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(54) Title: REGENERATED OXIDIZED CELULOSE BASED HEMOSTATIC MATERIALCONTAINING ANTIFIBROLYTIC AGENTS

(57) Abstract: In present invention oxidation method of viscon cellulose with NO₂ obtained H₃PO₄/HNO₃ is defined liquid and gas media. Regenerated oxidise cellulose (REOC in shorten term) contain in -COOH yields are standardised as 18.6 - 20.1 for textile, 19.8 - 21.5% for powder samples. Powder and textile (woven and fabric) products are impregnated 1.8-2.4% Ca⁺² ion, 0-1.1% Na⁺¹ ion, 0.8 - 1.5% tranexamic acid and 6-aminocaproic acid as antifibrinolytic. Obtained powder and gel products are impregnated Bi⁺³, Zn⁺² and Ag⁺¹ ions for antiseptic purposes. Only Bismuth of them is shown antibacterial effects. Also the aim of present invention is haemostat antimicrobial properties during impregnation of Rifampicin, Gatifloxacin, Doxycycline, Levofloxacin, Lincomycin, Clindamycin, Ciprofloxacin. Haemostatic properties are indicated for all products and antimicrobial properties are shown for some samples. Cytotoxicity, sensitivity and irritation properties are determined in compliance of Pharmacopeias.



REGENERATED OXIDIZED CELULOSE BASED HEMOSTATIC MATERIAL CONTAINING ANTIFIBROLYTIC AGENTS

5 TECHNICAL FIELD

Bleeding is foremost reason of death during medical operations and various traumas. The instant medical response and to stop the bleeding is critical for the treatment of patient. Traditional gauzes and bandaging is inadequate. Buffering with cellulose, thin and loose woven gauzes could not support the coagulation. Some absorbent as like "Bone powder, 10 ostene, gelatine foam, oxidise cellulose, micro fibrillar collagen, trombone, fibrin, chitosan, zeolite are in use for haemostatics' agents.

PRIOR ART

15 When search the mechanism of blood clotting, important 8 factors interfering biochemical reaction can be considered. To speed up the formation of fibrin is important to coagulate of incision and open wound. Therefore two effective process can be examined: inhibition of plasmin enzyme and providing the calcium ion flow. Calcium salts or fibrin formation with 20 anti-fibrinolytic compound and stabilisation of fibrin can be obtained (S. Samudrala, Aorn J. Inc. Sept. V.88 (3) 2008).

The basic aim of this study are to produce the bandage, pad, powder and gel with haemostatic and antiseptic features and release the medical market.

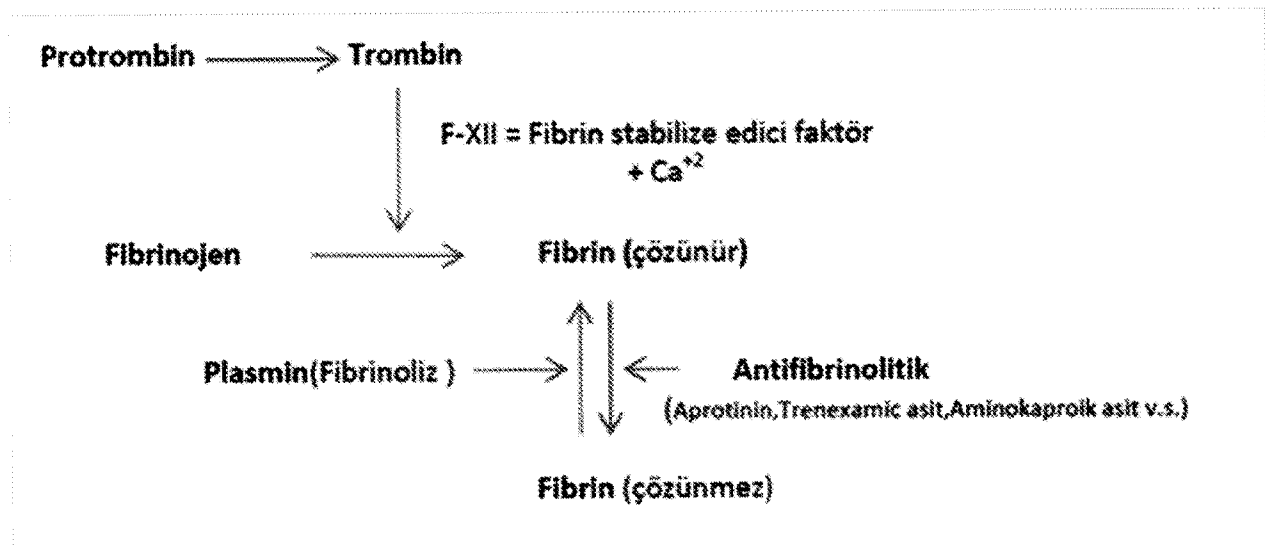
25 Dry cellulose based hemostatic bands or pads is impregnated by some substances formed solid, powder or gel, featured hemostatic and anti-septic, will be designed to control bleeding on traumatic and severe bleeding.

