<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Nano hemostat solution: immediate hemostasis at the nanoscale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>EllisBehnke, RG; Liang, YX; Tay, DKC; Kau, PWF; Schneider, GE; Zhang, S; Wu, W; So, KF</td>
</tr>
<tr>
<td><strong>Citation</strong></td>
<td>Nanomedicine: Nanotechnology, Biology, And Medicine, 2006, v. 2 n. 4, p. 207-215</td>
</tr>
<tr>
<td><strong>Issued Date</strong></td>
<td>2006</td>
</tr>
<tr>
<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10722/54239">http://hdl.handle.net/10722/54239</a></td>
</tr>
<tr>
<td><strong>Rights</strong></td>
<td>Nanomedicine: Nanotechnology, Biology and Medicine. Copyright © Elsevier Inc.; This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.</td>
</tr>
</tbody>
</table>
Nano hemostat solution: immediate hemostasis at the nanoscale

Rutledge G. Ellis-Behnke, PhD,a,b,c,* Yu-Xiang Liang, PhD, b,c David K.C. Tay, PhD, b,c
Phillis W.F. Kau, BSc,b Gerald E. Schneider, PhD,a Shuguang Zhang, PhD,d Kwok-Fai So, PhD,b,c,e

*Corresponding author. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
E-mail address: rutledg@mit.edu (R.G. Ellis-Behnke).

Received 22 August 2006; accepted 22 August 2006

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

Through the ages doctors have found ways to achieve hemostasis, beginning with the simple act of applying pressure, then cauterization, ligation, and clinically induced vasoconstriction [1-10], but nanotechnology brings new possibilities for changes in medical technology. Here we present a novel method to stop bleeding using materials that self-assemble at the nanoscale when applied to a wound. This method results in the formation of a nanofiber barrier that stops bleeding in any wet ionic environment in the body; furthermore, the material is broken down into natural l-amino acids that can be used by the surrounding tissue for repair.

Currently there are three basic categories of hemostatic agents or procedures: chemical, thermal, and mechanical [1,3,6,8,10-15]. Chemical agents are those that change the clotting activity of the blood or act as vasoconstrictors, such as thromboxane A2 [16], which causes vessels to contract thus reducing blood flow and promoting clotting [7,16,17]. Thermal devices commonly involve cauterization using electrodes, lasers [8,14], or heat. There are also agents that react exothermically upon application that may create an effect similar to a standard two probe cautery device [1,14]. Mechanical methods use pressure or ligation to slow the blood flow [3]. A combination therapy might use both chemical and mechanical means to produce a hemostat that adsorbs fluid and swells [18], producing pressure to slow the blood flow and allow clotting, or it may involve the introduction of fibrinogen, thrombin, and...
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...
dorsal laminectomy in anesthetized adult rats [22,23]. After opening the dura mater, we performed a right hemisection using a ceramic knife. Immediately after the cord hemisection, the femoral artery was cut to produce a high-pressure bleeder area of the cut for bleeding control. The controls received a saline treatment. The animals were allowed to survive for as long as 8 weeks as part of another experiment.

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 injury site, which also was effective. (Data are not shown and were not included in the analysis.) Controls were treated with a combination of saline and pressure with a gauge. All animals were killed 4 hours after the experiment.

Liver wound experiments

Rats were anesthetized and placed on their back, and the abdomen was opened exposing the liver (Figure 1, C). The left lobe of the liver was cut using a scalpel in the rostral-to-caudal direction, separating the two halves of the lobe (Figure 3, B) in the sagittal cut. With a 27-gauge needle, 100 μL of 1% or 2% NHS-1, NHS-2, or TM-3 solution was applied to the site of injury (Figure 3, B). Livers of the controls were treated with saline or cauterized. Cauterization was performed using a thermal cautery device and was applied to the entire surface of the 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).

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 were created 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 synthetic dry powder (obtained from the Massachusetts Institute of Technology Center for Cancer Research Biopolymers Laboratory, Cambridge, MA; the Zhang laboratory, and 3-DMatrix, Cambridge, MA) dissolved in an Eppendorf tube. The 1% NHS-1 solution was prepared by dissolving 10 mg of RADA16-I powder in 1 mL of autoclaved Milli-Q water (Millipore Corp., Billerica, MA), sonicated for as long as 5 minutes, and filtered. This was repeated with 20 mg/mL, 30 mg/mL, and 40 mg/mL to produce 2%, 3%, and 4% concentrations. NHS-2 and TM-3 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.
216(obtained from the Zhang laboratory) and stored in solution at room temperature, for 3 years before use, and it was performed as well as the newly mixed material.

