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Novel Porous Silica Granules for Instant Hemostasis

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For emergency bleeding control, there is a strong demand for topical hemostatic materials which could not only stop bleeding rapidly but also be carried and used conveniently. The aim of this work was to develop a novel type of porous silica material and investigated its hemostatic performance. The porous silica granules like-spherical were prepared via dry-mixing and wet-granulation with diameter of 0.40 mm-1.10mm. Granulation reinforced the infiltrating ability of porous silica materials with fluid and stabilized their capillary structure. Ability of rapid water absorption enhanced 130% for the porous silica granules compared to the mesoporous silica particles. In vitro coagulation studies showed clotting time of blood was shorten greatly from 150 seconds for mesoporous silica particles to 30 seconds for mesoporous silica granules at early stage of hemostasis. In vivo studies using rat injury model demonstratedthe granules' ability to aid in rapid hemostasis. The usability of silica material was improved significantly by granulation through enhancing its flowability and eliminating dust. This study suggested the porous silica granules were good candidate of hemostatic agent in clinical and family application.

Introduction

Hemorrhaging is one of the leading causes of death among military and civilian casualties. Stop bleeding is the basic task of trauma cases[1]. Hemostasis has three phases: stopping blood loss should occur quickly at primary phase while the final stage of debridement is a tedious process with carefully. Accelerating the clotting rate at primary stage of hemostasis is crucial to control massive blood loss[2, 3]. The body's natural mechanisms are not able to control massive hemorrhaging caused by major trauma or surgery, resulting in the requirement of hemostatic intervention. Depending on the type of injury and the capabilities at the site of treatment, we could choose hemostatic approach intravenously or topically. At hospitals, provided with conditions of storage and handling capabilities, active hemostatic agents which typically include one o rmore hemostatic active proteins such as thrombin, fibrin and collagen could be used both intravenously and topically[4-10]. Non-protein materials without the limitation of storage are widely used in emergency topically[3, 4, 10-12]. Mesoporous silica(MPS) has been paid much attention as hemostatic materials owing to the excellent biocompatibility and unique physicochemical properties (including large volume and surface area). The hemostatic mechanism of MPS was as follows: MPS could be soaked by blood via the hydrogen bonds formed between hydroxyl on the external surface of silica and water molecules in the blood. The external and internal mesoporous of MPS and voids between particles displayed the capillary phenomenon when it contacted with blood. By absorbing water in the blood and condensing the platelet and clotting factors, MPS achieved the hemostatic effect. But the MPS particles in the form of powder remained the following shortcomings. Firstly, particles floating on the surface of mass blood could not be immersed quickly in blood due to low density and poor flowability. Secondly, the poor compressibility of particles made it could not be controlled well when applying pressure with gauze after particles was poured on the bleeding site. Thirdly, particles both used in family and clinical could result in dust pollution which deteriorated the operating environment[13-16]. Lastly, it was difficult to debridement due to the thick callus formed easily when particles mixed with blood. And the difficulty in debridement also made the patients taking more pain. Beyond those, the most unfavourable problem was the voids between particles could be destroyed during the process of absorbing water and being pressured manually with gauze. Consequently, the hemostatic efficacy decreased which resulted from the decreasing capillary motions and it could not stop bleeding instantly at the first phase of hemostasis. So enlarging the contact surface and stabilizing the capillary structure were the most feasible methods to improve hemostatic efficacy.

Our lab had engaged in the study of MPS since the past years and the hemostatic properties of MPS particles had been evaluated[11, 12].The coagulation assay in vitro showed that the materials could achieve effective hemostasis through

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initiating the intrinsic pathway[12].On the basis of previous work, the aim of this research focused on the granulation of particles produced by our lab previously and investigating its procoagulant activity[17-28].In vitro coagulation studies and in vivo studies on rat surgical models were used to assess the hemostatic efficacy of the MPS granules. Fluid absorption behaviour was characterized via investigated the sample's uptaking amount of water.