30 To prevent the bleeding of open wound and incision, it is important to provide the formation of fibrin and the stabilisation of fibrin. The coagulation process and its steps are shown on Schema-1. Two important steps are inhibition of fibrinolysis enzymes and sufficient calcium ions flow is activated Factor XII. Therefore fibrin formation with Ca^{+2} ion and with anti-fibrinolytic (anti-plasmin) as like Aprotinine, 6-aminocaproic acid, 6-acetaminocaproic acid,

- 5 tranexamic acid, 4-aminomethyl benzoic acid and its stabilisation will be obtained (S. Samudrala, Aorn J.Inc. Sept. V88 (3) 2008).

This study also comprise to design the anti-microbial protective products together with its benefits as controlling of bleeding for hemostatic purposes.

10



Schema-1. Coagulation and fibrin formation

15

There are two steps for the hemostasis under the bleeding wounding, trauma.

- Primer hemostasis means that vascular wall established any reason is formation of blood platelet in the result of thrombocyte local activation and aggregation. After wounding, thrombocytes bound collagen protein fibres; glycoprotein-Ib and plasma factors is necessary for this reaction. The platelets aggregate with homogen mass unless the wound is big. Under the external effect neuromediator as like ceratone secrete and it causes the vasoconstriction and have the significant role for speeding up the coagulation. Shorter hemostatic time is the important point for this study.

- 25 Secondary hemostasis: Platelets formed at primer step is not enough for complete healing. To obtain sufficient hardness, fibrin formation in the other words coagulation thrombosis is required. For this formation there are intrinsic and extrinsic factors together with trombone generation on the platelet to change insoluble fibrin from soluble fibrin. The fibrin stabilisation factor is calcium ions at this step (Schema 1).

30

- 5 Changing soluble fibrin mechanism is essential to block fibrinolysis enzyme. It is the subject to add local effects along with anti-fibrinolytic compounds.

According to Morawitzs, for the coagulation there are 4 basic factors. "Farmasötik Kimya, Hacettepe Üniv. Eczacılık Fak. Yayınlar (2013), E.Mutschler, et.al. Arzneimittel wirkungen, 10 Wiessenschaftliche Verlag GmbH Stuttgart 9. Auflage(2008)"

- * Factor I Fibrinogen
- * Factor II Prothrombin
- * Factor III Thromboplastin
- 15 * Factor IV Calcium ion

It is well known that the result of thrombin reaction with fibrinogen is obtained the clot; the formation of fibrin and joining of 8 various factors ensure to stop the bleeding. All coagulation factors are the proenzyme excluding Factor III. To stop bleeding there is two 20 important points; first cellular which is blood platelet and second humoral factor which is formation of the fibrin. During trauma, on external and internal bleeding the formation of fibrin as humoral factor is mainly important.

The initial material and technology research shows there are not many alternatives for the 25 preparation of pad, gauze, powder and gel products which have hemostatic, antiseptic properties. Indeed some commercial brands for example: Hemcon, Anscare, Ankaferd Blood Stopper (ABS), Surcicel, Dettol, have either hemostatic or antiseptic feature. This study aim is the methods of preparing more useful hemostatic, anti-septic compound and certificate with its in-vitro in-vivo tests (or new tests if requires). In other words, anti-fibrinolytic, 30 hemostatic, hypoallergic (non cytotoxic), partial antimicrobial powder and pads for use in trauma and mid severe wound will be developed. Main aim is hemostatic time which will be in the range of 0.5 - 3.5 minutes.

To reach the aim mentioned above, formulation plan:

35

1. The formation of carrier matrix; the preparation of chitosan and oxidised cellulose based on hemostatic polymer
2. Impregnation of chemicals effective antimicrobial and anti-fibrinolytic on hemostatic polymer matrix material which is recorded on the literatures

- 5 3. Plaster this pad and packing against environmental effect
4. Sterilise by gamma radiation and compliance test for dermatologic, cytotoxic, microbiological at the university or accredited lab.
5. Determine the hemostatic time and antimicrobial minimal inhibition concentration (MIC) values in compliance with European Pharmacopeia in matrix material mentioned item 1.
- 10 6. Chemicals as antifibrinolytic effect plan to use is tranexamic acid and 6-aminocaproic acid and aprotinin.