219 Results

220 Hemostasis in a brain injury

221 We began our experiments in the brain, removing the 222 overlying skull and performing a complete transection of a 223 branch of the superior sagittal sinus in the brain of rats (n = 224 225) and hamsters (n = 15) (Figure 1, A). The areas were 226 treated with 20 µL of a 1% solution of RADA16-I (NHS-1) 227 self-assembling solution or with iced saline. In the groups Q13 Q14, hemostasis was achieved in less than 10 seconds in both hamsters and rats (Figure 2, A–D and Video 1, “Hemostasis in rat cortex with self-assembling peptide treatment”). Control group hamsters 231 and rats (n = 5) irrigated with saline bled for more than 3 minutes (Figure 4, A). A truncated iced-saline control

and subsequent treatment with NHS-1 is shown in Figure 233 B. Hemostasis in a spinal cord injury

234 Because blood has been shown to be toxic in neural tissue 235 [24] we wanted to know if the spinal environment was different from the brain. By quickly bringing bleeding under control secondary damage caused by surgery can be reduced. After laminectomy and removal of the dura, the spinal cord was hemisected at T2, from the dorsal to ventral aspect, and treated (n = 5) with 20 µL of 1% NHS-1. Hemostasis was achieved in just over 10 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 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 ± 1.0 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.
Hemostasis in a high-pressure femoral artery wound

The femoral artery of 14 adult rats was surgically exposed, transacted, and then treated with 200 \( \mu \)L 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 \( T \)\( _{\text{test}} \) \( P < 0.0001 \)).

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, (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 \( \mu \)L of 1\% NHS-1 269 solution bleeding ceased in less than 10 seconds (Figure 3, 270 A-D and Supplemental Video 3, “Sagittal cut of left liver 271 lobe using 1\% self-assembling peptide treatment”). In one 272 set of controls (\( n = 3 \)) bleeding stopped 90 seconds 273 (Figure 4, A) after cauterization of the wound; in the 274 saline-treated control animals (\( n = 3 \)) bleeding continued 275 for more than 5 minutes. Comparison of the cauterized and 276 saline-treated controls shows a significant difference 277 using the Tukey test with a 99\% confidence interval.

In the second experiment we severed a major branch of the 279 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 (\( n = 4 \)) control animals. We applied 400 \( \mu \)L of 4\% 283 concentration NHS-1, and bleeding stopped in 11 seconds 284 (Figure 3, E-J and Supplemental Video 4, “Transverse cut of 285 left liver lobe using 4\% self-assembling peptide treatment”). 286

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\( \mu \)m. 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\( \mu \)m. 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\( \mu \)m. 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\( \mu \)m.
Six 4-mm punch biopsies were made on the backs of each animal. Three punches were treated with 1% NHS-1 solution and the other three were left untreated, except for dabbing with cotton 292very 15 seconds until bleeding stopped. Punched wounds 300that bled for less than 10 seconds were excluded from the 301experiment. We applied a solution of 1% NHS-1 10 seconds 322after 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).

338Comparison of three different materials

339To learn more about the hemostatic properties and 340mechanism of action of NHS-1 (RADA-16), we repeated 341both the sagittal and transverse liver experiments, comparing 342them with two additional materials that are known to self-assemble and spontaneously form nanofibers: (1) RADA-12 343(NHS-2), 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 345bleeding models [25-31].

Making a sagittal liver cut in adult rats (n = 9) we applied 100 µL of 2% NHS-2 solution to the wound, and 350bleeding stopped in less than 10 seconds. In the cautery experiments (n = 3) controls (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 354assembled but did not achieve hemostasis; the animals 355continued to bleed until the experiment was terminated after 356more than 3 minutes.

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 369any concentration; 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 373achieving hemostasis.

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 hepatocytes 383and RBCs look to be intact with the assembled NHS-1 at the 384interface (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 was 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 392the blood and 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 395normal.
424other safety issues in animals[33].

