Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-7160

Reviewer A

Comment 1: I suggest describing in one sentence what means topical hemostatic agent in the introduction section

Reply 1: we have modified our text as advised (see Page 4, lines 8-10)

Changes in the text: Topical hemostatic materials applied locally to the bleeding wounds for hemostasis, currently play an important role in hemostasis during surgery.

Comment 2: Errors for the reference source makes it difficult to find tables and figures

Reply 2: we have added the missing reference (see Page 5, lines 2; Page 5, lines 21; Page6, lines 19; Page 11, lines 15; Page 23, lines 15; Page 23, lines 17)

Changes in the text:

1) Page 5, lines 2 : and blood clotting (Figure 1)
2) Page 5, lines 21 : as depicted in Table 1
3) Page 6, lines 19 : Table 1 summarizes the classification of hemostatic materials based on their main components.
4) Page 11, lines 15 : The recent research of modified chitosan or chitosan-based composite materials for hemostasis is depicted in Table 2
5) Page 23, lines 15 : as well as the recent development (Table 3)
6) Page 23, lines 17 : in different areas (Table 4).

Comment 3: Page 6 line 5. What authors mean by the term that polymers are still under development? There are many other polymers that in the future will be applied as hemostatic agents. It is more a matter of hemostatic material formulation instead of polymer development.

Reply 3: we have modified our text as advised (see Page 6, lines 5-7)

Changes in the text:

Synthetic hemostatic materials can be fabricated and formulated with other adjuncts industrially to improve their biocompatibility.

Comment 4: Page 9 line 7-8 Does CGX has higher rates of survival or cause higher rates of survival? I would like to underline the grammar of this sentence.

Reply 4: we have modified our text as advised (see Page 9, lines 12-15)

Changes in the text:

In a standard porcine trauma bleeding model, compared with QCG, the application of QCX could more effective reduce the mortality of the injured animals and achieved the superior immediate hemostatic effect, owing to the increasing gauze mass or greater quantities of active ingredient (43).

Comment 5: Page 21 line 10, the “discussion on” not is.

Reply 5: we have deleted these words and modified our text (see Page 21, lines 13-16)
Changes in the text:
Synthetic hemostatic materials are another type of hemostatic materials which can be produced industrially and can be formulated with other adjuncts to improve their biocompatibility, stability and clinical performance.

Comment 6: Page 23 and 24 (line 7)
Reply 6: we have added some additional references as advised (see Page 19, lines 22-23; Page 20, lines 1-4; Table2 Ref. 57; Table 2 Ref. 62)
Changes in the text:

1) doi.org/10.1016/j.actbio.2018.11.029: Recently, due to their controllable morphology and surface properties, nanofibrous materials have a wide range of applications in the biomedical areas, such as wound dressing, drug delivery, tissue engineering, and hemostasis.
2) doi.org/10.1016/j.biomaterials.2018.06.031: Xie et al. fabricated an injectable and superelastic nanofiber rectangle matrix ("peanut") coated with gelatin. It is demonstrated that the "peanut" presented effective hemostasis in a porcine liver injury model by absorbing water and accelerating clotting processes
3) https://doi.org/10.1016/j.msec.2020.111740: Table 2 Ref. 57
4) https://doi.org/10.1016/j.ijbiomac.2020.06.187: Table 2 Ref. 62

Reviewer B

Comment 1: some additional references should be added providing latest achievements in this field

Reply 1: we have added some additional references as advised (see Page 19, lines 22-23; Page 20, lines 1-4; Table2 Ref. 57; Table 2 Ref. 62)

Changes in the text:

1) https://doi.org/10.1016/j.msec.2020.111740: Table 2 Ref. 57
2) https://doi.org/10.1016/j.ijbiomac.2020.06.187: Table 2 Ref. 62
3) doi.org/10.1016/j.actbio.2018.11.029: Recently, due to their controllable morphology and surface properties, nanofibrous materials have a wide range of applications in the biomedical areas, such as wound dressing, drug delivery, tissue engineering, and hemostasis.
4) doi.org/10.1016/j.biomaterials.2018.06.031: Xie et al. fabricated an injectable and superelastic nanofiber rectangle matrix ("peanut") coated with gelatin. It is demonstrated that the "peanut" presented effective hemostasis in a porcine liver injury model by absorbing water and accelerating clotting processes

Comment 2: The article should contain more precise data about hemostatic agents obtainment or modification

Reply 2: we have added some additional contents as advised (see Table 2)

Changes in the text:

We mainly added the contents of 'Major components or process' in Table 2.