The product after present invention, features must be:

- * Hemostatic time will be 0.5 - 3.5 min.
- * Large antimicrobial effective spectrum
- 15 * Must be completed the in-vitro and in-vivo tests (hemostatic time of rat liver, cytotoxic effect, irritation, sensitisation, antimicrobial effect).
- * Must have hypo allergic feature
- * Soft and absorb the serum
- * Do not stick to the surface of the wound
- 20 * Proof the compliance with biodegradable standards via histopathological animal tests.
- * Determine the bio absorption time must be 2 - 5 days

Matrix formation:

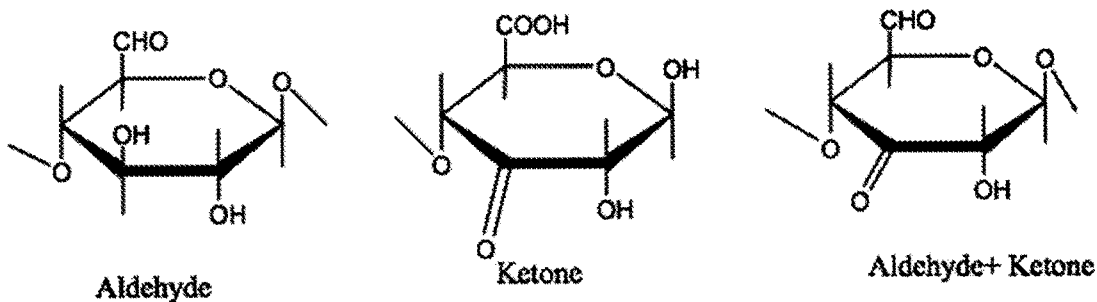
- 25 Until today hemostatic and antiseptic polymer matrix for use in medical purposes, had two basic targets:
 - Anionic matrix (oxidised cellulose and its derivatives)
 - Cationic matrix (Chitosan, gelatine, fibril collagen and its derivatives)

1.1. Anionic matrix polymer:

- 35 As a result of various natural cellulose derivate chemically, obtain hydroxyethyl cellulose, 2-hydroxypropyl cellulose, methyl-ethyl-cellulose and carboxymethyl cellulose. This cellulose derivatives obtain the substitution of OH groups on the glucose unit of the cellulose. These do not have any hemostatic feature and have water absorption, gel formation with micelle concentration. Oxidised cellulose is formed anionic hemostatic polymer in rate of carboxyl group on the 6. C which carry oxidise cellulose derivatives.

- 5 These products are called as poly anhydroglycuronic acid (PAGA). Polymeric mass carries carbonyl groups in yield 8-30% of carboxyl. In general linear polymer has 23.6 or 30% in this yield depends on its branches of glucose polymer. The predicted rate of glycuronic acid in carboxyl molecule is more than 80%. During oxidation partly occur ketone carbonyl at 3C. of aldehyde and glucose (Schema 2).

10



Schema-2: Oxide products after Cellulose oxidation

- 15 Aforesaid carbonyl have maximal 5% aldehyde carbonyl as request. More than this yield is not applicable for hemostatic polymer. In general desirable yield is less than 2.3%. After cellulose nitrate oxidation, anionic matrix contain maximal 0.5% bound nitrogen. In general desirable rate is less than 0.2%.
- 20 The size of carbohydrate polymer molecule size is 1×10^3 - 3×10^4 daltons. Regenerated cellulose molecule size is 1×10^3 - 5×10^3 that is bigger than linter cellulose. Viscon cellulose molecule size is the range of 5×10^3 - 1.5×10^4 dalton. Ideal size is 1500 k.dalton. Carbonyl amount of cellulose polymer is 12 - 26% of whole molecule. Glycuronic acid is 95% of it. In the molecule for the decreasing of aldehyde rate (if 0.6 - 1.5%) and increasing the yield of
- 25 carboxyl is done by raw oxidised cellulose re-oxidised with hydrogenperoxide. Oxidised cellulose (which carry 16 - 22% carboxyl) and its salts as an anionic matrix are used for hemostatic purpose " W.H.Ashton, US 3 364 200 (1968)".

1.2. Cationic matrix polymer:

30

Cationic matrix (polypeptide gelatine, fibrillar, collagen, 2-amino-D-glucose as polysaccharide and 2-acetamido-D-Glucose as chitosan) is used as hemostatic covers. They all are biocompatible hemostatic but some patients had occur allergic and erethitic reaction. Chitosan chitin is obtained by de-asetilation which 85% gain is necessary condition for

35 hemostatic purpose.

