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Oxidized regenerated cellulose-based hemostat with microscopically gradient structure

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ABSTRACT

Partially water-soluble oxidized regenerated cellulose carboxylate sodium (ORC-Na) materials have been prepared by controlled neutralizing oxidized generated cellulose (ORC). The carboxyl were converted into sodium carboxylate as evidenced by FT-IR, and carboxyl content decreased from 18.41% to 0.98%, with enhancing water solubility of ORC-Na to form gel, and SEM–EDX revealed that the sodium carboxylate groups presented in a gradient distribution from the exterior to the interior of fiber. ORC-Na introduced a new hemostatic mechanism, i.e., forming gel to mechanically seal off the crevasses of vessels. Due to its excellent water solubility and 5.23% carboxyl, ORC-Na-3 possessed optimum hemostatic efficiency and demonstrated a capability to stop bleeding within shortest time (102 and 138 s) with the least blood loss (0.886 and 1.006 g), and implantation test showed ORC-Na-3 could be absorbed in less than 2 weeks with no pathological response remaining. In conclusion, ORC-Na-3 is an efficient hemostat with optimum biodegradability.

1. Introduction

Oxidized regenerated cellulose, which could be obtained by partial oxidation of the primary hydroxyl groups on the anhydroglucose rings to produce the monocarboxyl cellulose, is a kind of natural and topical biomaterials. Within the range of 16–24% carboxylic acid content, all the ORC materials represent an important class of biocompatible and bioabsorbable polymers, and they have been available in a sterilized knitted fabric or powder form for use in human beings to stop bleeding (Zhu, Kumar, & Banker, 2001), which have been proved to hold an excellent bio-security.

ORC was prepared and studied for the first time in the late 1930s. Nitrogen dioxide (Camy, Montanari, Rattaz, Vignon, & Condoret, 2009; Foglarova, Prokop, & Milichovsky, 2009; Yackel & Kenyon, 1941; Zimnitsky, Yurkshtovich, & Bychkovsky, 2004), the initial kind of oxidants, which was used for the selective oxidation of the primary hydroxyl groups in the cellulose, was reported in the 1940s. So far, both gaseous and liquid nitrogen dioxide oxidation processes have been commercialized for many years. Recently, the preparation (Praskalo et al., 2009; Saito, Okita, Nge, Sugiyama, & Isogai, 2006; Yin, Koschella, & Heinze, 2009) and modification (Zhu et al., 2001; Zimnitsky, Yurkshtovich, & Bychkovsky, 2006) for oxidized generated cellulose are still of significant interest.

Johnson & Johnson has pioneered an industrial scale oxidation process using nitrogen dioxide to manufacture ORC absorbable haemostat (Domb, Kost, & Wiseman, 1998) – Surgicel (Alpaslan, Alpaslan, & Oygur, 1997; Ashworth & Whear, 2003; Breech & Laufer, 2000; Loescher & Robinson, 1998; Sharma & Malhotra, 2006; Sharma, Malhotra, & Pundir, 2003). In recent decades, commercial Surgicel absorbable hemostatic agent has been widely applied in various surgeries and played an important role on stopping the bleeding.

Although this hemostatic material is broadly applied due to its excellent properties, the commercial ORC has also shown several inherent disadvantages. For example, the hemostatic property of this material is relatively poor and has a low biodegradability. With a carboxyl content ranging from 16% to 24% and a pH approximately of 3.1, ORC would damage nervous system if this material is implanted in the human body. Many researchers have made great efforts to improve the hemostatic performance and to overcome other shortcomings (Watt, Harvey, & Wiseman, 2003), but significant improvement on the hemostatic performance is still limited (Harvey, Leeuwen, Hyland, & Aitken, 2001). Stilwell et al. reported

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an enhanced hemostasis property in the calcium-modified hemostat with a low neutralization degree by using calcium acetate monohydrate/H_2O-IPA to neutralize ORC, the interference with the biodegradability was unfortunately noticed (Saferstein, Wolf, Kamp, Linsky, & Wiseman, 1992; Stilwell, Whitmore, & Saferstein, 1996). The basic salt of a weak acid/aqueous solution and the alkali aqueous solution can be utilized to neutralize ORC, but the resulting materials are distorted from their original morphology with little integrity and show a very weak strength.

In this paper, partially water-soluble oxidized generated cellulose sodium materials are prepared by a controlled neutralization method with strong base/ethanol, and the hemostatic efficiency and biodegradability are investigated. The new material can maintain the original physical form with suitable tensile strength for practical usage. Especially, the water-soluble groups present a gradient distribution, so that the materials can adopt different hemostatic mechanism to arrest the bleeding during the whole hemostasis procedure. Through animal experiments, the excellent hemostatic performance and high biodegradability of the novel materials have been thoroughly proved and analyzed.

2. Materials and methods

2.1. Materials

Regenerated cellulose filaments, used as the starting material, were obtained from Xinxiang City, Henan Province, China. Nitrogen dioxide (AR, 99.999%, w/w) was purchased from Summit Specialty Gases Co., Ltd., Tianjin City, China. Carbon tetrachloride (AR, 99.5%) and sodium hydroxide (AR, 96%, w/w) were supplied by Shuang Shuang Chemical Co., Ltd., Yantai, China. Ethanol (AR, 99.7%, w/w) was purchased from Fu Yu Chemical Co., Ltd., Tianjin, China. Surgicel®, a kind of commercial oxidized generated cellulose, was obtained from Johnson & Johnson Medical Limited.

2.2. Preparation of partially water-soluble oxidized generated cellulose materials

Prior to oxidation, regenerated cellulose filaments were knitted with knitting machine to obtained single layer weft-knitting rectangular fabric. ORC weft-knitting fabric was prepared by using the method described previously (Ashton, Philadelphia, & Moser, 1968). The detailed preparation process was performed as follows. First NO_2 was dissolved into CCl_4 to prepare 20% (wt) NO_2/CCl_4 oxidant solution, then added regenerated cellulose knitting fabric into a round bottomed flask containing mentioned oxidant in a proportion of 1:42.6 (g/ml) (weft-knitting fabric: oxidant). Stirred constantly, kept the reaction temperature at 19.5 °C and oxidation duration was 40 h. After the reaction, washed the product thrice with CCl_4, and then washed the product thrice with the aqueous solution containing 50% (v/v) ethanol followed by washing the product thrice with 100% ethanol. Finally, ORC was frozen-dried at −50 °C in vacuum for 48 h.

