (T2) was identified before performing a

Fig 1. Schematics of surgical procedures. Rostral is up and caudal is down in all figures. A, Dorsal view of the rat brain. The blue lines depict the blood vessels superficial to the cortex. The boxed area corresponds to location of the lesion and treatment. B, Drawing of ventral view of the lower limb of a rat with the femoral artery in red and sciatic nerve in yellow. C and D, Drawings of a ventral view of rat with abdomen open. Overlying structures have been removed exposing the liver. The lobe was transected with a cut (depicted in red) in both sagittal (C) and transverse (D) directions.
Fig 2. Complete hemostasis in brain and femoral artery. The pictures are time-lapse images at each stage of the experiment for brain (A - D) and femoral artery (E - H). A - D, Adult rat cortex hemostasis. Part of the overlying skull has been removed in an adult rat, and one of the veins of the superior sagittal sinus is transected and treated with 1% self-assembling NHS-1. A, The brain and veins of the superior sagittal sinus (SSS) are exposed. B, Cutting of the vein (arrow). C, Bleeding of the ruptured vein (arrow). D, The same area 5 seconds after application of the self-assembling NHS-1 to the location of the cut (arrow) as seen under the clear NHS-1. E - H, Rat femoral artery hemostasis. Exposure of the neurovascular bundle in the thigh showing the sciatic nerve (*) in each panel. E, Femoral artery and vein exposed. F, Cutting of the artery (arrow). G, Bleeding, masking the artery completely and sciatic nerve partially. H, The same area 5 seconds after application of the self-assembling peptide to the cut (arrow). Note that there is complete hemostasis in the area formed by NHS-1 (covering the entire picture) as it self-assembles in the presence of blood and plasma, revealing the underlying structures. Complete hemostasis was achieved in 10.6 ± 4.1 seconds, significantly different from 367.5 ± 37.7 seconds in controls irrigated with saline (P < 0.0001). Scale bars represent 1 mm.

Femoral artery cut experiment

Rats were placed on their backs, and the hind limb was extended to expose the medial aspect of the thigh (Figure 1, B). The skin was removed, and the overlying muscles were cut to expose the femoral artery and sciatic nerve. The femoral artery was cut to produce a high-pressure bleeder (Figure 2, F). With a 27-gauge needle, 200 µL of 1% NHS-1 solution was applied over the site of injury. In two cases we applied the dry powder of NHS-1 to the site of injury. In another group of 28 adult rats the same procedure was followed for the liver, which was cut transversely (Figure 3, D). With a 27-gauge needle, 400 µL of 1%, 2%, 3%, or 4% NHS-1 or TM-3 solution was applied to the site of injury (Figure 3, H).

In another group of anesthetized adult rats the liver was exposed, and a 4-mm punch biopsy done from the ventral aspect through the liver to the dorsal surface of the left liver lobe. The resulting core was removed from the liver, after which one of three treatments was applied. For the treatment group 200 µL of 3% NHS-1 solution was applied to the site of injury, whereas in the controls either saline was applied or cauteryization of the exposed liver surface was carried out. The superficial material was then wiped clear of the injury site. The abdominal incision was closed, and the animals were allowed to survive for as long as 8 weeks.

Skin punch experiment

In anesthetized adult nude mice using aseptic precautions, a 4-mm punch was used to create three wounds on each side of the back of the animal. On one side of the animal the wounds created were treated with 1% NHS-1 solution, and the wounds on the opposite side were left untreated to provide a control. The punch biopsies were made through the full thickness of the skin. If the wound did not bleed for 10 seconds the punch would be excluded from the data analyzed. All procedures were videotaped, and the analysis consisted of reviewing the tapes. The animals were allowed to survive for as long as 2 months. If animals involved in any of the above experiments appeared to experience any discomfort they were euthanized.
Transmission electron microscopy sample preparation

In the brain and liver of anesthetized adult rats a 1% or 2% NHS-1 solution was injected immediately after making a cut, and the treatment site was sampled. Samples were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB) for 4 hours. The samples were washed in 0.1 M PB three times for 10 minutes each at 4°C and embedded in 2% agar; blocks were postfixed in 4°C 1% osmium tetroxide for 2 hours and then washed in buffer three times for 10 minutes each at 1924°C. The sample blocks were dehydrated in ethanol, infiltrated, and embedded in pure epon with Lynx EM 194 tissue processor. Ultrathin 70-nm sections were cut (Reichert-Jung ultra cut) and collected on no. 200 mesh grids. Sections and grids were stained with uranyl acetate and lead citrate and examined under a Philip EM208S transmission electron microscope.