423some other hemostatic agents, and no systemic coagulation or
422material found no pyrogenicity, which has been found with
421blood products, collagens, or biological contaminants that
420may be present in human- or animal-derived hemostatic
419agents such as fibrin glue[1,8,10,14,20]. They can be applied
418directly onto, or into, a wound without the concern that the
417material may expand, thus reducing the risk of secondary
416tissue damage as well as the problems caused by constricted
415blood flow. In our previous brain studies[21] we looked for
414evidence of the production of priam-like substances or fibril
413tangles in animals that had the material implanted in their
412brain for as long as 6 months but found none. Furthermore,
411the breakdown products of NHS-1 are amino acids, which
410can be used by the body as tissue building blocks for the
409repair of the injury[21]. Independent third-party testing of the
408material found no pyrogenicity, which has been found with
407some other hemostatic agents, and no systemic coagulation or
406other safety issues in animals[33].

405The exact mechanism for the hemostasis reported here
404is not fully understood, but we have uncovered several
403clues. First, we know that the hemostasis is not explainable
402by clotting. Blood clots are produced after injury, but do
401not begin to form until 1 to 2 minutes have elapsed, de-
400pending upon the status and coagulation history of the
400patient[6,12,34].

400Second, the electron micrographs show no evidence of
400platelet aggregation at the interface of the material and wound
400site. That arginine inhibits platelet aggregation suggests that
400the arginine in NHS-1 plays a role in this effect[4,35-37];
400this seems to be consistent with our data. The NHS-1 and
400NHS-2 solutions appear to self-assemble into a barrier,
400stemming the flow of blood and facilitating the movement of
400adjacent cells to repair the injured site[21].

400Third, in our experiments the NHS-1 and NHS-2 solutions
400easily filled in and conformed to the irregular shapes of the
400wounds before assembling, as shown in the electron micro-
400graphs. We believe this tight contact is crucial to the
400hemostatic action because of the size of the self-assembling
400peptide units. The micrographs also showed that the material
400does not cause the RBCs to lyse, apparently protecting them
400from normal degradation when exposed to the air.

400Fourth, we do not believe that the hemostasis can be
400explained by gelation kinetics. One would think that a stiffer
400gel would be more effective for higher pressure bleeds; how-
400ever, we found the opposite to be true. TM-3, which is
400from the same family of peptides as NHS-1 and NHS-2, and
400is the stiffest of the three self-assembling peptides tested, did
400not arrest bleeding; it fractured at the tissue interface and
400within the resultant gel. We surmise that TM-3 may have
400fractured because of (1) the pulsations of the liver and (2) the
400inability of the material to flex with the tissue as blood
d molded through the organ. This is similar to the fracturing
400of an artery when grown in a laminar flow environment and
400then transplanted to a pulsed environment. The cells line up
d056400along the direction of flow, unlike the natural helical coil
400[38-41] seen in a pulsed environment, which allows for a
400expansion and contraction, without splitting, as blood
d056400moves though the artery. Conversely, NHS-1 and NHS-2
400were able to flex with the tissue.

400Finally, NHS-2, the most pliable of the three materials,
400seemed to perform identically to NHS-1, probably as a
400result of their similar structure and modulus.

400With this discovery the ability to speedily achieve
d056400hemostasis will reduce radically the quantity of blood needed
d056400for surgery of the future. As much as 50% of surgical time
400can be spent packing wounds to reduce or control bleeding. The
400NHS solutions may represent a step change in 473technology and could revolutionize bleeding control during
400surgery and trauma; however, they still require clinical testing
400before they can be used in humans.

Acknowledgments

477The authors wish especially to thank both Dr. Ed
478Tehovnik (Brain and Cognitive Sciences Department, 479Massachusetts Institute of Technology, Cambridge) and 480Dr. Chi-Sang Poon (Health Sciences and Technology
481Department, Massachusetts Institute of Technology, Cam-
482bridge) for their valuable editing assistance. Thanks also to
483Dr. W.W. for assistance in performing the surgery.