Next step, immersed ORC weft-knitting fabric above in 150 ml NaOH/ethanol solution in an Erlenmeyer flask, with a mole ratio (n_{NaOH}:$n_{carboxyl\ group\ of\ ORC}$) of 1/4, 1/2, 3/4, and 1/1, respectively. Then sealed the flask with a rubber stopper and shocked under a reciprocating oscillator at 25 °C for 24 h (the corresponding products were labeled as ORC-Na-1, ORC-Na-2, ORC-Na-3, and ORC-Na-4). After the reaction, the products were washed three times with 80% (v/v) ethanol aqueous solution, and then washed twice with pure ethanol. After that, the samples were frozen-dried at −50 °C in vacuum for 72 h. The final ORC-Na single layer weft-knitting rectangular fabrics were sealed and kept in dry condition at about 2–7 °C.

2.3. Structural analysis

Fourier transfer infrared (FT-IR) spectroscopy was obtained in the KBr pellet mixture form from a Nicolet-Nexus 670 spectrophotometer. Monofilaments were embedded with epoxy resin, and then scanning electron microscopy (SEM) observations and energy dispersive X-ray (EDX) elemental analysis were obtained in a Hitachi S-4700 SEM.

2.4. Determination of carboxyl content

The carboxyl content was measured according to United States Pharmacopeia (USP23-NF18). Briefly, a calcium acetate solution with a mass fraction of 2% was prepared. Then chopped fibers (0.5 g) were immersed in 50 ml 2% calcium acetate solution for 15 h, and then phenolphthalein indicator was added and titrated with standard 0.1 M NaOH. The volume of the consumed NaOH was corrected by the blank. The carboxyl content was determined using the equation

$$-\mathrm{COOH}\ \ (\%) = \frac{N \times V \times \mathrm{MW}_{-\mathrm{COOH}}}{m} \times 100\% \quad (1)$$

where N referred to the concentration of NaOH solution; $\mathrm{MW}_{-\mathrm{COOH}}$ was the consumed volume of NaOH which was corrected by the blank, $\mathrm{MW}_{-\mathrm{COOH}}$ was the molecular weight of carboxyl group and m was the weight of the testing sample.

2.5. Water-soluble property

Surgicel and ORC-Na fabrics were cut into pieces of suitable sizes and then the lower part of the fabrics was immersed in 37 °C water. Five seconds later, the samples were taken out and their morphological changes were studied.

2.6. Hemostatic evaluation

All procedures were complied with the AAALAC Guide for Care and Use of Laboratory Animals and the hemostatic efficiency was tested in two different models.

2.6.1. Rabbit ear artery model

After the anesthesia of intraperitoneal injected pentobarbital sodium, the area of middle auricular artery of a rabbit was prepared and sterilized, and was made to bleed by transverse cut of the vessel using a scalpel blade. As the blood flowed out from the wound, a piece of conventional sterile gauze was used to absorb the blood immediately. And then one layer of the testing samples was covered on the wound with an applied 3 N force, and the hemostatic situation was observed every 30 s until the bleeding was controlled and the hemostatic time was recorded.

2.6.2. Hepatic trauma model

Rabbits were fixed on the autopsy table and anaesthetized, opened the abdomen and exposed its liver. One of the anterior lobes was chosen in each animal and the tissue was scooped out using a curved iris scissor. The wound was induced using a scalpel on the lobe, measuring approximately 1.0 cm × 1.0 cm × 0.3 cm. As the injury started bleeding, absorbed the blood with conventional sterile gauze immediately. And then a layer of testing knitting fabric was placed on the wound with an applied force of 3 N. The hemostatic situation was also monitored every 30 s and the hemostatic time was recorded.

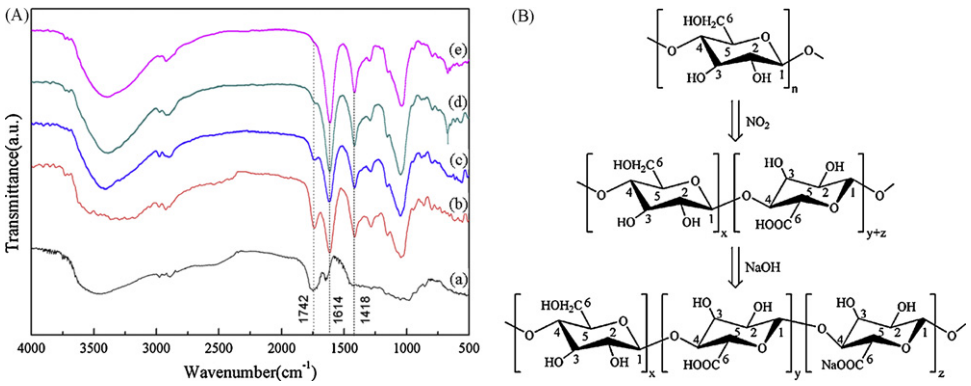

Fig. 1. Characterization of the materials: (A) FT-IR spectra of (a) ORC, (b) ORC-Na-1, (c) ORC-Na-2, (d) ORC-Na-3, (e) ORC-Na-4, and (B) molecular structures.