Preparation of the self-assembling solutions

The NHS-1 solution was prepared using RADA16-I 200 synthetic dry powder (obtained from the Massachusetts Institute of Technology Center for Cancer Research 202 Biopolymers Laboratory, Cambridge, MA; the Zhang 203 laboratory, and 3-DMatrix, Cambridge, MA) dissolved in 204 an Eppendorf tube. The 1% NHS-1 solution was prepared by dissolving 10 mg of RADA16-I powder in 1 mL of 206 autoclaved Milli-Q water (Millipore Corp., Billerica, MA), 207 sonicated for as long as 5 minutes, and filtered. This was 208 repeated with 20 mg/mL, 30 mg/mL, and 40 mg/mL to 209 produce 2%, 3%, and 4% concentrations. NHS-2 and TM-3 210 dry powders (made by the Massachusetts Institute of Technology Center for Cancer Research Biopolymers Laboratory, Cambridge, MA) were prepared using the same method. The time of preparation did not affect the action of the solution. We also tested some material that was prepared 215

Fig 3. Rat liver hemostasis. This series of pictures is of an adult rat wherein the skin covering the intraperitoneal cavity was excised, exposing the liver. A - D, Sagittal cut. A, The left lateral lobe received a sagittal cut completely transecting a portion of the liver lobe. B, The liver is separated (arrow). Note the profuse bleeding. C, The two halves are allowed to come back together, and the bleeding continues (arrow). D, The 1% NHS-1 solution was applied, and the extent of the incision was visible under the transparent assembled NHS-1 (arrow). Complete hemostasis was achieved in 8.6 ± 1.7 seconds, statistically significant when compared to 90.0 ± 5.0 seconds when cauterization was applied, or 301.6 ± 33.2 seconds if irrigated with saline. E - J, Transverse cut. This series of pictures is of a transverse cut to the left lateral lobe in an adult rat. E, The exposed intact liver. F, Applying a transverse cut in the lobe (arrow). G, Profuse bleeding produced when a major branch of the portal vein is cut (arrows). H, Treatment with self-assembling NHS-1. Note the complete cessation of bleeding (in 10.3 ± 0.5 seconds using 2% concentration; 10.0 ± 1.0 seconds and 11.0 ± 1.0 using 3% and 4%, respectively) seen under the clear assembled NHS-1 (arrow). I, 2 minutes after treatment and after the superficial self-assembling NHS-1 has been removed (arrows) to show the extent of cut. J, Bleeding had ceased 15 minutes after NHS-1 treatment. Scale bars represent 1 mm.
Results

Hemostasis in a brain injury

We began our experiments in the brain, removing the overlying skull and performing a complete transection of a 223branch of the superior sagittal sinus in the brain of rats (n = 22415) and hamsters (n = 15) (Figure 1, A). The areas were 225treated with 20 ± L of a 1% solution of RADA16-I (NHS-1) 226self-assembling solution or with iced saline. In the groups 227Q13(Q12) treated with NHS-1 hemostasis was achieved in less than 10 228seconds in both hamsters and rats (Figure 2, A-D and 229Q14(Q13)Supplemental Video 1, “Hemostasis in rat cortex with self- 230assembling peptide treatment”). Control group hamsters 231(n = 5) and rats (n = 5) irrigated with saline bled for more 232than 3 minutes (Figure 4, A). A truncated iced-saline control 233and subsequent treatment with NHS-1 is shown in 234Supplemental Video 2 (“Saline control and treatment with 235Q15Q14Q13 NHS-1 self-assembling peptide in rat cortex.”) Student’s t-test for 236two independent samples in both hamsters and rats showed 237highly significant differences (P < .0001).