5

For use in medical operation and external bleeding is subject to prepare intermolecular complex (IMC) from all these matrix. This invention aim is to use PAGA as a basic matrix, prefer to take either costing or toxicity side effects. Final product is dual effected medical material featured both flexible, absorbable, biocompatible hemostatic and antimicrobial.

10

Present invention try to determine the fabrication production method of powder, band, spanch, gel forms and IMC complex solution' s preparation which specified above.

15

For powder formulation PAGA is quite sufficient. It is possible to prepare gel formulation with Gelatine and Chitosan. Biocompatible formulation is possible to add Ca and Na salts. Plaster band and spanch form production make difficulties to add these salts. Therefore latest studies start to use nonwoven textile or polymeric film. Animal hemostatic test and human tests are parallel relatively (0.99/0.01 - 0.01/0.99 statistically) for PAGA and this is its primer advantage. Secondly its hemostatic degree is measurable biologically. On the other hand its hemostatic time, bio-absorption time and immune modulative compliance values are measurable. The measurement techniques are written on pharmacopeias.

20

PAGA is compatible with polymeric cationic matrixes and use with. Cationic matrix groups are:

25

1. Biocompatible, with nitrogen, synthetic oligomer and polymer
 - a. Acrylamide and metacrylamide polymer and co-polymer, natural polysaccharides (gum-, quargum-, hydroxypropyl triammonium chloride etc.
 - b. Synthetic and semi-synthetic polyamino acid polilizin, polyarginine, ...-poli (N-(2-hydroxethyl) DL-asparamid and synthetic anti-fibrinolytic, hexadimethrine bromide (pyoben)
2. Natural and semi-synthetic peptides, gelatine, collagen, protamine, fibrinopeptide and its derivates.
3. Chitin as natural anti-aminoglucan and its fractions, its de-asetile derivatives are chitosan, microbial origin arthropods "crabs"

30

In this study these groups are not included because their measurement and standardisation have the difficulties and their cost is high.

35

As numerous literature and patent studies are recorded, PAGA matrix compounds/solutions was prepared with specified three groups above and could not reach satisfied results correspond to speed hemostatic and allergic and cytotoxic properties, stability at animal trials. IMC specified at 1a and 1b is tested preferably but only film and plaster had technic

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5 efficiency while working on non-woven, film, plaster and pad forms. Indeed prototype
production (hemostatic and cover) is prepared for external wounding. We concentrate only
oxidised cellulose production and its method standardisation on our invention. Second and
third group cationic matrix are used in micro operations and need aseptic and automated
systems to produce therefore this study focus on the low-cost production of biodegradable,
10 biocompatible oxidised cellulose and its validation.

Regenerated oxidised cellulose (REOXC) is natural topic hemostatic biomaterial commonly
used. It is formed that the selective oxidation of 6C. primer alcohol groups on cellulose
polymer. As a result of general oxidation, -COOH group is obtained and its range is 16 - 24%
15 of total polymer. "Yadong Wu et al. Carbohydrate Polymers; 88 (2012) 1023 - 1032". This
product is comply to produce as sterilisable powder or textile manufacturing. This product is
seen as powerful acute hemostatic and also it is known as great biosafety material in
reference to "Zhu I.H. et.al. Journal of Pharmaceutics (2001), 223, 35 - 47".

20 As it is known REOXC was produced by NO₂ oxidation of cellulose on industrial level at
1930's (Lit. 5,7,13,14). Selective oxidisation was done by "Yackel, E.C. and Kenyon, W.O. US
2 232 990" at 1940's and analyse the oxidation kinetic and the rate of NO₂ / cellulose. The
result of them is shown at Table-1. The more gas level in a unit volume is the more
oxidation level of the cellulose.

25

Table-1.

72 hours, NO₂/Cellulose ratio and oxidation relation (Previous Method)

NO₂-Cotton concentration factor	-COOH %
0.60	13.26
0.75	13.38
0.90	15.84
1.20	19.80
1.50	21.80

5

Many researcher have studied with different oxidants of this product and one of them is Proskulo et. al. carbohydrate Polymers 77, 791-798 (2009). Production trials by using inert gas instead of pure gas is first done at 1968 (US 3 364 200) by Johnson and Johnson Company. 8-13% oxidation in 16 hours, 18-22% in 72 hours was got to use 20% NO₂ gas in CCl₄, Freon-113, Freon-11 that is written US 6 627 749 B1, WO 2007/085364.