Experimental

The HLSY-10 high shear mixer granulator (Shanghai Xinyi, China) was used for dry-mixing and wet granulation process. As pre-set proportion, MPS particles(hereafter also referred as MPS or particles, synthesised according to the previously report[29]), sodium pyrophosphate,clay and polyurethane (PU, Mingguang Chemical, China) were put into the mixing bowl with shape of cylindrical, a three-bladed impeller and an auxiliary chopper. The impeller rotated at a speed of 500 rpm for 3 min to make the components mixing well and forming MPS mix (hereafter also referred as particles). Then deionized water (hereafter also referred as DI water) was added by dropping into dry MPS mix and the chopper rotated at a speed of 1500 rpm until formed granules like-spherical. Obtained wet granules were dried at 60^{o}C until the weight was constant.

Characterization of MPS materials

An JSM-6360LV scanning electron microscope was used to image morphology of MPS,MPS mix, outer surface and inner cross-section of G_{44} and G_{44}. MPS mix was the mixture of MPS particles, clay and sodium pyrophosphate. G_{44}(hereafter also referred as granules) was the granules from MPS mix containing PU which functioned as pore former. G_{40} (hereafter also referred as granules) was the granules from MPS mix without PU. Packing density of four samples were measured.

Sorption capacity test

MPS worked as hemostatic materials by promoting aggregation of platelet and clotting factors through absorption water in blood. The capacity of water absorption was investigated via gravimetric test which was crucial to its hemostatic performance. 100 mg of MPS, MPS mix, $G_{40}$and G_{44} were pre-weighed and recorded as W_d. Weighed particles and granules were portioned into pre-weighed glass vials, following by the addition of 1 mL of water, and placed on a bench top rotator. At time intervals of 10s,30s, 60sand 120s, vials were removed from the rotator and drained of excess fluid by pipette before being weighed. The weight of the samples was determined and recorded as W_w. All experimental groups were run in triplicate. The sorption capacity of the particles/granules was calculated using the following equation.

Fluid Absorption Capacity (g/g)=$(W_w-W_d)/W_d$

where: Fluid absorption Capacity was the fluid mass that one gram of samples absorbed.

In vitro test of blood coagulation

MPS, MPS mix, G_{40} and G_{44} with same volume were placed into plastic tubes, respectively. Refrigerated rabbit blood was allowed to reach room temperature. Pre-setting amount of rabbit blood was added into plastic tubes containing samples, respectively. The same volume of rabbit blood to plastic tube served as the control group. Sample in plastic tube was recalcified to a 10 mM $CaCl_2$ final concentration using a 0.2 M $CaCl_2$ stock. The tube was then rotated for10 sec and set up vertically on the lab bench. Tube was inverted every 10 sec until the blood aggregates completely ceased to flow. The coagulation aggregation was imaged both before and after washing with deionized water.

In vivo test

Low flowability of MPS and dust were two important problems of all which should be solved for the widely application. Granulating was designed to enhancing the flowability and eliminating the dust. The hemostatic efficacy, flowability and dust of MPS/G_{40} were investigated by observing the action process with blood. An incision of 1cm in length and 0.2cm in depth was made with a surgical scalpel on the edge of rabbit's ear and maintained the wound bleeding for 10 sec 0.2g of MPS/ G_{40} was poured onto the incision site, then the laceration was compressed with gauze for 10 seconds. An incision of ~2cm×2cmin length and ~0.5cm in depth was made with a surgical scalpel in the lobe of the rabbit's liver. After 10 sec of bleeding, using gauze removed the blood around the incision, 1g of MPS/ G_{40} was poured onto the laceration and the incision was inspected visually after 3 min. At the conclusion of each experiment, rats were euthanized by exsanguination while under anaesthesia. This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85e23 Rev. 1985) and was approved by the Research Center for Laboratory Animal of Shanghai University of Traditional Chinese Medicine.

Results and discussion

The morphology of silica granules

Fig.1 A showed the color of MPS mix changed to light yellow from white of MPS and G_{40}, G_{44} were light yellow. The diameter of G_{40} and G_{44} ranged from 400μm to1100 μm with an average value of 650 μm. Fig.1 B displayed the packing density improved greatly to 270 mg/mL for G_{40} and 160 mg/mL for G_{44} from about 60 mg/mL for MPS and MPS mix.Fig.1D-G were the images of morphology of MPS (D), MPS mix (E), G_{40}(F) and G_{44}(G). Fig.1D-E showed granulation additives dispersed well around MPS which meant the good compatibility of clay and sodium pyrophosphate with MPS. The size of voids including crevices and holes both on the surface and inner part of G_{40} was about1- 2μm and that of G_{44} was about 3-5 μm.

