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ORIGINAL RESEARCH

In-situ Electrospinning for Intestinal Hemostasis

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Introduction: During routine surgery, rapid hemostasis, especially the rapid hemostasis of internal organs, is very important. The emergence of in-situ electrospinning technology has fundamentally solved this problem. It exhibits a high speed of hemostasis, and no bleeding occurs after surgery. Thus, it is of great significance. The use of sutures in some human organs, such as the intestines and bladder, is inadequate because fluid leakage occurs due to the presence of pinholes.

Methods: Three types of large intestine wounds with an opening of about 1 cm were investigated. They were untreated, treated by needle and threaded, and treated by hand-held electrospinning, respectively.

Results: The results show that hand-held electrospinning technique effectively prevented the exudation of fluids in the intestinal tract. The average diameter of the nanofibrous membrane was about 0.5 μm with hole of several micrometers. It can be elongated 90% without breakage. The hand-held electrospinning device could be used with nitrile gloves, preventing the risk of infection caused by exposed hands.

Discussion: This work can provide a reference for future animal experiments and clinical experiments. However, safety should be investigated before application.

Keywords: electrospinning, hemostasis, nanofibers

Introduction

Whether in war, common traffic accidents, criminal activities, surgical accidents, or other harmful accidents, uncontrolled bleeding leads to a large number of deaths.[1] Although blood transfusions can delay death and extend surgery times, in large-scale disasters, the lack of blood supply is still a common difficulty, especially for rare blood types. Therefore, the development of rapid hemostasis will save the lives of patients. Trauma and internal injuries are two common wound types. For trauma, hemostasis methods include tourniquet hemostasis, filling compression, ligation, and suturing, which can effectively treat an arterial hemorrhage.[2,3] For internal injuries, additional hemostasis method is still required to assist the surgery.[4] For internal injuries, the complex soft tissue injuries, dense local microvascular groups and capillaries lead to prolonged operation times. Consequently, it is necessary to rely on some wound treatment methods, such as electrocoagulation,[5] ultrasonic coagulation,[6] and fusion.[7] Electrospinning has recently been developed for hemostasis of internal injuries. Electrospinning has forms of coaxial,[8] modified coaxial,[9] tri-axial,[10] side-by-side[11] and multiple-fluid processes,[12] and the processes are becoming more and more complicated for preparation.[13,14] However, facile and ease preparation is always popular for real applications. By using in situ electrospinning, using which method the nanofibers are deposited directly on the wound

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by electrospinning, and the nanofibers can fit well with the wound surface. A thin layer of degradable material can be used to complete the hemostasis of the wound surface without post-operative bleeding and maintain low toxicity. There have been reports of hemostasis in the liver,[15] meningeal closures,[16] and the like[17,18] using this method. In contrast, separate spinning uses electrospinning technology to first obtain the fiber membrane and then use it as a wound dressing. This requires additional fixation and the fiber membrane will have to be thick to support itself, which limits its application in visceral hemostasis.

Typical materials in the electrospinning process include cyanoacrylates (CA),[15] chitosan,[19] alginate,[20,21] polycaprolactone (PCL),[22] and other similar materials.[23–25] Chitosan and alginate are difficult to use directly in human organs to stop bleeding because they are soluble in water, and PCL shows advantages over CA due to its lower toxicity. PCL has been approved by the US Food and Drug Administration (FDA) for drug delivery vehicles with low biotoxicities. In addition, its melting point is only 60°C, so it can be deposited by melt electrospinning to avoid solvent evaporation.[26,27] Furthermore, its solvent, acetone, is one of the three solvents that the medical field allows to be directly used in the human body (water, alcohol, and acetone), so the deposition of PCL using electrospinning technology is a good choice for wound treatment.[28]

Human intestines have their own special hemostasis requirements. Because the intestines often contain undigested fluids, especially when the postoperative digestion is weak, fluid foods create fluid in the intestines. Therefore, the sealing requirements for intestinal wounds are more demanding. Conventional sutures can lead to leakage of the liquids at the pinholes. Under stress conditions, the leakage will be more pronounced, requiring a fast and effective wound closure to prevent the exudation of fluids in the gut. In this work, we show the use of electrospinning technology for rapid nano-enclosures of the intestine, which establishes a foundation for future biological experiments.