Comment 3: some sections are described very poorly - for instance chitosan application as hemostatic agents.

Reply 3: The application of chitosan has been further introduced (see Page 11, lines 4-15)

Changes in the text:

Currently, several FDA-approved chitosan-based hemostatic products are commercially available, including HemCon® Bandage, Celox® and TraumaStat®. HemCon® Bandage uses freeze-dried chitosan as raw material. Because of the bandage compression and the hemostatic properties of chitosan, effective hemostasis is achieved within 2 minutes. However, HemCon® Bandage needs to be removed within 48 hours and is difficult for deep or small wounds due to its rigidity. Celox®, the main ingredient of chitosan particles, avoids the shortcoming of rigidity and performs better in the effect of hemostasis compared with HemCon® Bandage. Moreover, Celox® is also suitable for patients with coagulation dysfunction. TraumaStat®, a hemostatic gauze synthesized from chitosan, silicon dioxide and polyethylene, has larger specific surface area, which significantly increases the contact area with the wound. And TraumaStat is more flexible to apply to various types of wounds (53). The recent research of modified chitosan or chitosan-based composite materials for hemostasis is depicted in Table 2.

Comment 4: The repetitions should be removed e.g. abstract (lines 2-3, 3-4); page 5, 1-2, 26, pages 9-10, 14 line 5, 19 page lines 16-17, 21 and so on

Reply 4: we have modified our text as advised

1) the repetition of abstract in future part have been rewritten (see Page 23, lines 19-23)

2) page 5, line 26 and page 14, line 5 have duplicate content, which have been deleted (see Page 6, lines 3-5; Page 14, lines 11-14)

3) pages 9-10 have duplicate content, which have been modified the text (see Page 6, lines 1-3; Page 10, lines 4-9)

4) we have modified the text in page 19, line 16-17, 21 as advised (see Page 19, lines 15-21)

Changes in the text:

1) Despite the great potentials of the improved hemostatic materials for clinical use, few of them have been translated from the laboratory study to clinical application. As we know, fabrication of the novel products and continuous optimization of their hemostatic properties are still of great significance for controlling bleeding and reducing mortality. Therefore, future research should be directed at combining the different materials and technologies to screen out an ideal hemostatic product

2) Page 6, lines 3-5: Biological-derived hemostatic materials have excellent hemostatic performance by directly increasing clotting substances, whereas the high-cost, immune reactions and viral infections hamper the widely used of these materials.

Page 14, lines 11-14: Biologically derived hemostatic agents can directly increase coagulation factors at the topical injury site, activate blood clotting and thereby present the outstanding hemostatic efficacy. These materials participating at the end of the coagulation cascade to form a fibrin clot are called active agents, such as thrombin and fibrinogen.

3) Page 6, lines 1-3: Polysaccharide based hemostatic materials, composed of the natural carbohydrates, draw attention to the researchers for their bioabsorbable features and low cost.

Page 10, lines 4-9: Polysaccharides, the form in which most natural carbohydrates occur, have a branched or linear molecular structure. With the characteristics of low cost, abundant resources, good biocompatibility and no risk of immune responses, polysaccharide-based materials have been extensively investigated for the application on hemostatic agents, such as chitosan, starch, cellulose and alginate. However, the limited hemostatic efficacy hampers their widely use. With the development of the chemical and physical methods, more and more polysaccharides-based hemostatic materials have been achieved.

4) FloSeal, the liquid form of gelatin-based products, contains a proprietary of the gelatin matrix and topical thrombin and is prepared by mixing them immediately prior to use. FloSeal has a wide range of clinical applications not only because of its advantage of a tamponade effect, but also its advantage of the liquid form: flexible to any irregular wounds, resulting in intimate contact of FloSeal with the site of bleeding. Furthermore, FloSeal can be safely used in limited surgical spaces, in which high concentrations of thrombin achieve effective hemostasis (96).