Hemostasis in a spinal cord injury

Because blood has been shown to be toxic in neural tissue 239[24] we wanted to know if the spinal environment was 240different from the brain. By quickly bringing bleeding under 241control secondary damage caused by surgery can be reduced. 242After laminectomy and removal of the dura, the spinal cord 243was hemisected at T2, from the dorsal to ventral aspect, 244and treated (n = 5) with 20 ± L of 1% NHS-1. Hemostasis 245was achieved in just over 10 seconds. In the saline controls 246(blood was achieved in just over 10 seconds. In the saline controls 247blood continued until normal clotting occurred at 75.5 248seconds for NHS-1–treated cases (in red), saline controls (in blue), and cautery controls (in yellow). A. In the brain cut, durations were measured from the start of application of self-assembling NHS-1 to the completion of hemostasis after transection of the veins leading to the superior sagittal sinus in the brain of adult rats. Complete hemostasis was achieved in 8.4 ± 2.1 seconds. In the saline controls bleeding continued until 227.0 ± 36.6 seconds. In the hamster brain cut, complete hemostasis was achieved in 9.0 ± 1.8 seconds. In the saline controls bleeding continued until 187.6 ± 34.7 seconds. In the femoral artery cut, complete hemostasis was achieved in 10.5 ± 4.1 seconds. In the saline controls bleeding continued until 367.5 ± 37.7 seconds. In the liver sagittal cut, complete hemostasis was achieved in 8.6 ± 1.7 seconds. In the cautery control (yellow), bleeding continued until 90.0 ± 5.0 seconds, and the saline controls bled for 301.6 ± 33.2 seconds. B. Liver 4-mm punch biopsy. A 4-mm core was removed from the left liver lobe, and the hole was treated with NHS-1, heat cautery, or saline. Treatment with 3% NHS-1 brought about complete hemostasis in 9.7 ± 1.2 seconds. In the cautery controls (yellow) bleeding continued for 81.2 ± 6.7 seconds, and the saline controls bled for 204.3 ± 49.6 seconds. C. Skin 4-mm punch biopsy. A 4-mm punch biopsy was made on the backs of nude mice. The biopsy extended through the dermis, and the core was removed. Care was taken not to disrupt the underlying muscle. The three wounds on one side were treated with 1% NHS-1, and complete hemostasis was achieved in 6.4 ± 1.5 seconds. On the opposite side of the animal the wounds were not treated. Bleeding continued until normal clotting occurred at 75.5 ± 16.3 seconds. D. Concentration response curves of NHS-1 and TM-3. The left lateral liver lobe received a transverse cut severing a portion of the liver lobe and branch of the portal vein. A higher concentration of NHS-1 (open circles) is more effective in achieving hemostasis in 11.0 ± 0.5 seconds, 10.0 ± 1.0 seconds, and 10.3 ± 0.5 seconds, respectively. The 1% NHS-1 solution required 86.6 ± 20.8 seconds at the area of the most severe bleeding. TM-3 (diamonds) was not effective at any concentration, in the saline controls bleeding continued until 377.5 ± 85.0 seconds, and one animal died. Time (seconds) is shown on the x-axis, concentration on the y-axis.

216(obtained from the Zhang laboratory) and stored in solution 217at room temperature, for 3 years before use, and it 218performed as well as the newly mixed material.

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Hemostasis in a high-pressure femoral artery wound

The femoral artery of 14 adult rats was surgically exposed, transacted, and then treated with 200 μL of a 1% solution of NHS-1 or iced saline and packing (Figure 2, E-H). In the treated rats (n = 10) about 10 seconds elapsed before hemostasis occurred (Figure 4, A). The controls (n = 4) continued to bleed for more than 6 minutes. The difference in times to achieve complete hemostasis was highly significant (Student’s t-test P < .0001).

Hemostasis in highly vascularized liver wounds

Using a group of 76 rats, we performed three different liver cuts: (1) a sagittal (rostrocaudal) cut (Figure 3, A and B) to test NHS-1 in an irregular-shaped laceration wound, 264(2) a transverse (lateral-medial) cut involving the transection of a major branch of the hepatic portal vein to intensify bleeding (Figure 3, E-J), and (3) 4-mm punches through the liver lobe to observe the material in uniform wounds.

In the first liver experiment we made a sagittal cut in the 268 left lobe (n = 8); upon treatment with 100 μL of 1% NHS-1 bleeding ceased in less than 10 seconds (Figure 3, 270 A-D and Supplemental Video 3, “Sagittal cut of left liver lobe using 1% self-assembling peptide treatment”). In one set of controls (n = 3) bleeding stopped 90 seconds after cauterization of the wound; in the saline-treated control animals (n = 3) bleeding continued for more than 5 minutes. Comparison of the cauterized and the saline-treated controls shows a significant difference using the Tukey test with a 99% confidence interval.

In the second experiment we severed a major branch of the portal vein while making a transverse cut in the left lobe to 280 test NHS-1 in an environment with a high flow rate. Four 281 concentrations of NHS-1 were tested (n = 12) along with 282 saline-treated control animals (n = 3) bleeding continued for 283 more than 5 minutes. Comparison of the cauterized and 276 the saline-treated controls shows a significant difference 277 using the Tukey test with a 99% confidence interval.