Experimental Section

Poly-ε-caprolactone (PCL, Mn=70,000–90,000, ACROS) was purchased from Sigma Aldrich, and acetone was purchased from the Sinopharm (Shanghai). The PCL powders were dissolved in acetone, and the PCL mass fraction was 15%. The solution was stirred for 3 h using a magnetic stirrer to obtain a clear, uniform solution. Approximately 1 mL of the solution was loaded into a 5-mL syringe using a 0.7 mm inner diameter injection needle. Wound treatment was performed by hanging a hand-held electrospinning device manufactured by the Junada company 3–5 cm from the organ. The inverter of the hand-held devices can boost battery voltage to 10 kV. The liquid supply rate is controlled at about 1mL/h. To examine the morphologies of the PCL electrospun fibers, scanning electron microscopy (SEM, TM-1000, Hitachi) was used. The stress–strain behaviors of the PCL electrospun fibers were tested using an Instron 3300 Universal Testing System. In order to study the effect of intestinal closure, we performed a simulation experiment using a pig large intestine. The fresh intestine was cleaned first, and then large intestines were treated in three different ways. A scalpel was used to cut wounds approximately 1 cm long in each section. For the first way, a group sections of intestine were kept open. For the second way, a needle thread suture was used to close a group sections of intestine. The electrospinning technology was used for wound closure as the third way of treatment, and an additional section was also closed using electrospinning technology for demonstration. The wound treatment time was approximately 10 s.

Results and Discussion

Electrospinning technology is an emerging hemostasis technology that uses high-voltage electrostatic traction to extract polymer chains from a solution to form a nanofiber membrane. The fibers are often in the range of a few hundred nanometers in diameter with many voids. At present, there are two methods for using the electrospinning technique for hemostasis treatment. In one method, a soft, porous dressing is prepared using electrospinning technology, which is then used to cover the wound for hemostasis. This method allows nanoparticles and drugs to be loaded in the fibers, and thus, the fibers are versatile. However, this dressing technique is similar to the traditional dressing method, and it has difficulty stopping the bleeding of internal organs. Recently, the Long Yunze Group of Qingdao University and the Junada Company began to use portable electrospinning equipment to treat wounds in situ and achieved positive results.[15,29] This was because the fibers could be attached to the surface of the organ better during in-situ electrospinning. Since the electrospun fibers are soft and have small diameters, the distance between the first fiber layer and the surface of the organ is usually only approximately 100 nm, which allows polar interactions and van der Waals forces to

produce strong adsorptive forces and allows the fibers to resist hydrostatic pressure.[16]

Figure 1 shows a photograph of this electrospinning technique. A hand-held electrospinning device (HHE-1, Junada) was used in the experiments. This device does not rely on a fixed power supply. It can generate several thousand volts using only two AAA dry batteries and complete the electrospinning process. The device uses the human body as a wire, but since the high-voltage system uses less charge, it does not affect the user. This does not require additional wires, so the operation is simple. The device only requires the spinning solution to be inserted into a common 5-mL syringe. The organ to be treated was held in the left hand (a pig's large intestine in this study), the hand-held electrospinning device was held in the right hand, and the power was turned on by the right thumb. The spinning process could be completed by advancing the syringe, and the precise dressing of the wound could be achieved by controlling the spinning area by controlling the needle position, requiring less material (only a few tens of microns thick after deposition), which could greatly reduce the immune response and material degradation toxicity.[30] In general, the electrospinning process encounters polarization problems during fiber deposition, and thus, a conductive circuit is required. In this study, the electrospinning process could be completed even with nitrile gloves. This may have been due to the thinness of the nitrile gloves, which could conduct small currents at high voltages. This finding indicated that the use of a hand-held electrospinning device can be accomplished while wearing nitrile gloves, preventing the biofouling caused by bare hands.

Figure 1 Handling of the intestinal incision by the hand-held electrospinning device.

Figure 2 shows an SEM photograph of an electrospun PCL nanofiber membrane. The coarser fiber diameters were mostly 1–1.2 μm, while the finer fibers diameters were mostly 200–400 nm. The coarser fibers could provide mechanical strength as a skeleton. The finer fibers approached the nanometer scale, thereby producing stronger van der Waals or polar interactions with the biological tissue, allowing the fibers to be well adhered to the surface of the organ.