10

J&J Co. have still supplied to the world, to produce oxidised cellulose by NO₂ oxidation which is validated by oxidation process. REOXC (Surgicel®) as absorbable hemostatic is marketed. Both clinically and during the operations, it is important hemostatic of external wounding.

15

Although these important properties REOXC has disadvantage. Either partly anti-microbiological or biocompatibility or biodegradability is problematic. However it is written on "X. Zhou, X. Huan, US 2010 /0298264A1" that degradation time is 5 - 7 days, absorption time is 8 - 15 days, -COOH yield is 8-18%, hemostatic time is 2 min; this statement is related with -COOH groups carried the product. In our study if the yield of -COOH is 8 - 12%, increasing at absorbability and degradability is determined. -COOH yield is 22 - 24% cause sensitivity and irritability to nervous system, it is disadvantage and undesired status. "Watt et al. EP 1325754A1". The best hemostatic property -COOH yield must be limited "Stilwell et. al USP 5,484,913". To increasing absorbability and degradability, Ca⁺² and Ca-salts can be added to free -COOH.

20

25

In this invention Ca-ReOxicell (REOXI-C, Sample3) powder which pH is 4.0 - 4.5 is prepared and both hemostatic effect and biodegradable features is getting better. To produce powder form is easy but band and cotton-spunch producing is hard to provide this specs. (saferstein, L., et.al. (1992) US 5,134,229; US 5,134,229; US 5,484,913).

30

In this study and invention, REOXC is prepared with Ca-asetate, Na-asetate and Ca, Na salts. We determined via animal tests that hemostatic properties are decreasing while Na amount and solubility in water is increasing.

35

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2. Detailed explanation of the invention:

15

2.1. Oxidised Cellulose Production (REOXC)

Naturally present cellulose are natural cotton and linter cotton finer. The cellulose rate of cotton is 88 - 92% and for linter cotton is approx. 94 - 97%. In recent years regenerated synthetic polymer have been used. Regenerated viscon cellulose (Regenerated cellulose REC) called viscon cellulose is the most important one. Natural semi-synthetic carbohydrate polymer is D-glucose polymer. After 1-4-D-Glucose 6C. primer alcohol group oxidation, occur -COOH group and oxidised cellulose. During REOXC production following oxidation methods (as is known) have been worked:

- 25 a. Hypochlorite oxidation
b. Persulphate oxidation
c. TEMPO (1-oxyl -2,2,6,6-tetramethylpiperidin) catalyse hypobromide oxidation
d. NO₂ oxidation
- 30 Oxidation productivity is 5 - 11% while working on these methods a. Hypochlorite oxidation and its modification b. radicals oxidation with sodium persulphate "WO 2007/085364, WO 95/07303". Ketone rate at C2-3 status of glucose is high while going the reaction kinetically and thermodynamically. It is recorded that NaIO₄ and C2-C3 bound breaks to occur dialdehyde form "WO03/020191, DD 275 696A1. EP 1 122 237 A1". On watered media
- 35 reaction, small molecular oligopolymer occur at side reaction by hydrolysis the cellulose polymer. To prevent this, on the c. 1-oxyl- 2,2,6,6-tetramethylpiperidin (TEMPO) oxidation method, when radical oxygen carrier is used carboxyl percent is increasing a little, and this reaction is working at pH 10 - 11 and watered media.

- 5 By this way hydrolysis of cellulose is precluded and selective primer alcohol oxidation is done. But this application is not sufficient for industrial production and oxidation productivity is low. "Santo T. et. al. Carbohydrate polymer, 2005, 61, 414-419., X. Zhou, X. Huan US 2010/0298264A1. WO 95/07303."
- 10 d. With fourth oxidation method, selective oxidation of primer alcohol groups are occurred. This method is applicable for all cellulose types. It is controllable reaction under the conditions of thermodynamic and kinetic. Oxidation is worked on both reaction in water and organic phase. Reaction in water was recorded 55-70% HNO₃, 5-20% H₂SO₄ ve 1% NaNO₂ and for 17 hours 12-16% -COOH is obtained and dispersed 6 times water at the first time:
- 15 Wanning H. US 2 758 112 (1956). At 2003 Kumar, V., Yang T. Carbohydrate Polymers, 48, 403-412 (2002) Kumar, V. US 6 627 749 B1 recorded that -COOH amount is 25.6% max. by using phosphoric acid at 75 - 92% efficiency instead of sulphuric acid. In present invention four different kinetic oxidation at 12., 24., 36., 48. hours is done at max. temperature in 260 C. 1:1, 1:2, 1:4, 2:1, 4:1 mix proportions of H₃PO₄ / HNO₃ is tested. The best oxidation is
- 20 handled at 1:2 acid mixture, for 48 hours and 23.5% -COOH yield.