Fig 5. Electron micrographs. This series of TEM images shows the interactions of NHS-1 with liver, cortex, and red blood cells. A, The left lateral lobe was treated with NHS-1, and the tissue was taken shortly after treatment. Note the hepatocyte and its nucleus (HN). There is a red blood cell (RBC) between the assembled NHS-1 (N-1) fields. Scale bar represents 2 μm. B, A closer look at the interface of the RBC and the material. Scale bar represents 50 nm. C, In the liver the RBC do not appear to mix with the NHS-1. Scale bar represents 1 μm. D, Application of 1% NHS-1 solution to a cut in the cortex. Note the close interface with the axons (Ax). Scale bar represents 0.2 μm. E, In another part of the brain the interface between the RBC and the NHS-1 appears to be similar to that in the liver. Scale bar represents 0.1 μm.
We duplicated the test successfully with 400 μL of both 3% NHS-1 and 2% TM-3 solution; bleeding ceased in 10 and 28910.3 seconds, respectively (Figure 4, D). When 400 μL of 2901% NHS-1 was applied, bleeding continued for more than 29160 seconds (Figure 4, D). The controls, however, bled for longer than 6 minutes. The dose response shows that treatment 293results using 3% and 4% NHS-1 are nearly the same as with 294the 2% concentration. Furthermore, in the 2%, 3%, and 4% 295concentration treatment cases complete hemostasis was 296maintained after removing the excess assembled NHS-1 297material (Figure 3, I and J). We found that the higher blood 298pressure/flow rate transverse liver cut required a concentra- 299tion of 2% NHS-1 or higher to bring about complete 300hemostasis in less than 15 seconds. A significant difference 301was found between the NHS-1–treated and control groups 302using analysis of variance (ANOVA). When each treatment 303group was compared to the control group those differences 304were also significant; a Tukey test showed a 99% confidence 305interval. There was no significant difference when the 306various NHS-1 concentrations were compared, except for 307the 1% NHS-1 solution treatment group.

In the third experiment using adult rats (n = 45) we 308punched 4-mm holes through the left lateral lobe and then 309treated the area with 3% NHS-1, saline, or heat cautery to 310bring about hemostasis (Figure 4, B). In the experimental 311group (n = 15) we applied a solution of 3% NHS-1 after 312injury and hemostasis was achieved in about 10 seconds, 313whereas the saline controls (n = 15) required 3.5 minutes to 314stop bleeding. In the heat cautery control group (n = 15) 315cessation of bleeding took more than 60 seconds, inclusive of 316appling heat to cauterize the inside surface of the punch. We 317allowed the NHS-1–treated animals to survive for as long as 318months with no detrimental effect on the tissues. Using 319ANOVA there was a significant difference between the 3% 320NHS-1 treatment and the controls (P < .0001). In addition, 321the Tukey test showed that each group was significantly 322different from the other with a 99% confidence interval.

Hemostasis in skin punch biopsies

Six 4-mm punch biopsies were made on the backs of each 325of 23 anesthetized adult nude mice for a total of 138 punches. 326The punches were treated with 3% NHS-1 solution and the 327other three were left untreated, except for dabbing with cotton 328every 15 seconds until bleeding stopped. Punched wounds 329that bled for less than 10 seconds were excluded from the 330experiment. We applied a solution of 1% NHS-1 10 seconds 332after injury (n = 23), and hemostasis took less than 33310 seconds; the controls (n = 23) continued to bleed for 334more than 60 seconds (Figure 4, C). The bleeding times were 335averaged for each side of the animal, and the Student’s t-test 336for paired samples showed a significant difference between 337the treatment and control side of the animal (P < .0001).

Comparison of three different materials

To learn more about the hemostatic properties and 339mechanism of action of NHS-1 (RADA-16), we repeated 340both the sagittal and transverse liver experiments, comparing 341them with two additional materials that are known to self-assemble and spontaneously form nanofibers: (1) RADA-12 342(TM-3), a sequence variation of NHS-1, and (2) EAK-16 344(TM-3), a different sequence in the same family of self-assembling peptides used to determine if the material’s length and stiffness altered its hemostatic effectiveness in 347bleeding models [25-31].