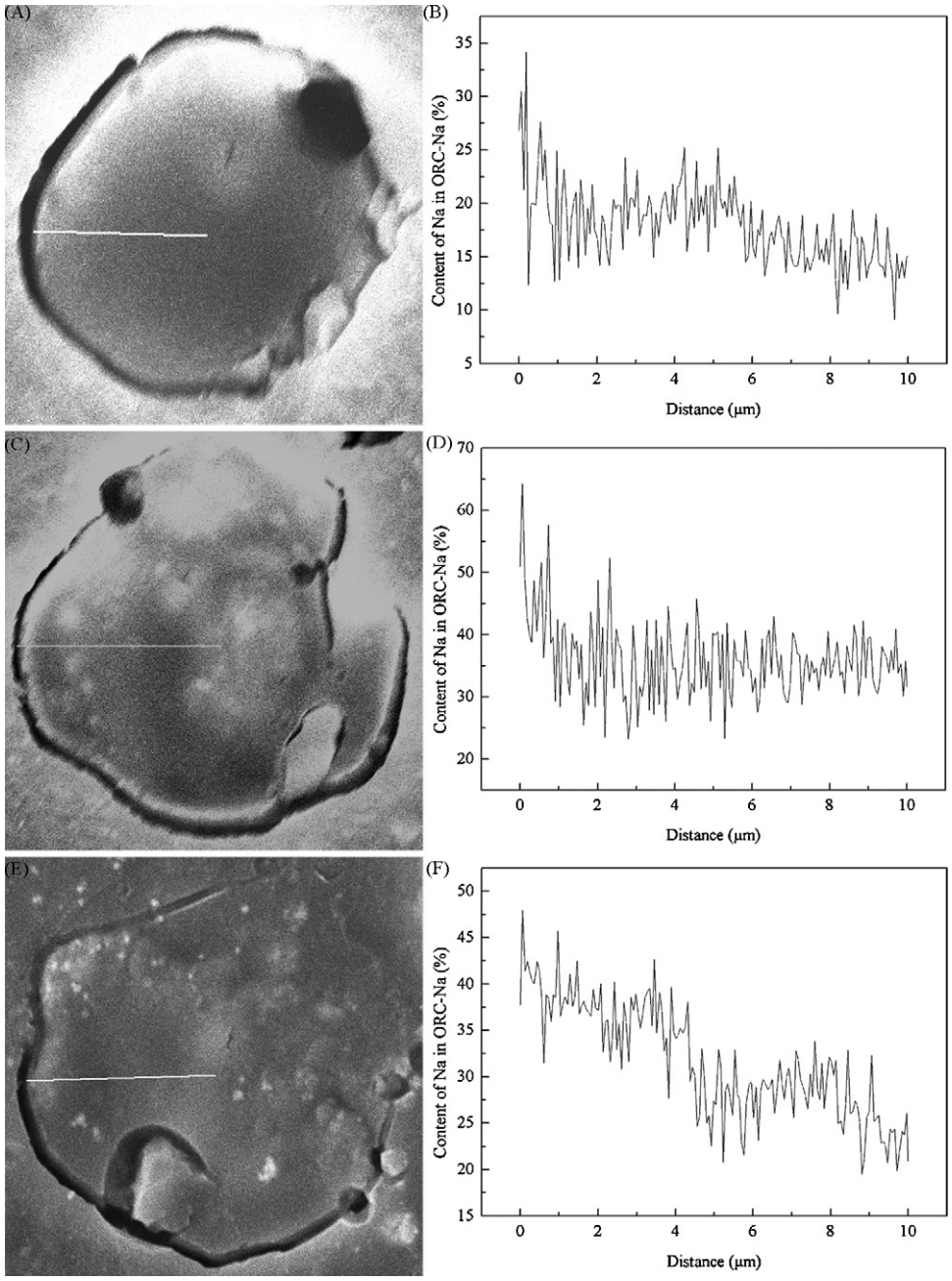

Fig. 2. Na element distribution along the radial direction in ORC-Na-3 monofilament.

2.7. Tests for biodegradability and local effects after implantation

The commercial Surgicel (weft-knitting fabrics) in aseptic packaging and the prepared single layer ORC-Na weft-knitting fabrics were cut into discs with 10 mm diameter, and then sterilized by gamma ray at a certain irradiation dosage of 18 kGy. All procedures were performed under sterile conditions.

Adult SD rats weighing around 224–255 g were prepared for conventional operation in this experiment, and no particular gender was prescribed for this test. After the anesthesia of intraperitoneal injected pentobarbital sodium, the operational area was sterilized, and then a piece of testing weft-knitting fabrics disc was implanted flat across the implantation site of subcutaneous dorsal skin and six implantation sites were needed at 2–3 cm interval at the back of one animal. Then, 20,000 U/kg gentamicin was injected intramuscular once. For every kind of implanting materials, three animals were used per implantation period and the implantation periods were varied from 1, 2 to 4 weeks. All the animals were intensively cared until the implanting terminals were reached. At each termination of the experimental period, the animals were sacrificed with overdose pentobarbital sodium. The implants and the surrounding tissues were retrieved for macroscopic observation and histopathological evaluation. The tissue blocks around the implants were fixed by 20% formalin. After gradient ethanol dehydrated, the samples were paraffin embedded, sectioned and stained with hematoxylin and eosin (HE).

2.8. Statistical analysis

All data were expressed as mean ± standard deviations (SD). Statistical comparisons were performed using SPSS 11.0 software. Differences were considered significant for $P < 0.05$.

3. Results and discussion

3.1. Analysis of structures

Fig. 1A shows the FT-IR spectra of the synthesized ORC and different ORC-Na materials. The absorption peak at 3471 cm^{-1} corresponds to the stretching vibration of O—H, and the twin peaks at 2974 and 2891 cm^{-1} are induced by the asymmetrical and symmetric stretching vibration of $—CH_2$, respectively. The peak around 1742 cm^{-1} is due to the stretching of C=O, and the peak at 1653 cm^{-1} (in curve a) is related to the O—H bending vibration of the adsorbed water, while the 1049 cm^{-1} absorption peak is contributed to the stretching vibration of C—O—C. ORC and different ORC-Na materials are observed similarly in FT-IR spectra, except the characteristic carbonyl peak (—COOH) at 1742 cm^{-1}, whose intensity reduced in the order ORC > ORC-Na-1 > ORC-Na-2 > ORC-Na-3 > ORC-Na-4, consistent with the decrease in the carboxyl groups in the products. The presence of the new double bands at around 1614 and 1418 cm^{-1} are assigned to the asymmetrical and symmetrical stretching vibration of $—COO^-$, which are significantly