Table-2: 48 hours kinetic NO₂ oxidation reaction control (previous method)

Reaction No	Cotton fiber (g)	H ₃ PO ₄ /HNO ₃ 70 ml (v/v)	NaNO ₂ (g)	% -COOH	% Yield
1	5	1:4	1	21.4	92
2	5	1:2	1	23.5	90
3	5	1:1	1	20.5	88
4	5	2:1	1	21.6	84
5	5	4:1	1	20.8	86

The relation between kinetic control and reaction period, the performance at 19 - 21° C is listed as on **Table-3:**

Period (Hour)	-COOH %	Performanc e%
12	8.0	81
24	13.4	79
36	17.4	78
48	18.4	75

5

This table explain us that consisted water after oxidation is the result of hydrolysis and the performance go down. In this invention after serial trials, 5 g cellulose polymer and 0.9 g NaNO₂ add in 85% H₃PO₄ / 67% HNO₃ (1:1.5) 55 - 60 ml mixture and 36 hours oxidation is done. Test result is 20.4 - 21.5% efficiency of -COOH and for hemostatic, standard the method is validated.

10

At 2007 on US 0054880 instead of liquid phase reaction, perfluorine hydrocarbon solvent is used during the oxidation studies directly mixing with NO₂. W. O. Kenyon et. al. US 2423 707, Ind. and Chem. 41, 2 (1949) recorded that direct NO₂ using prevents the hydrolysis. If use the CCl₄ as a solvent, low productivity and low oxidation is obtained. This solvent has high toxic effect to liver, that is why limited usage. Freon-113 and Freon-11 fluorine hydrocarbon is used as carrier solvent on Ashton et. al, US 003364200. After 16 h. oxidation process, derive the oxide cellulose contents 8 - 13% -COOH. G. Vasnitsky US 4 347 057 Reaction optimisation is done by fluorine hydrocarbon and its derivatives. Main results is here that do not exist "-CH-" structure causes radical forms in NO₂ solvents. Starting this point of view our aim is to prevent carbene ion and radical forms by adding hydrogen otherwise 6. -CH₂OH can not be oxidation. Boardman et. al. US 5 180 398 reformed oxidation is very important and they informed volatile hydrocarbon is not have negative effect for ozone layer in atmosphere. Kosowski et. al. US 5 914 003 is not successful to test hydrofluoroether, because the presence of "-CH-".

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All these studies show us that micro crystal cellulose, fiber cellulose, woven cellulose, non-woven cellulose (cotton, linter cotton, viscon cotton, rayon, lyocell, fibril cotton and its string) are relatively compatible for oxidation. The efficiency factors are cellulose impurity of material, linear or branched glucose polymer unites.

- 5 In this invention topic oxidations are re-search kinetic and thermodynamic applications and oxidation ratio to reach the cost effective product. Primarily considering different records on the literature, 12 - 72 h. as kinetic tests and -200C and +60 o C liquid gas phases as thermodynamic tests and controlled productivity, purity level trials is done. Studies in this invention, optimal concentration range preferably is 25:5 - 25:15 cellulose material using
 10 and NO₂ gas weight. Ideal reaction temperature determined is 18 - 26 o C. We observed the same result as literature recorded that -COOH yield is max. 12 - 14.5%. Cellulose/NO₂ gas mixture ratio preferably keep the rate of 25:10 - 25:14.5 in this invention. We found suitable test conditions are that gas outlet temperature for gas saturation is 45 - 50 o C, reactor (oxidation vessel) temperature is 19 - 26 o C and reaction period is 150 - 170 h.
- 15 Reactions which conditions are improved, realised this mechanism as above explanation.
 Figure: 1

2. Short explanation of figures

Figure: 1. Gas phase oxidise cellulose production reaction system

- 20 **A:** NO₂ gas production, 2 l reactor with coil
B: Fiber or woven material reactor, 5 l
C: (On the cooling 0 - 3 ° C liquid circulates) **B** vessel cooled by circulating occur N₂O₃ gas, every 5 h, 2 l aeration, N₂O₄ circulate and recover in **A** vessel.
D: Tap is for pressure control
 25 **E:** The tap of gas aspiration on the reactor

Figure 2. With qualitative method, 5 different textile studied. Under anti-microbial control, obtained photos: **(1)** REOXC-2, **(2)** REOXC-1, **(3)** REOXC*CaNaT, **(4)** PA-RB, **(6)** Textile

- a. *S. aureus* ATCC 6538 strain, 24 h incubation
 30 b. *E. coli* ATCC 25922 strain, 24 h incubation

Figure-3. Photograph of Hemostatic test example

5 **3. The preparation of formulation**

In present invention 3 different formulation is prepared for hemostatic, antiseptic biocompatible samples preparation.