Making a sagittal liver cut in adult rats (n = 9) we applied 348100 μL of 2% NHS-2 solution to the wound, and 350bleeding stopped in less than 10 seconds. In the cautery 351controls (n = 3) bleeding continued for more than 35290 seconds (P < .0001). Upon repetition of the experiment 353in adult rats (n = 8) using 100 μL of 2% TM-3, the material 354was not effective; however, the higher blood pressure and flow 355rate after the transverse liver cut required a concentration of 3562% or higher of NHS-1 to stop bleeding in less than 35715 seconds.

The increased blood flow from the portal vein after 358making a transverse liver cut allowed us to perform another 359dose response experiment in which we compared various 360concentrations of NHS-1 (1% to 4%) and TM-3 (1% to 3%) 361with controls (Figure 4, D). All concentrations of NHS-1 362were effective; however, the higher blood pressure and flow 363rate after the transverse liver cut required a concentration of 3642% or higher of NHS-1 to stop bleeding in less than 36515 seconds.

TM-3 is a stiffer gel; 1% TM-3 is similar in stiffness to 3663% NHS-1. We tried three different concentration levels 368(1%, 2%, and 3%) and found that TM-3 was not effective at 369lower concentrations; the assembled material fractured and the 370TM-3–treated animals continued to bleed regardless of the 371concentration used. There was actually no significant 372difference between TM-3 and the controls (Figure 4, D) in 373hemostasis.

Interface of NHS-1 and tissues

Still looking for mechanism clues as well as further 376understanding of the relationship of the NHS-1 blood/tissue 377interface in both the brain and liver, we also examined the 378treated tissues using transmission electron microscopy 379(TEM), interested in learning how the red blood cells (RBCs), 380platelets, tissue, and the ECM interact with the material. 381

We applied 1% NHS-1 to a liver wound and immediately 382harvested the tissue. In the electron micrograph the hepato- 383cyte and RBCs look to be intact with the assembled NHS-1 at 384the interface (Figure 5, A). When applied shortly after injury, 385the material appeared to stop the movement of blood from the 386vessels without detrimental effects to the liver’s RBCs; there 387was also no evidence of lysing (Figure 5, B). Furthermore, 388there was no evidence of platelet aggregation [32] at the 389blood/NHS-1 interface (Figure 5, C) when samples were 390taken at various time points after treatment.

In the brain we found a very tight interaction between 392NHS-1 and the neural tissue (Figure 5, D). We observed no 393RBCs and no evidence of platelet aggregation in the 394assembled NHS-1. The RBCs that were present appeared 395
Our study demonstrates that hemoastasis can be achieved in less than 15 seconds in multiple tissues as well as a variety of different wounds. This is the first time that nanotechnology has been used to stop bleeding in a surgical setting for animal models and seems to demonstrate a new class of hemostatic agent that does not rely on heat, pressure, platelet activation, adhesion, or desiccation to stop bleeding. NHS-1 and NHS-2 are synthetic, biodegradable [10,19] and do not contain any blood products, collagens, or biological contaminants that may be present in human- or animal-derived hemostatic agents such as fibrin glue [1,8,10,14,20]. They can be applied directly onto, or into, a wound without the concern that the material may expand, thus reducing the risk of secondary tissue damage as well as the problems caused by constricted blood flow. In our previous brain studies [21] we looked for evidence of the production of prion-like substances or fibril tangles in animals that had the material implanted in their brain for as long as 6 months but found none. Furthermore, the breakdown products of NHS-1 are amino acids, which can be used by the body as tissue building blocks for the repair of the injury [21]. Independent third-party testing of the material found no pyrogenticity, which has been found with the ability of the material to flex with the tissue as blood flowed through the organ. This is similar to the fracturing of an artery when grown in a laminar flow environment and then transplanted to a pulsed environment. The cells line up 400Q23 along the direction of flow, unlike the natural helical coil [38-41] seen in a pulsed environment, which allows for expansion and contraction, without splitting, as blood moves through the artery. Conversely, NHS-1 and NHS-2 were able to flex with the tissue.

Finally, NHS-2, the most pliable of the three materials, seemed to perform identically to NHS-1, probably as a result of their similar structure and modulus.

With this discovery the ability to speedily achieve hemoastasis will reduce radically the quantity of blood needed during surgery of the future. As much as 50% of surgical time can be spent packing wounds to reduce or control bleeding. The NHS solutions may represent a step change in technology and could revolutionize bleeding control during surgery and trauma; however, they still require clinical testing before they can be used in humans.

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