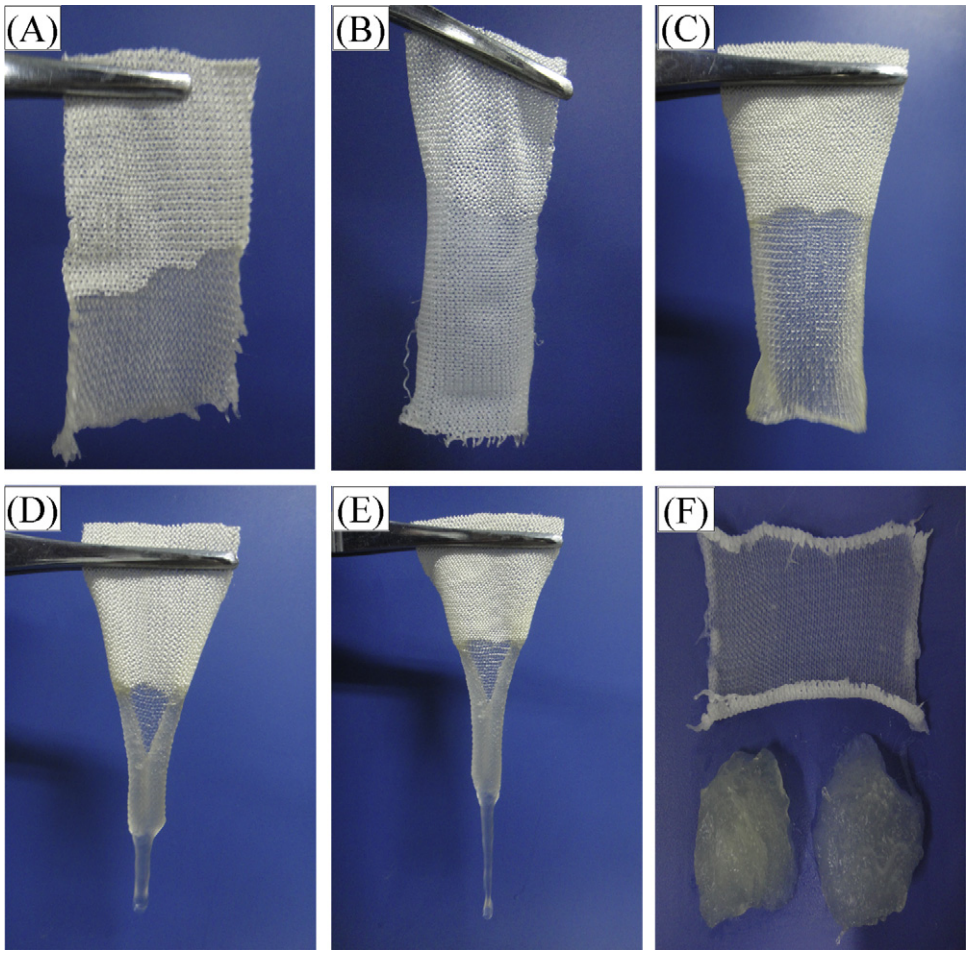

Fig. 3. The digital pictures of (A) commercial Surgicel, (B) ORC-Na-1, (C) ORC-Na-2, (D) ORC-Na-3, (E) ORC-Na-4, and (F) Surgicel [*up*], ORC-Na-3 [*down-left*], ORC-Na-4 [*down-right*] after immersed in H_2O for 30 s.

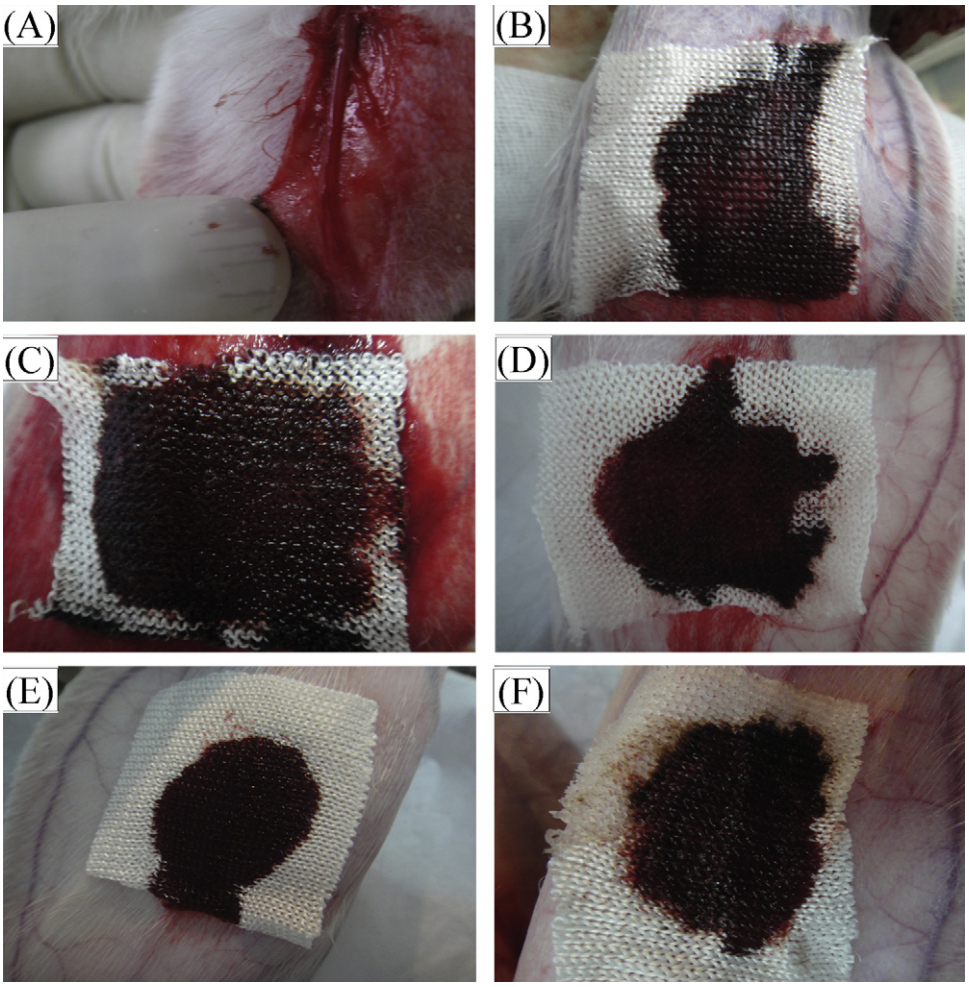

Fig. 4. Photographs of (A) injury site, and the hemostatic evaluation of (B) commercial Surgicel, (C) ORC-Na-1, (D) ORC-Na-2, (E) ORC-Na-3, and (F) ORC-Na-4 on the middle ear artery model.

enhanced with increasing the amount of NaOH (from b to e in Fig. 1A).

FT-IR analysis indicate that ORC has reacted with NaOH, producing oxidized regenerated cellulose sodium salts (ORC-Na), and the content of sodium carboxylate groups raises with increasing the used NaOH in controlled neutralization system. The reaction of ORC with NaOH/ethanol is shown in Fig. 1B. Besides a few residual carboxyl groups, numerous carboxylate sodium structures appear in the molecule as the neutralization reaction between the —COOH groups in ORC with NaOH progresses.