- Powder formulation (Pulver: samples coded **PA**)
- Woven or textile formulation (samples coded **REOXC**)
- 10 - Gel formulation (samples coded **GA**)

Every three formulation have the matrix material which has 18.6 - 20.2% -COOH groups of regenerated oxidised cellulose (REOXC). Every three formulation plan to add 0 - 0.2 % gelatine as 90 - 100% gelation, 0 - 3.2% Ca⁺² ion as blood coagulation, 0 - 0.9% Na⁺¹ as speeder for biodegradation and 0.1-1.1% antiplasmin, antifibrinolytic 6-aminohexanoic acid, 15 4-aminomethyl cyclohexanecarboxylic acid (trenexamic acid) or aprotinin. Easy biodegradation of oxide cellulose is carried certain rated Ca⁺² and Na⁺¹ ion. If Na ion is more than 1%, it causes low haemostat feature and haemostat time decreasing is determined. It is determined via in vivo test that using 0.1 - 1% trenexamic acid as antiplasmin is provided the haemostat stabilisation (Table 7 - 11). However Vladimir N.F. and 20 Vlademir R, US 2008/0181936 recorded that 2,3-dialdehit cellulose carrier matrix obtained NaIO₄ oxidation, this studies had used chlorhexidine, AgNO₃ and lysozyme as anti-microbial; trenexamic acid, 6-aminocaproic acid as haemostat stabiliser. In present invention we have used first time Bismuth Oxidise cellulose compound as antimicrobial properties instead of Ag-oxidised cellulose, AgNO₃, chlorhexadine. We tested to add siprofloksasin, gatifloxacin, 25 levofloxacin, rifampin, lincomycin, clindamycin, doxisiklin as anti-microbial to have good synergy with Bismuth Oxidise and also it is tested at the first time. In this study siprofloksasin, gatifloxacin, rifampin and doxisiklin as antibiotic have been preferred. Hemostatic-antiseptic formulation is studied on preferably 1.2 - 2.4% bi-oxidise cellulose and 1-2.4% antibiotic.

30 On basic matrix of powder formulation it is predicted to use that REOXC which is haemostat and partly antiseptic and 1.2 - 1.8% Ca ion for speeder of the haemostat and 6-aminohexanoic said or 4-aminomethyl cyclohexanecarboxylic acid for decreasing the fibrin solubility. Samples prepared have 0.2 - 1.2% ratio. If the product will use for internal wound, 0.5 - 1.1% Na ion must be added. Woven and textile REOXC formed band impregnated 35 calcium is done firstly by calcium acetate as mentioned on US2008/0181936 and sodium impregnation is done by sodium acetate. Standardisation problem of sodium rates is noticed therefore in present invention we have prepared sodium-2ethykhexanoat on organic phase

5 and it is used for impregnation sodium quantitatively. After adding calcium and sodium and drying the band, antiplasmin is impregnated to textile. For antiplasmin preparation, 6-aminohexanoic acid or 4-aminomethyl cyclohexanecarboxylic acid is solving in 40 - 55% isopropanol:water mixture. Specific antifibrinolytic compound have been determined by HPLC methods in accordance with European and US Pharmacopeia.

10

3.1. Oxidised cellulose product' s standardisation

NMR studies: On H1-NMR - C13-NMR studies show us the existence of -COOH and aldehyde after polymer CDCl₃ is solved in and alcohol and carboxyl group persilation with
15 trimethylsilyl chloride / hexamethyldisilazane.

3.2. Carboxyl quantitative determination:

It is recorded USP at 1995. 0.5 g sample (with P₂O₅ is dried in vacuum oven at 500 C for
20 1.5 h) is taken. Mix with 50 ml 2% calcium acetate for 30 min. Mixture titrated by using 0.1N NaOH (std) and phenolphthalein indicator. Amount of NaOH used in reaction is verified as blank. Using following formula carboxyl determine:

$$\text{Carboxyl group (w/w\%)} = (N \times V \times \text{MW}_{\text{-COOH}}) / \text{sample weight (mg)} \times 100$$

N: NaOH normality = 0.1 N

25 V: used NaOH amount (ml) in the reaction

$$\text{MW}_{\text{-COOH}} = 0.9$$

3.3. Aldehyde quantitative determination:

30 2 g oxidise cellulose sample (with P₂O₅ is dried in vacuum oven at 500 C for 1.5 h) get the reaction with 0.2 M 100 ml NaCl and adjust pH= 2.9 - 3.01 with 0.1 M acetic acid. Place the sample on room temperature and dark for 72 h. Filter and wash with water to separate the acid. The wet sample is dried by air and place in dark room till the determination of carboxyl amount which is done mentioned at 3.2. Primary carboxyl value (at 3.2) subtract this
35 carboxyl value and result is aldehyde value.