The stress–strain curve is an important indicator of the tensile properties of a material (Figure 3). On the one hand, when used on an organ, uncontrollable organ stretching occurs during the operation. On the other hand, the material itself must be able to withstand a certain external force without being destroyed. We determined through multiple stress–strain curve tests that the material broke at approximately a 90% elongation, and the fiber film could withstand stress of about 1 MPa before breaking. Compared with similar nanofibers, the fibers possessed fairly good stretching properties.[31] Meanwhile, the fibers were stronger than similar nanofibers.[32,33] The stress–strain curve shows that the PCL fiber membrane prepared by electrospinning had a reliable mechanical strength and was suitable for medical experiments. Organs such as the digestive tract and bladder typically have special treatment requirements. Since the inside of the organ contains a stress-carrying liquid, simple suturing tends to introduce a liquid seepage risk. Since PCL nanofibers had good hydrophobicity properties and the electrospun fibers themselves tended to form intact fiber membranes, they can be used in such a situation. To examine this, we conducted exploratory experiments using a pig's large intestine. A wound and closure are shown in Figure 4A and B, respectively. The test setup is shown in Figure 4C.

For the convenience of observation, we diluted ink with deionized water to simulate intestinal fluid, injected 3 mL of liquid into the three-stage intestine, and suspended the sample for a specified time. After 3 min, liquid exudation was observed. As evident in Figure 5(A,D,G),

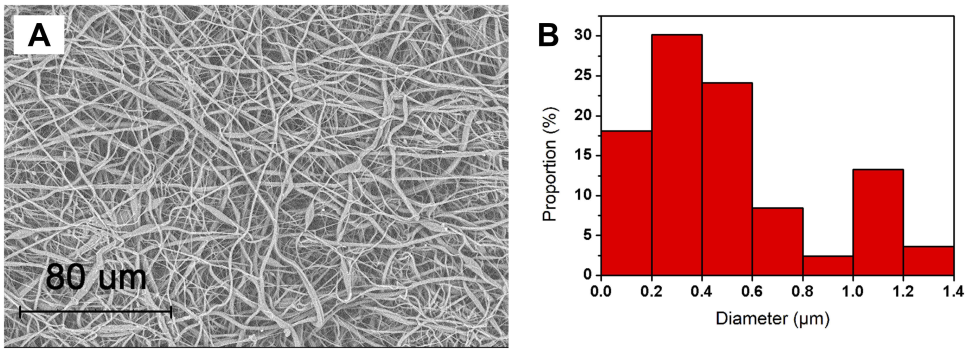

Figure 2 (**A**) Scanning electron microscope image and (**B**) fiber diameter distribution of the electrospun poly-ε-caprolactone fibers.

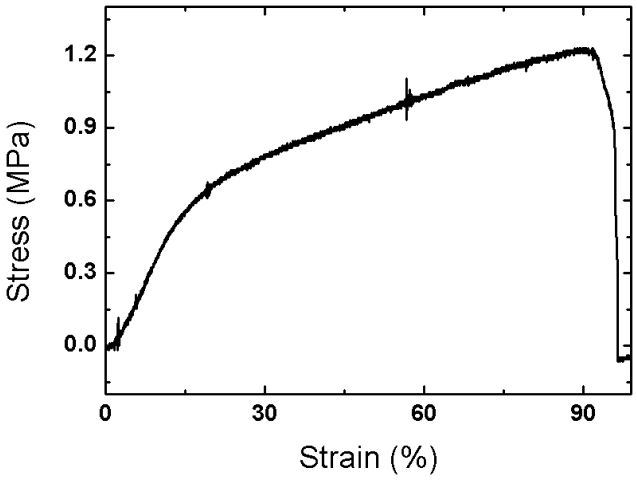

Figure 3 Stress–strain curve of electrospun poly-ε-caprolactone nanofiber membrane.

a large amount of liquid flowed out of the untreated slit. After suture stitching, the amount of fluid outflow was reduced relative to the untreated wound, but there was still liquid outflow, and most of these fluids flowed out from the pinholes, as shown in Figure 5(B,E,H). In sharp contrast to the former two samples, no liquid outflow occurred for the wound closed by the electrospun fibers, and thus, no droplets dripped into the beaker. However, a small amount of liquid seeped out over a long period of inactivity. Careful observation revealed that the liquid did not seep from the vicinity of the wound, so it may have originated from the liquid exudation effect of the intestine itself, as shown in Figure 5(C,F,I).