3.2. Carboxyl content

To quantificationally analyze the neutralization degree of ORC, the residual carboxyl content in different ORC-Na materials have been determined. With increasing the ratio of $n_{NaOH}/n_{carboxyl\ group\ of\ ORC}$, the carboxyl content of ORC-Na reduced steadily, 18.41% for ORC, 14.09% for ORC-Na-1, 9.18% for ORC-Na-2, 5.23% for ORC-Na-3, and 0.89% for ORC-Na-4, respectively. The reaction between carboxylic acid and NaOH aqueous solution is known to occur quite easily, and the carboxyl groups can totally and rapidly take part in the reaction when the amount of NaOH is sufficient. In this study, the carboxyl groups still existed in the material (ORC-Na-4) even if the molar ratio of NaOH to —COOH was 1:1. This phenomenon can be explained by considering the different reaction sites in the fiber, skin and core, i.e., crystalline region and amorphous region. The distinct difference in the reactivity and the accessibility of the carboxyl groups in skin and core, leads to the easy and rapid reaction of the initial stage near the fibril surface, however, the reaction in the core of the fiber proceeds very difficultly.

3.3. Graded Distribution

Take three filaments in ORC-Na-3 as the examples (in Fig. 2A, C and E), and Fig. 2B, D and F show the distributions of Na element along the radial direction of ORC-Na-3 fibers. Along the line on the cross section of the ORC-Na-3 monofilament, the content of Na element becomes less and less. In other words, the distribution density of —COONa group appears to be gradually reduced from the surface to the core along the radial direction. As the regenerated cellulose is attacked by nitrogen dioxide, the oxidation reaction occurs on the surface and then gradually into the interior of the fiber, thus results in higher carboxyl content on the surface than that in the interior of the fiber. And this is similar to the micro reaction process between NaOH and carboxyl groups in the ORC. That is to say, the distribution density of the sodium carboxylate groups in the fiber became lower from the skin to the core along the radial direction.

3.4. Water-soluble property

Fig. 3 reports the water-soluble property of ORC and all the ORC-Na materials. ORC is able to dissolve in alkaline solution easily, however, it only shows swelling behavior in water (Fig. 3A).

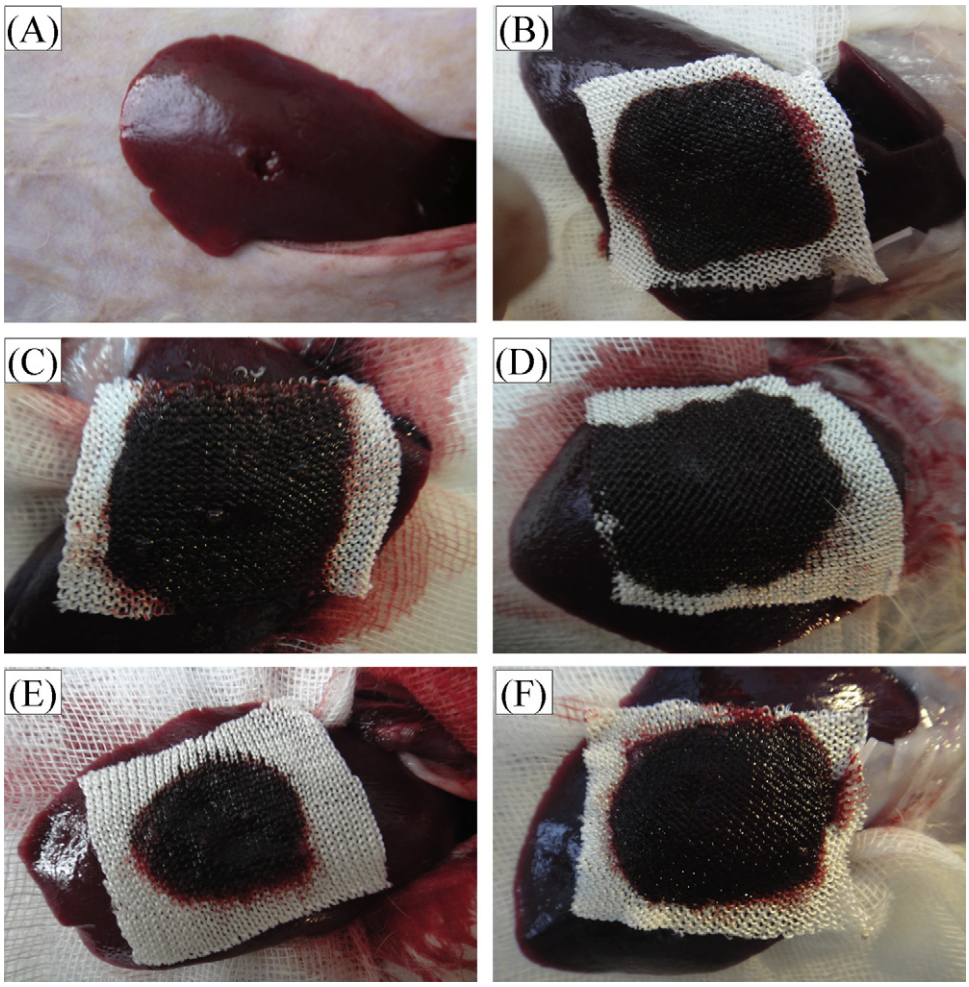

Fig. 5. Photographs of (A) injury site, and hemostatic evaluation of (B) commercial Surgicel, (C) ORC-Na-1, (D) ORC-Na-2, (E) ORC-Na-3, and (F) ORC-Na-4 on a hepatic trauma.

Instead, ORC-Na-1 is similar to ORC after absorbing water (Fig. 3B), and slight gel quickly appears on the surface of ORC-Na-2 knitting fabrics (Fig. 3C) as soon as the materials absorb water. Bulk of the materials on the surface start to form gel immediately and only few fibril structures are observed (Fig. 3D and E) after ORC-Na-3 and ORC-Na-4 knitting fabrics contact with water. Even more, ORC still maintains its knitting form after immersed in water for 30 s, ORC-Na-3 and ORC-Na-4 knitting fabrics on the other hand totally lose their original physical forms and generate transparent gel bulks after the gauzes are soaked with enough water (Fig. 3F). This is due to the controlled neutralization reaction with NaOH/ethanol, the sodium carboxylate (—COONa) groups have been introduced into the materials, which are water-soluble. Thereby they are able to lead the ORC-Na materials to be partially water-soluble subsequent with different gel degree. Further more, sodium carboxylate groups and carboxyl groups are graded distributed, which is induced by this controlled neutralization method, i.e., the content of sodium carboxylate groups gradually decreases from the surface to the interior, meanwhile, carboxyl content increases. Consequently, these ORC-Na materials are able to possess different water soluble levels, and the dissolution begins from the surface and then deep into the interior with different rates.