Comment 5: The key word "Application" should be removed or completed with the missing word
Reply 5: we have removed “Application” from the keywords (see Page 3, lines 10)
Changes in the text:
Keywords: Topical hemostatic materials; Hemostatic mechanism; Hemostatic products; Prospect

Comment 6: Line 21 - of various blood components, such as platelets, coagulation factors, cells and so on. -- the sentence should be corrected - platelets are also cells
Reply 6: we have deleted “platelets” from the sentence (see Page 4, line 23)
Changes in the text:
of various blood components, such as coagulation factors, cells and so on.

Comment 7: Line 23 - add missing ref
Reply 7: we have added the missing figure (see Page 5, lines 2)
Changes in the text:
and blood clotting (Figure 1)

7) Page 6, lines 19 : Table 1 summarizes the classification of hemostatic materials based on their main components.
8) Page 11, lines 15 : The recent research of modified chitosan or chitosan-based composite materials for hemostasis is depicted in Table 2
9) Page 23, lines 15 : as well as the recent development (Table 3)
10) Page 23, lines 17 : in different areas (Table 4).

Comment 8: page 5 Line 20 missing ref, line 23-25 should be rewritten, line 26 misspelling
Reply 8:
1) we have added the missing reference (see Page 5, line 21)
2) we have modified the text as advised (see Page 6, lines1-3)
3) we have changed the word “homeostatic” into the right one “hemostatic” (see Page 6, line 3)
Changes in the text:
1) as depicted in Table 1.
2) Polysaccharide based hemostatic materials, composed of the natural carbohydrates, draw attention to the researchers for their bioabsorbable features and low cost.
3) Biological-derived hemostatic materials

Comment 9: page 6 Line 3 (grammar), line 14 missing ref, 15 grammar
Reply 9:
1) we have modified our text as advised (see Page 6, lines 5-7)
2) we have added the missing reference (see Page 6, line 19)
3) we have modified our text as advised (see Page 6, lines 19-20)
Changes in the text:

1) Synthetic hemostatic materials can be fabricated and formulated with other adjuncts industrially to improve their biocompatibility.
2) Table 1 summarizes the classification of hemostatic materials based on their main components.
3) Table 1 summarizes the classification of hemostatic materials based on their main components.

Comment 10: page 7 Line 16
Reply 10: we have modified our text as advised (see Page 7, line 20-23)
Changes in the text:
For increasing survival in the prehospital setting, a flexible mesoporous zeolite-cotton hybrid hemostat was synthesized, which possesses various advantages, such as meso-/micro-porosity, quick blood coagulation and stability. The combination of cotton and zeolites can achieve rapid hemostasis and save life in emergency conditions, especially in the case of insufficient first aid measures (36).

Comment 11: page 9 line 7
Reply 11: we have modified our text as advised (page 9, line 12-15)
Changes in the text:
In a standard porcine trauma bleeding model, compared with QCG, the application of QCX could more effective reduce the mortality of the injured animals and achieved the superior immediate hemostatic effect, owing to the increasing gauze mass or greater quantities of active ingredient (43).

Comment 12: pages 10, line 11
Reply 12: we have modified our text as advised (page 10, lines 15-17)
Changes in the text:
(1) Aggregation of red blood cells (RBCs). Positively charged glucosamine on chitosan can attract negatively charged RBCs to agglutinate, and thereby promote coagulation, which is independent of the classical coagulation cascade (49);

Comment 13: page 12, line 1 soluble in what (author probably meant water or aquatic solutions)
Reply 13: we have modified our text as advised (page 12, lines 7-10)
Changes in the text:
Natural cellulose is insoluble in water but oxidized cellulose (OC), an absorbable oxidation product of cellulose, can be either regenerated to form organized fibers (oxidized regenerated cellulose, ORC), or remain non-regenerated with unorganized fibers prior to oxidation (oxidized non-regenerated cellulose, ONRC).

Comment 14: page 13, line 1, 21
Reply 14: we have modified our text as advised (page 13, lines 7-9; page 14, lines 1-6)
Changes in the text:
1) Although SURGICEL® family is the effective and bioabsorbable hemostatic

materials, it still brings some safety concerns when applied in the clinical treatment, such as cord compression, granuloma/neoplasia and so on (69).
2) It has been demonstrated that divalent or polyvalent cations, such as Ca2+, crosslink soluble sodium alginate into insoluble alginate. Once calcium alginate (CA) contacts with blood, Ca2+ act as a procoagulant to activate the coagulation process. In fact, alginate does not possess inherent hemostatic property in the classical sense, but alginate wound dressings provide a physiological moist microenvironment, prevent bacterial infection at the injured site and facilitate the wound healing (72,73).