Fluidsorption capability

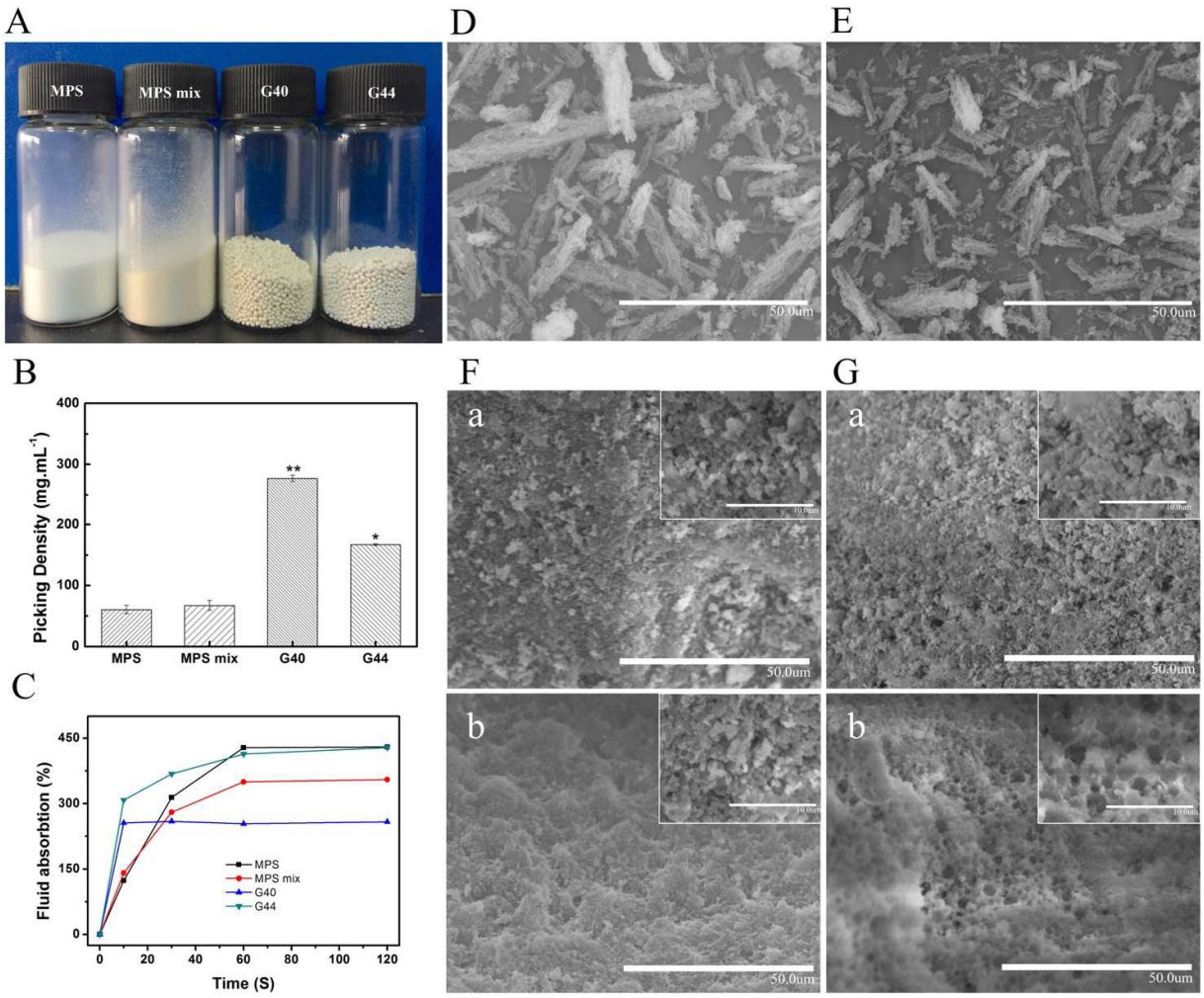

Fig.1 A: photos of MPS, MPS mix, G40 and G44; B: packing density of samples; C: water absorbability of samples; D-G: SEM of MPS, MPS mix, G40 and G44(a: outer surface, b: cross section);*$p<0.0008$, **$p<0.0003$: significantly different from control.*