3.5. Hemostatic evaluation

After placing commercial Surgicel on the bleeding sites of the middle ear artery, Fig. 4, and of the hepatic trauma on the liver lobes of rabbits, Fig. 5, the knitting fabrics absorbs blood onto its surface, turn to be dark brown or black immediately, and then gradually formed a clot (Figs. 4B and 5B). Finally, the bleeding is stopped in 126 and 176 s in different models with 1.035 and 1.875 g blood loss.

As ORC-Na knitting fabrics are applied on the two trauma models, the blood quickly penetrates on the surface of the knitting fabrics, subsequently ORC-Na generate gel. Both the formation rate and the gelatinous degree are observed to concordant with the water solubility of the materials. Few seconds later, the materials form the dark brown clots adjacent to the injury, which arrest the bleeding (Figs. 4C–F and 5C–F).

Toward to commercial Surgicel, the hemostatic time of ORC-Na materials increase gradually first then decrease in the two trauma models, and the ORC-Na-3 material exhibits the shorter mean hemostatic time (102 and 138 s) and less blood loss (0.886 and 1.006 g) than those of any other testing materials.

In order to explain the experimental data of the hemostatic evaluation above, the hemostatic mechanism should be analyzed comprehensively. The commercial Surgicel gauze, a kind of oxidized regenerated cellulose, has the ability to collect red blood cells and results in the plasmatorrhexis of red blood cells with the subsequent delivery of acid hematin, leading to the hemostatic action of Surgicel (ORC), which is in part due to the styptic action from the low pH value (~3) of the material (Saferstein et al., 1992; Stilwell et al., 1996). Thereby, the amount of carboxyl groups determines the hemostatic efficiency, in other words, the higher carboxyl content contribute the higher hemostatic activity. Furthermore, the

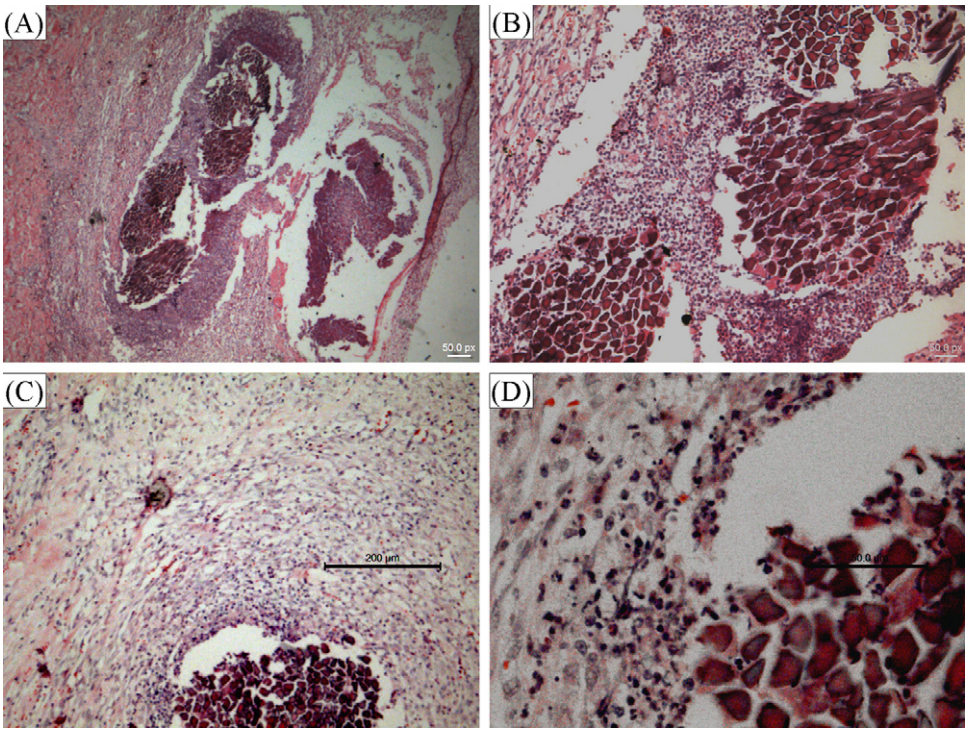

Fig. 6. Photomicrographs of subcutaneous implantation site for 1 week: (A) view of the area demonstrating degrading Surgicel, (B) magnified view of area demonstrating degrading Surgicel, (C) view of the area demonstrating degrading ORC-Na-3, and (D) magnified view of area demonstrating degrading ORC-Na-3.

hemostatic performance of the commercial Surgicel also depends on a cooperation of local chemical activity and mechanical action, i.e., activation of platelets and formation of an artificial clot by combining the carboxyl groups with Fe^{3+} ions, but these hemostasis procedures work slowly.

While ORC-Na retain the hemostatic as commercial Surgicel, which is based on the residual carboxyl groups, it also introduce a new hemostatic mechanism relied on the sodium carboxylate groups, i.e., it could immediately forms a gel to mechanically seal the crevasses of blood vessels to arrest bleeding as it contacts