We know that electrospun fibers have different mechanical properties due to their different thicknesses. Therefore,

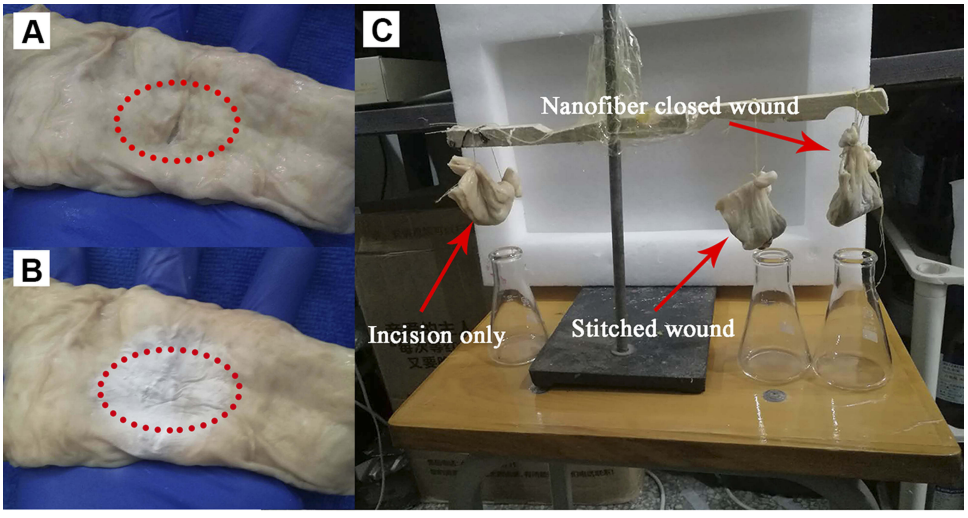

Figure 4 Incision closure for electrospinning demonstration and initial state of the experiment. (**A**) The wound produced by cutting a pig's large intestine using a scalpel. (**B**) The wound after treatment using the hand-held electrospinning device for 10 s. (**C**) A photograph of the initial experimental setup. The pig's large intestine with only the incision was hung at the left end. The middle sample was the intestine after suturing using a needle and thread. The sample on the right end was the intestine closed using the hand-held electrospinning device.