MPS, MPS mix, G_{40} and G_{44} were employed for the study of water absorption for 120 sec at ambient temperature. Fig.1 C showed the water absorption amount increased with increase in time for all samples. For the period shorter than 10 sec, the water absorption rate for granules groups including G_{40} and G_{44} were much higher than that of particles groups containing MPS and MPS mix, which showed granules' ability of rapid absorption at early stage. Up to 60 sec, the water absorption amount closed to equilibrium for all samples, the maximum water absorption amount of MPS was 430% and the minimum that of G_{40} was 260%. The water absorption amount for G_{44} with pore enlarged by pore former was 300% and higher than that of G_{40}. At early stage of particles contacting with water, particles floated on the water surface due to buoyancy resulting from its low density and poor flowability. The water absorption rate was low because only a small percentage of voids between particles, which were on the contacting surface, functioned as capillary tubes. The water absorption amount increased when more voids functioned as capillary action. The particles aggregates containing water absorbed by capillary action sank and surrounded by water when the weight of aggregates was over the buoyancy. The Equilibrium water absorption amount of MPS was the highest after most of MPS particles with high surface area were infiltrated by water and functioned as capillary. So the water absorption rate of particles groups was low at early stage but its equilibrium water absorption amount was high. For granules groups including G_{40} and G_{44}, because of the porous structure both outer and inner of the granules, the high density and good flowability of spherical shape made them immersed quickly in fluid while pores played a role of capillary tube, so the granules displayed the initial rapid ability of water absorption. The ability of early water absorption enhanced 130% for granules groups compared to particles groups. The results indicated that granulating additives mixed well with MPS particles and the