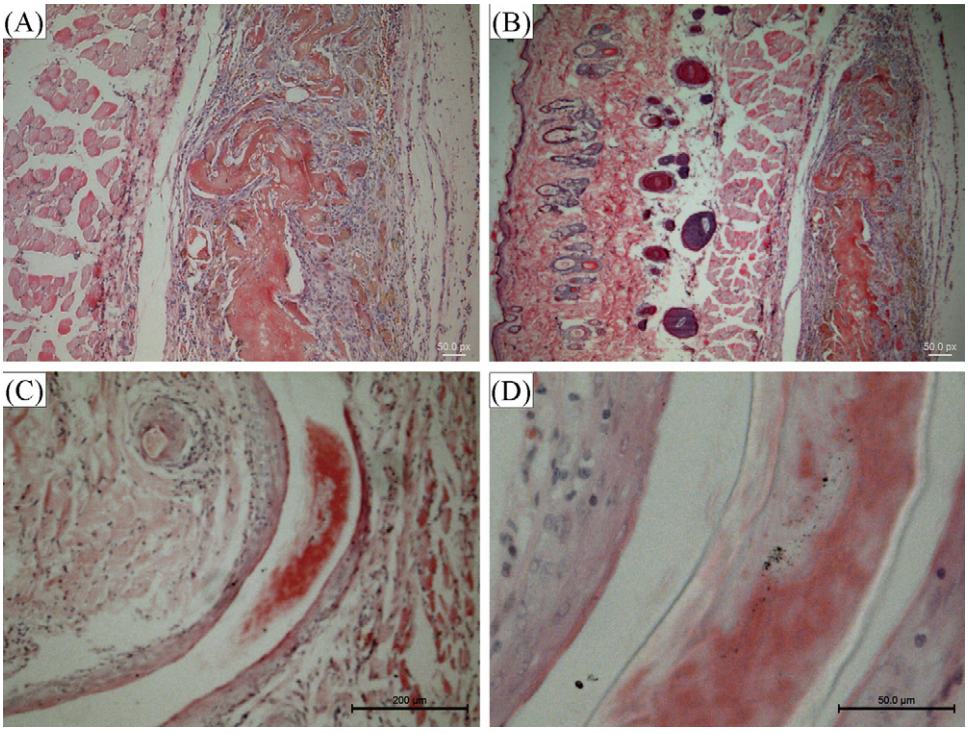

Fig. 7. Photomicrographs of the subcutaneous implantation site for 2 week: (A) view of the area demonstrating degrading Surgicel, (B) magnified view of the area demonstrating degrading Surgicel, (C) view of the area demonstrating degrading ORC-Na-3, and (D) magnified view of the area demonstrating degrading ORC-Na-3.

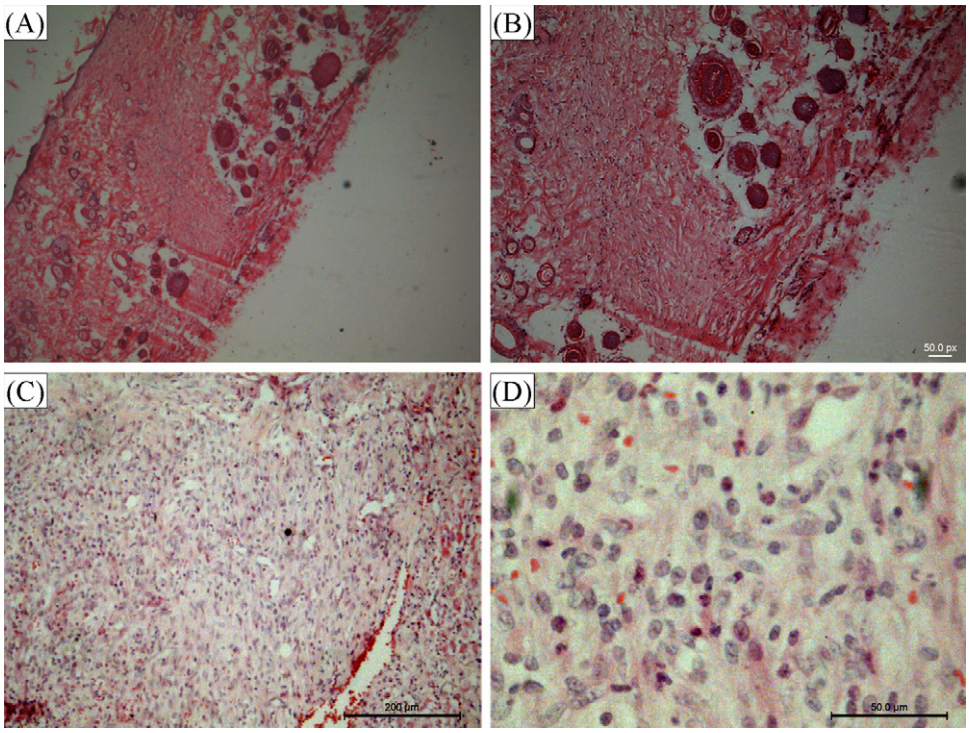

Fig. 8. Photomicrographs of subcutaneous implantation site for 4 week: (A) view of the area demonstrating degrading Surgicel, (B) magnified view of the area demonstrating degrading Surgicel, (C) view of the area demonstrating degrading ORC-Na-3, and (D) magnified view of the area demonstrating degrading ORC-Na-3.

with blood. In ORC-Na-1 (*Hemostatic time*: 226 and 244 s, *Blood loss*: 1.974 and 2.998 g) and ORC-Na-2 (*Hemostatic time*: 186 and 198 s, *Blood loss*: 1.591 and 2.355 g), the contents of sodium carboxylate structures are so lower that the gel is inconspicuous. The efficiency of the new hemostatic mechanism cannot make up the loss of the carboxyl hemostatic efficiency induced by the controlled neutralization. As the controlled neutralization degree rises, such as ORC-Na-3 (*Hemostatic time*: 102 and 138 s, *Blood loss*: 0.886 and 1.006 g) and ORC-Na-4 (*Hemostatic time*: 124 and 168 s, *Blood loss*: 1.004 and 1.566 g) the gel effect is good enough to meet the carboxyl loss, even exceed the contribution made by the equivalent carboxyl groups, and then enhanced the hemostatic efficiency, meanwhile, rapid hemostasis and gel effect would reduce the blood loss. However, when the gel effect reaches a certain extent, further improvement on the neutralization degree could not enhance the hemostatic efficiency; instead, it would even be worse with the loss of more carboxyl groups. Thus, ORC-Na-4 takes a longer time to stop the bleeding than ORC-Na-3 and lose more blood.

ORC-Na materials, with the controlled distribution of sodium carboxylate and carboxyl groups, exhibit different hemostatic mechanisms through progressive stages. As soon as the materials touch the blood, they absorb the blood and start to be gelatinous immediately, and then the gelatinous materials jam the crevasses of blood vessels to arrest the bleeding. This hemostatic mechanism is mainly dominant in the initial stage of bleeding. As majority of the materials become gel, more and more carboxyl groups are exposed and in touch with blood, so the hemostatic effect like the commercial Surgicel starts to work. Further, due to the graded distribution of different groups and the appropriate dissolution rate, ORC-Na knitted fabrics could maintain the whole framework even when it has partly formed gel. The knitted fabrics are able to provide the platforms to absorb and assemble platelets, and then activate thrombus to stop the bleeding.The commercial Surgicel has been evaluated as a hemostat on the bone regeneration in clinical studies, and Surgicel has been found to cause an intense detrimental inflammatory reaction when it is implanted into the bone defects, due to its low pH and swelling effect as it become hydrated with blood component (Krishnan, Mohanty, Umashankar, & Lal, 2004). However, ORC-Na with partially water-soluble ability can reduce or eliminate the swelling effect, and the acidity of the material has been relatively decreased and even close to be neutral. Therefore, ORC-Na is anticipated to be applied as hemostat for demic sensitive sites and would not lead to the clinical damages. Moreover, the weak acidity of ORC-Na allowed it to be accompanied with some acid-sensitive biologic or drugs simultaneously to promote the hemostatic activity further.