References

484[1] Alam HB, Burris D, DaCorta JA, Rhee P. Hemorrhage control in the
4841909;35.
484novel approach to endoscopic therapy for esophageal perforation. Disch
484[4] Dunser MW, et al. Does arginine vasopressin influence the coagula-
484tion system in advanced vasodilatory shock with severe multiorgan
484Obstet 1915;21:452.
484p. 335-52.
484[7] Pallapies D. Vasotive drugs with an effect on the prostaglandin 504
[15] Konturek SJ, Pawlik W. Physiology and pharmacology of prosta-
[16] Konturek SJ, Pawlik W. Physiology and pharmacology of prosta-
[17] Greer IA. Therapeutic progress—review XXVIII. Platelet function and
[19] Sabel M, Stummer W. The use of local agents: Surgicel and
[20] Bhanot S, Alex J. C. Current applications of platelet gels in facial
surgical application: an experimental study. Eur J Cardiothorac Surg
relationship of the bleeding time test with parameters of surgical
[22] Wu W. Expression of nitric-oxide synthase (NOS) in injured CNS
[23] Yick LW, So KF, Cheung PT, Wu WT. Lithium chloride reinforces the
regeneration-promoting effect of chondroitinase ABC on rubrospinal
Interactions of the alkyl-ether-phospholipid, platelet activating factor
(PAF) with platelets, neural cells, and the psychotropic drugs
self-complementary oligopeptide to form a stable macroscopic

[26] Zhang S, et al. Self-complementary oligopeptide matrices support
[27] Holmes TC, et al. Extensive neurite outgrowth and active synapse
formation on self-assembling peptide scaffolds. Proc Natl Acad Sci
USA 2000;97:6728-33.
biological materials through self-assembly of peptides and proteins.
[29] Zhang S. Fabrication of novel biomaterials through molecular self-
Control of self-assembling oligopeptide matrix formation through
systematic variation of amino acid sequence. Biomaterials 2004;
[31] Caplan MR, Moore PN, Zhang S, Kamm RD, Lauffenburger DA. Self-
assembly of a β-sheet protein governed by relief of electrostatic
repulsion relative to van der Waals attraction. Biomacromolecules
[33] Zhang S, Zhao X, Spirio L. In: Elisseeff PXMaJ, editor. Scaffolding
in tissue engineering. Boca, Raton, FL: Taylor & Francis/CRC Press;
[34] Moore Jr JE, et al. A device for subjecting vascular endothelial cells to
both fluid shear stress and circumferential cyclic stretch. Ann Biomed
[35] Finlay HM, McCullough L, Canham PB. Three-dimensional collagen
formation on self-assembling peptide scaffolds. Proc Natl Acad Sci
Endosc 2004;60:327-33.
[38] Carr Jr ME. Monitoring of hemostasis in surgical application: an experimental study. Eur J Cardiothorac Surg
[39] Ives CL, Eskin SG, McIntire LV. Mechanical effects on endothelial
[40] De Caterina R, et al. Bleeding time and bleeding: an analysis of the
relationship of the bleeding time test with parameters of surgical
[41] Sipkema P, van der Linden PJ, Westerhof N, Yin FC. Effect of cyclic
axial stretch of rat arteries on endothelial cytoskeletal morphology and
organization of human brain arteries at different transmural pressures.
[42] Zhang S, et al. Self-complementary oligopeptide matrices support
[43] Holmes TC, et al. Extensive neurite outgrowth and active synapse
formation on self-assembling peptide scaffolds. Proc Natl Acad Sci
USA 2000;97:6728-33.
biological materials through self-assembly of peptides and proteins.
[45] Zhang S. Fabrication of novel biomaterials through molecular self-
Control of self-assembling oligopeptide matrix formation through
systematic variation of amino acid sequence. Biomaterials 2004;
[47] Caplan MR, Moore PN, Zhang S, Kamm RD, Lauffenburger DA. Self-
assembly of a β-sheet protein governed by relief of electrostatic
repulsion relative to van der Waals attraction. Biomacromolecules
in tissue engineering. Boca, Raton, FL: Taylor & Francis/CRC Press;
[50] Lind SE. The bleeding time does not predict surgical bleeding. Blood
1991;77:2547-52.
Antithrombotic effect of l-arginine in hypertensive rats. J Physiol
2005;570:55-64.
[52] Wang WT, Lin LN, Pan XR, Xu ZJ. Effects of L-arginine on the func-
tion of platelet aggregation during hepatic ischemia/reperfusion in-
[53] Wang YY, Yang YZ, Dong M, Liu XY, Deng SQ. Inhibition of platelet
[54] Finlay HM, McCullough L, Canham PB. Three-dimensional collagen
axial stretch of rat arteries on endothelial cytoskeletal morphology and
both fluid shear stress and circumferential cyclic stretch. Ann Biomed
[57] Ives CL, Eskin SG, McIntire LV. Mechanical effects on endothelial