Comment 15: page 15, line 2, 15
Reply 15: we have modified our text as advised (page 15, lines 7-8; page 15, lines 18-19)
Changes in the text:
1) rhThrombin has similar efficacy to animal- or human-derived plasma thrombin, but has a significantly lower risk of immunologic impact.
2) Besides two primary components, other active components usually added to fibrin sealants are Ca2+ and anti-fibrinolytic agents like aprotinin (80).

Comment 16: page 17, line 8
Reply 16: we have modified our text as advised (page 17, line 8-12)
Changes in the text:
At least 29 types of collagen have been identified so far and classified primarily according to their structures, over 90% of which in the body is the type I. The excellent characteristics of collagen include easy extractability, low cost, good biocompatibility and so on, which make collagen an attractive biomaterial for developing the medical products and therapeutical devices (89).

Comment 17: page 18, line 5, 9
Reply 17: we have modified our text as advised. (page 18, line 4-5; page 18, lines 6-12)
Changes in the text:
1) The powder has a series of excellent characteristics, such as easy-and ready-to-use with good biocompatibility (resorbed within 4 weeks) (27).
2) Oxidized microcrystalline cellulose (OMCC), a new type of oxidized regenerated cellulose, is regarded as an affordable and effective hemostat with properties of biological safety, low cost and excellent hemostatic effect. Researchers had fabricated a composite containing the single-collagen sponges and OMCC, to improve the hemostatic ability of the collagen. The results have shown that the composite could reduce the lengths of the activated partial thromboplastin time (APTT) and thrombin time (TT) in vitro, and presented a rapid hemostatic effect in vivo (92).

Comment 18: page 19, line 4, 23
Reply 18: we have modified our text as advised (page 19, line 2-5; page 20, line 6-7;)
Changes in the text:

1） Gelatin materials can not only absorb many times of their own weight of blood, but

also concentrate clotting factors and platelets at the site of injury. After absorbing water, gelatin materials swell, which in turn provides a compressive effect in the wound site. Additionally, it can provide a structural matrix for clotting (94).

2）Microfibrillar collagen, made from purified bovine collagen and applied as dry loose flour form, is one of the most widely used topical hemostatic agents.

Comment 19: page 21, re-write lines 10-16
Reply 19: we have modified our text as advised (see page 21, lines 13-16)
Changes in the text:
Synthetic hemostatic materials are another type of hemostatic materials which can be produced industrially and can be formulated with other adjuncts to improve their biocompatibility, stability and clinical performance. However, the cytotoxicity and non-biodegradability from the synthetic hemostatic materials are the potential problems, which should be noticed in the future development.

Comment 20: page 22, line 18
Reply 20: we have modified our text as advised (page 22, line 17)
Changes in the text:
forming the adhesive film that glues the wounds and holds the apposed edges together.

Comment 21: page 23 Future - whole section must be re-written
Reply 21: we have modified our text as advised (page 23-24)
Changes in the text:
Despite the great potentials of the improved hemostatic materials for clinical use, few of them have been translated from the laboratory study to clinical application. As we know, fabrication of the novel products and continuous optimization of their hemostatic properties are still of great significance for controlling bleeding and reducing mortality. Therefore, future research should be directed at combining the different materials and technologies to screen out an ideal hemostatic product.
Firstly, due to the limited hemostatic performance of a single-component hemostatic material, composite hemostatic materials containing two or more active hemostatic agents should be fabricated to improve their hemostatic performance.
Secondly, the comprehensive performance of hemostatic products should be improved with up-to-date technology. Besides the excellent hemostatic performance, the products would be more widely used with other advantages, such as antibacterial, anti-inflammatory, healing promotion and so on.
Thirdly, tissue-specific hemostatic materials and products should be developed. Novel hemostatic products should be specific for different tissue wounds, such as different shapes and depths of wounds.
Lastly, substantial interdisciplinary research efforts are necessary to conduct robust studies for fabricating an ideal hemostatic dressing, such as establishing and perfecting animal models of trauma, inventing effective hemostatic devices and conducting clinical trials in multidisciplinary.