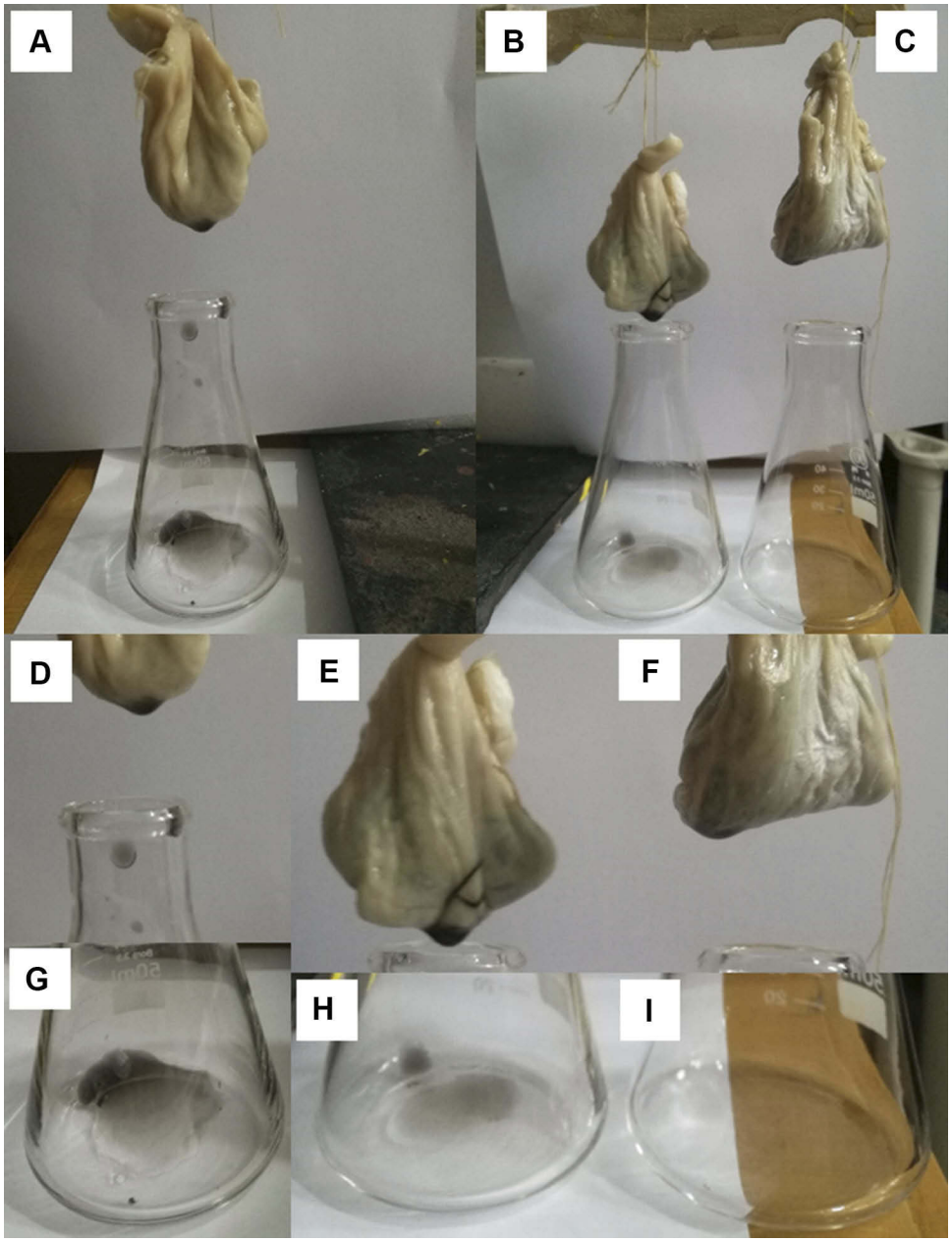

Figure 5 Demonstration of the leaking of the wound at 3 min. Leakage of the large intestine (**A**) with only the incision, (**B**) from the intestine treated by needle and thread, and (**C**) from the intestine treated by electrospinning. (**D**–**F**) Suspended liquid droplets corresponding to (**A**–**C**), respectively. (**G**–**I**) Fallen and collected droplets corresponding to (**A**–**C**), respectively.

if softer fibers are needed, the hand-held electrospinning device can be placed farther away from the wound, so that the length of the straight fluid jet is larger and the fiber diameter is smaller,[9,14] so softer. Reducing the deposition time also improves the flexibility of the fiber membrane. If stronger fibers need to be deposited, the hand-held device can be placed closer to the wound. At this time, the length of the straight fluid jet is smaller and the fiber diameter is larger,[9,14] so it is stronger. Increasing the deposition time also increases the strength of the fiber membrane. The control of fiber morphology can also be used in drug-loaded multifunctional hemostasis. For example, for cancer resection, chemotherapeutic drugs can be loaded to control recurrence. To reduce the release rate of chemotherapeutic drugs, thicker fibers are required, which requires larger deposition distance.

In addition, unlike the skin and liver, the large intestine has its own unique needs for hemostasis. When the skin is hemostatic, the dressing can be fixed on the surface of the skin with an adhesive tape, so no matter the traditional

hemostatic method, ex situ electrospinning hemostasis and in situ electrospinning hemostasis are good choices. For liver hemostasis, in situ electrospinning has begun to show advantages, as it does not require fixation and thus has better practical value. For the large intestine to hemostasis, since the large intestine has only a thin layer, electrocoagulation and so on will damage the large intestine and cause secondary leakage. Therefore, electrospinning can hardly be replaced in this case.

Discussion about safety. Acetone is a commonly used medical solvent, so the the relevant solution can be strictly prepared during the aseptic operation in the laminar flow surgery room, thereby ensuring the sterility of the used solution. In the in-situ electrospinning process, because the fibers are nanoscale, acetone will be completely volatilized during the flight, the deposited PCL is solid, and the solvent residue can be ignored. This experiment is an in vitro model experiment, so there is no clinical safety data. If the method is to be used clinically, relevant certifications must be developed in accordance with the ICH guidelines before use.[34]

Conclusions

We used a hand-held electrospinning device to seal an intestinal incision for the first time to simulate intestinal hemostasis. Experiments showed that the in-situ electrospinning had a good sealing effect on the intestinal incision and could prevent the exudation of the intestinal fluid. In addition, our experiments showed that hand-held electrospinning devices can be used while the operator is wearing nitrile gloves, which is of great significance for the clinical use of hand-held electrospinning devices. This research laid the foundation for future animal experiments and clinical trials.

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Disclosure

The authors report no conflicts of interest in this work.

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