3.6. Biodegradability and local effects after implantation

Histopathological examinations of the experimental tissue sites of the subcutaneous implantation show different responses of the commercial Surgicel at different implanted periods and the ORC-Na-3 has also be chosen to be examined due to its excellent hemostatic efficiency.

The liver samples implanted with the commercial Surgicel have been stained to purple brown on most of the sections, Fig. 6A and B. Some cells are observed to have grown on the implantation samples. Around the Surgicel samples, obvious capsules composed of many fibroblasts, lymphocytes, eosinophils and tissue cells are observed and the thickness of these capsules varies from dozens to hundreds of micrometers.

ORC-Na-3 application sites show that small pieces of articles can be observed on a few areas and have been stained to light purple to red color (Fig. 6C and D). (For interpretation of the references to color in this text, the reader is referred to the web version of this article.) About 200–400 nm thick layers of cells capsule are found somewhere. In these cell layers, many macrophages, neutrophils and new-formed capillaries are found, and in some places, un-absorbed bleeding is obvious. Also on some other sections, there are no obvious tissue reactions or the testing articles.

In sites where the commercial Surgicel was applied for 2 weeks, the testing samples are found still on the most of sections. Around

them, the capsules composed of fibroblasts, lymphocytes and tissue cells are noticed, and the thickness of them varies from dozens to hundreds of micrometers, Fig. 7A and B. Whereas for ORC-Na-3, there are not obvious tissue reactions observed on the implanted sections, Fig. 7C and D.

As the implanted period lasted for 4 weeks, for both the commercial Surgicel and ORC-Na-3 samples, neither the residual testing samples nor the obvious anaphylactic tissue reactions could be observed in the implantation sites, Fig. 8.

For further explaining the histopathological results, it is important to note the in vivo degradation mechanism of Surgicel (ORC) and ORC-Na. When implanted in vivo, the commercial Surgicel turns to yellowish brown and loses much of its original tensile strength with increasing the implantation period. From the histological examination in this paper, the commercial Surgicel appears to be absorbed within a period of 2–4 weeks. Early studies also showed that the materials were completely absorbed within about 1 month and degraded into many oligomeric carbohydrate breakdown products, majority of which were glucose and sodium salt of the glucuronic acid metabolized via well-established pathways. A biodegradation mechanism is proposed, which start with a degradation procedure due to the weak basic pH of blood. This degradation depends on the ketone groups, which are formed due to the oxidation reaction at C2 and C3 during the preparation of ORC. These groups on the enolization yielded enol, rendering the adjacent glucosidic groups sensitive to β-elimination. Once the degradation is initiated, the degraded fragments are generated and further degrade due to the formation of new carbonyl groups, and the process of degradation will continue along the molecular chains. And then the macrophage digestion of the oligosaccharides is carried out with hydrolytic enzymes (Domb et al., 1998).

Subcutaneous implantation has demonstrated that ORC-Na-3 could be completely biodegraded within 2 weeks without traces of a foreign body reaction remaining, which indicates it have a significant higher biodegradability than the commercial Surgicel. Similar to the commercial Surgicel, the biodegradation process of ORC-Na-3 is consistent with an initial chemical degradation followed by the enzymatic processes to produce nontoxic products. What is more, due to the additional water-soluble property, ORC-Na-3 start to form a gel when it imbibed blood and its surrounding tissue fluid, and then the gelatinous materials will be eroded as fragments and dissolve in the surrounding fluid in a shorter time. Thus, the distribution of small fragments in vivo is broadened so that more enzymes will have an effect on carrying out the enzymatic processes, which could accelerate ORC-Na to degrade.Overall, compared with the commercial Surgicel and other ORC-Na materials, ORC-Na-3 hemostat represents an outstanding hemostatic efficiency and a high biodegradability, and also suggests a wide application scope, which may give it a competitive edge.

4. Conclusions

The results show that the novel partially water-soluble oxidized regenerated cellulose carboxylate sodium (ORC-Na) materials with different carboxyl content have been obtained through the controlled neutralization of oxidized regenerated cellulose (ORC) with NaOH/ethanol. FT-IR spectroscopy demonstrates that partial carboxyl groups in the ORC have been converted into sodium carboxylate groups, and the residual carboxyl content in ORC-Na materials decreases gradually 18.41%, 14.09%, 9.18%, and 5.23–0.98% with increasing the ratio of n_{NaOH}:$n_{\text{carboxyl group of ORC}}$, leading the materials to represent more and more excellent water-soluble performance with subsequent formation of the gel. Significantly, SEM–EDX analysis reveals that the content of Na element becomes less and less from the surface skin to the core of the fiber, suggesting that the carboxylate sodium groups exhibit a graded distribution from the outside to the inside in the ORC-Na fibers. Therefore, ORC-Na materials could adopt different hemostatic mechanisms at different bleeding stages, one is provided by the residual carboxyl groups in these partially water-soluble materials, and the other is based on the water-soluble carboxylate sodium structure, ORC-Na is able to form gel by absorbing blood and then seals off the crevasses of blood vessels to stop bleeding. Experimental data indicate that the hemostatic efficiency of ORC-Na materials rely on not only the residual carboxyl content but also the carboxylate sodium structures. As the carboxyl content is neutralized to 5.23%, i.e., ORC-Na-3 represented an excellent hemostatic performance, its mean hemostatic time is the shortest about 102 and 138 s with the least blood loss in rabbit ear artery and hepatic trauma models, separately, which is 24 and 38 s shorter than that of the commercial Surgicel. On basis of biodegradability mechanism including chemical degradation and enzymatic processes, the subcutaneous implantation test showed that the complete biodegradation of ORC-Na was no longer than 2 weeks without local reactions due to its additional water-soluble property, whereas the commercial Surgicel needs 2–4 weeks.

Disclosure

Surgicel® absorbable haemostat, a kind of commercial oxidized regenerated cellulose, was manufactured by Johnson & Johnson Medical Limited.

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