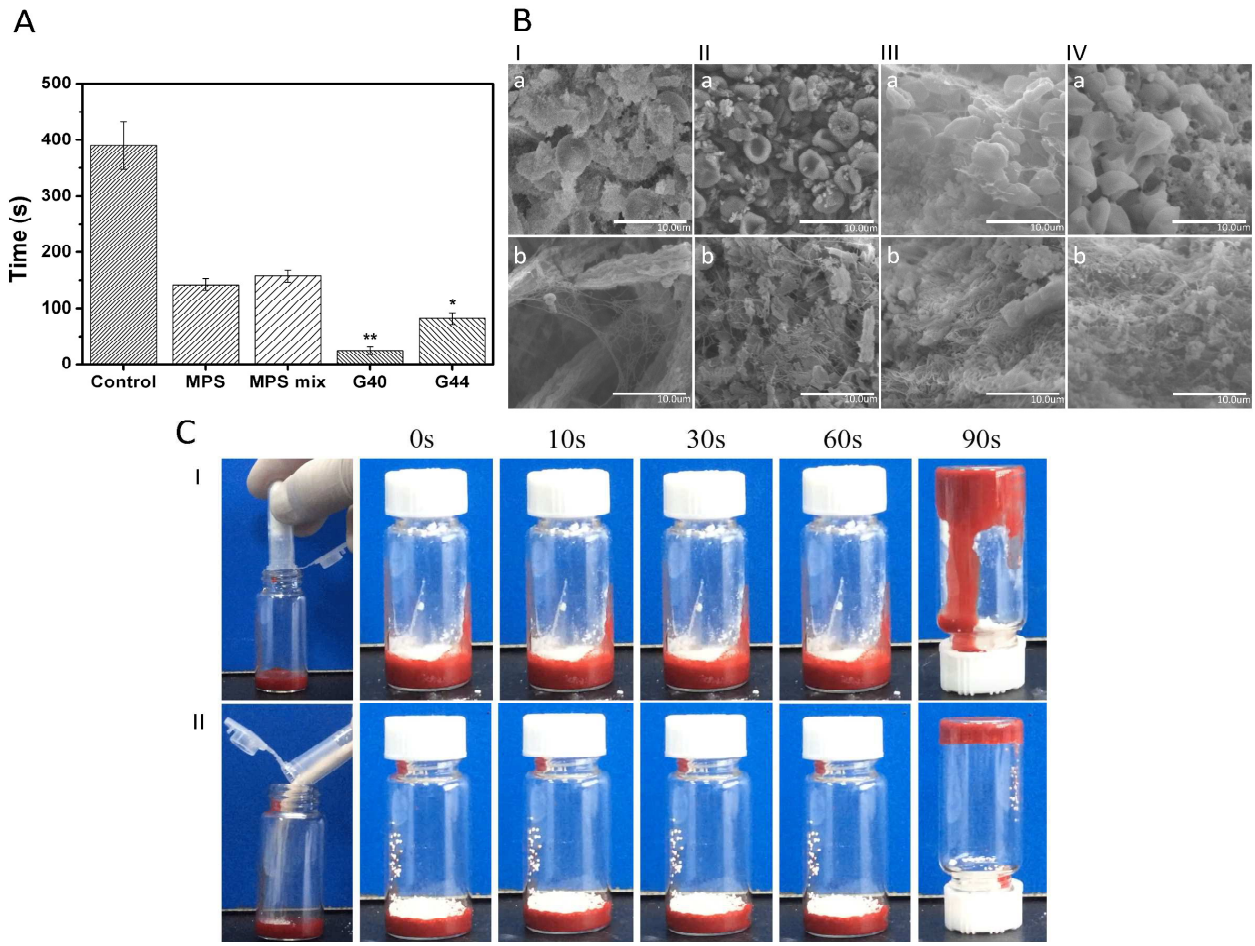

Fig.2 Blood coagulation test in vitro. A: Coagulation time as percent of control; B:SEM of cross-section of clots of samples with blood; (I) a:clot of MPS with blood; b: clot washed with DI water; (II) a:clot of MPS mix with blood; b: clot washed with DI water; (III) a:clot of G_{40} with blood; b: clot after washed with DI water; (IV) a:clot of G_{44} with blood; b: clot washed with DI water; C:Photographs of state of blood adding into vials containing nanoparticles/granules; (I) MPS with blood; b: (II) G_{40} with blood. *$p<0.04$, **$p<0.03$: significantly different from control.*

adding of PU, worked as pore former, enlarged the pores' size of granules. The equilibrium water absorption capacity of particles group was higher than that of granule group. G_{44} with pore enlarged by pore former was with higher equilibrium water absorption than G_{40}.The higher density eliminated the dust pollution and the similar-spherical shape improved the flowability of silica materials.

Blood coagulation in vitro

Coagulation time was measured in glass to clarify the coagulation efficacy of samples in vitro. The blank was as control group. Fig.2A showed the coagulation time (CT) of particles groups including MPS and MPS mix was shorter over 50% than control group. Furthermore, the CT of granules groups including G_{40} and G_{44} was shorter than particles groups. Especially, CT of G_{40} was only 5% that of control group which displayed the excellent hemostatic performance of G_{40}.

The result of CT was not consistent with that of water absorption, which indicated materials with high equilibrium water absorption capacity did not function as good hemostatic materials. Fig.2 B presented the morphologies of blood clot after particles (Fig.2B I-II) and granules (Fig.2B III-IV) sinking in blood for 10min. There were extremely more accumulations of red blood cells and platelets within the cross-section surface of clots of granules groups (2B III-a, 2I V-a) compared to that of particles groups (Fig.2BI-a, 2II-a). After washed with DI water, blood clots for granules groups maintained the clots' shape which could block the bleeding wounds. There were protein strands (2BIII-b, 2IV-b) with high density which enwinded and wrapped the blood with granules together. For particles groups, there were only a small amount of fibrous protein strands (Fig.2B I-b, 2II-b) and clot's shape did not maintained with space of large hole. That indicated the poor strength of clots and could be rushed away by the bleeding of wound site. The amount of fibrin strand formed on granules (2B

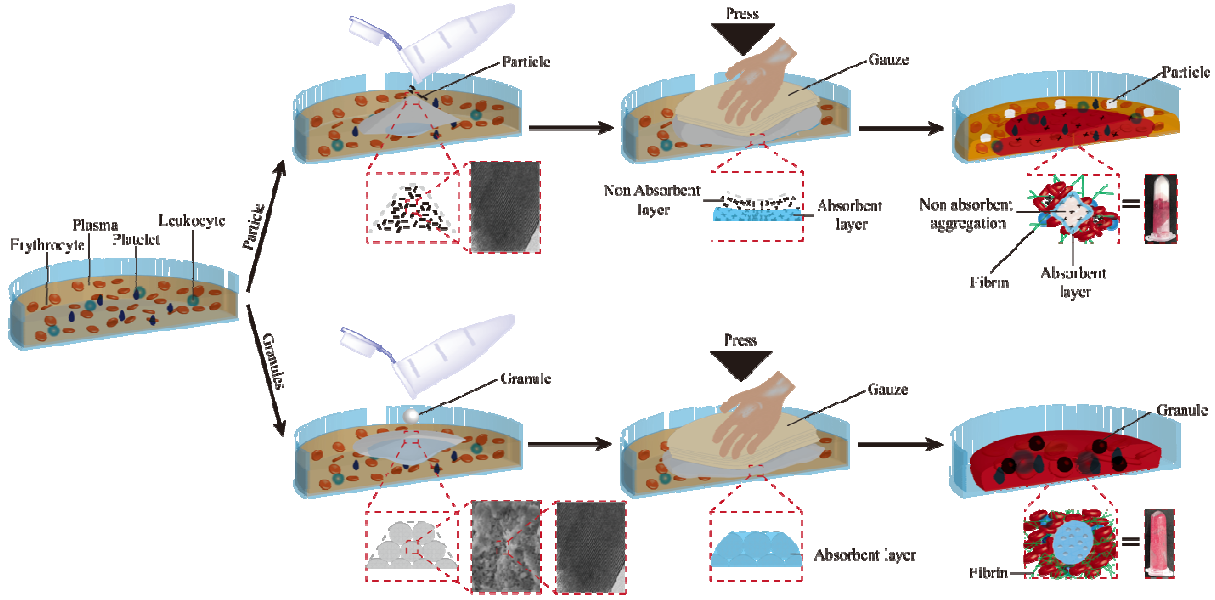

Fig.3 Processing illustration of particles/granules contacting with blood.

Ⅲ-b, 2IV-b) was also significantly higher than that of particles groups (Fig.2B I-b, 2II-b).

Considering the hemostatic efficiency, economic cost and manufacturing of four samples, MPS and G_{40} were chosen for further hemostatic evolution. Fig.2 C showed visually the changing process of blood acting with silica materials and displayed visually the hemostatic rate of MPS and G_{40}.The MPS particles(marked I) floated over the blood and the blood clots formed about 150 seconds after it was added into vials containing blood while the G_{40} granules (marked II) could contact with blood completely when it were adding into vials containing blood and the blood clots formed within 30 seconds. The increasing density and flowability made the granules infiltrated instantly in blood when they were putting into blood. After adding into the plasma, the particles did not absorb water in blood with floating above in plasma while the granules were infiltrated by plasma instantly. It was delightful the coagulum formed significantly faster for G_{40} than MPS through the formation of a blood/granules clots aggregate. The results was consistent with that of analysis above (Fig.2 B). Fig.3 was the schematic illustration of the interaction of particles/granules with blood. Pressing manually particles which poured into blood, they flew over and sealed the slits between particles because of the unstable aggregation structure with loose packing. The only outer surface of particles aggregation formed the absorbent layer via absorbing water in blood while inner part present were dry particles without absorbing water. A little visible components of blood like platelet and red blood cells were concentrated on the surface of particles and formed platelets plug. For granules, the stable cavity and slit on the surface and inner part of the granules were filled with water instantly when they contacted with blood, all silica materials were immersed in the blood completely and formed the absorbent layer. The visible component like platelet and red bloods aggregated around the granules and a great number of fibrin strands formed (Fig.3).

Hemostasis in vivo

Fig.4 was photographs of bleeding incisions on rabbit's ear (Fig.4 A-C, skin wound)and liver(Fig.4 D-E, wound inside body). For G40 group, the granules were evenly dispersed

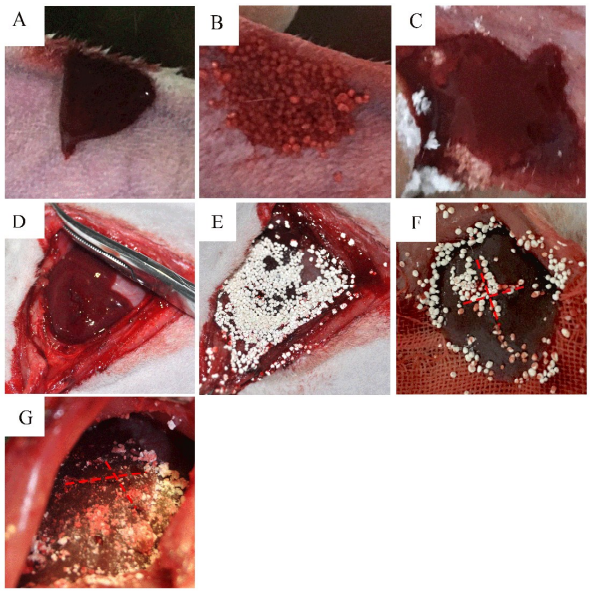

Fig.4 Hemostasis test in vivo. A, Photograph of ear showing bleeding. B, Photograph after 10 sec application of granules on ear. C, Photograph after 10 sec application of particles on ear. D, Photograph of liver incision. E, Photograph after 10 sec application of granules on liver. F, Photograph after 3 min application of granules on liver with less G_{40}. G, Photograph after 3min application of particles on liver.The red dotted line indicated the incisions position.

around the incision site and the surface of incision site was dry with liquid in blood being absorbed (Fig. 4B). The wound bleeding had been stopped. However, for MPS group, part of the particles drifted away, the wound was still bleeding and moreparticles should be supplemented at the incision site (Fig.4C). G_{40} worked well for surface wound. The hemostasis function of G_{40} was also demonstrated on internal wound (Fig.4E) of rabbit. Because of the deeper incisions on liver,G_{40} with good flowability flowed into the bleeding site and were embedded easily between incisions, absorbed water in blood and sealed the bleeding vessels. The process that G_{40}workedincluding embedded into incision-absorbed water-sealed bleeding vessel were more obviously on Fig. 4F. While for particles group, sealing could not be observed between incisions (Fig 4G). Compared to MPS group, G_{40} displayed the improved ability of stop bleeding instantly.

Conclusion

The mesoporous silica granules were prepared from mesoporous silica particles via wet granulation. The flowability, water absorption capacity and in vitro coagulation of particles/granules were investigated. Granule's hemostatic efficiency in rabbit's ear and liver injury models were also evaluated. granulation stabilized the capillary structure and increased the density and flowability of the particles. The early water absorption rate was accelerated greatly for granules which be helpful to the primary phase of stop bleeding. The shape of granules increased hemostatic efficiency of mesoporous silica by enhancing the capillary motion of silica to blood and enlarging the contact areas of silica with blood. Granules absorbed water in plasma, concentrated platelet and red blood cells quickly and consequently reduced the bleeding time of rabbit' wound. The improved flowability of silica materials made the granules dispersed evenly on the bleeding site. At the same time, the granulation of particles eliminated the dust which was harmful to operating environment. The results showed mesoporous silica granules were a promising hemostatic agent.

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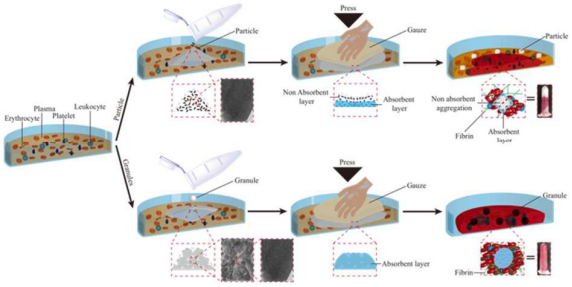

Granulation was one of the most feasible methods to enlarge the contact surface and stabilize the capillary structure for improve hemostatic efficacy. The usability of silica material was improved significantly by granulation through enhancing its flowability and eliminating dust. This study suggested the porous silica granules were good candidate of hemostatic agent in clinical and family application.