5 3.4. Molecule weight determination:

SBP cellulose samples processed liquid ammonia or unprocessed, solve in 1 M cupriethylenediamine diluted from 3 M stock solution (Prolabo, France). Viscometric average polymerisation degree of cellulosic samples (DP_v) is calculated by this formula (**Rinaudo**
 10 **1968**);

$$[\eta] = 0,891DP_v^{0,936}$$

Molecular weight of cellouronic acid is determined by using HPSEC. Sodium salt (1 g/l concentration) of cellouronic acid is solved in water, and filter 0.22 μ m and injected to
 15 Shodex OH park SB 804 Hq and SB 805 HQ in Alliance GPCV 200 (Waters Tech., MA, USA) system. 0.2 M $NaNO_3$ is used as diluter. And then the solution is analysed using 3 detectors (differential refractometer, multicapillary viscometer, multi angle laser light scattering DSP-F (Wyatt Tech. CA, USA) and specific index $dn/dc=0.1512$ at 25o C. Obtained oxidise cellulose polymer is $5 \times 10^3 - 1.5 \times 10^4$ dalton determined.

20 3.5. Carbonyl determination:

0.5 g oxidise cellulose sample (with P_2O_5 is dried in vacuum oven at 50 ° C for 1.5 h) put in 250 ml flask. 50 ml hydroylxamine hydrochloride put in another 250 ml flask. One of flask must have drain tap and connect each other. Flasks are connected to water pump. Close tap and hydroylxamine hydrochloride solution pour **REOXC'** s flask. Place the mixture at 50 ° C
 25 for 2 h. Cool down the room temperature, take 25 ml and titrate to 0.1 N HCl pH 3.2. Another 25 ml solution titrate for blank. Calculate the carbonyl using this formula:

$$\text{Carbonyl (\%w/w)} = MW_{co}(B-S)/10 (\text{Weight of sample}) \times 100$$

B and S is ml, and amount of 0.1 N HCl consumed Blank and Sample

For hydroxamine hydrochloride preparation, 50 g Hydroxamine is solved in HCl 120 ml 1N
 30 NaOH and add on 1000 ml water for dilution. Generally ketone carbonyl does not exist in **REOXC** is produced by NO_2 oxidation ($CO < 0.01$).

3.6 Elementary analysis:

Carbon, hydrogen, nitrogen and sulphur elemental determination:

They are done by Ankara University, Pharmaceutical Fac. Instrumental Analysis Lab. Analysis
 35 result especially for nitrogen is given.

5 **Na, Ca, Zn, Bi, Ag element determination:**

Using Atomic Absorption method they are done by Hacettepe Univ. Analitik Chemistry Dept.

3.7. Antifibrinolytic "trenksamikacid and 6-aminocaproin acid" determination:

Qualitative analyses is used HPLC method which recorded on EU Pharmacopeia 5.0 (2005).

10 **3.8. Cytotoxicity, biosensitivity and irritation tests:**

Hemostatic, antispetic REOXC products must be biocompatible therefore test cytotoxicity, biosensitivity and irritation values and must be in certain limits. **WO 2010/086616, USP-XXI and EU Pharmacopoeia 5.0 (2005).**

These test of products are done by Hacettepe University Pharmacy Fac. Pharmacology Dept.

15 **Cytotoxicity:**

Used standards are TS EN ISO 10993 medical device evaluation, TS EN ISO 10993-1 evaluation and trial, TS EN ISO 10993-5 in-vitro cytotoxicity assay, TS EN ISO 10993-12 Sample and reference material preparation.

20 Test method: (extract test) original extract contact with single layer cells 50 - 80% plating during 24 h.

Culture media: Eagle (non polar)

Cell line: CCL 81 (Vero African Monkey Kidney Cell Line)

25 Extraction method: 0.2 g/ml test sample with media placed in incubator 5% CO₂ (V/V) at 37° C for 24 h. Negative control with nitrocellulose; positive control with natural rubber latex, reactive control with culture media w/o serum was done.

Evaluation criteria: After 24 h. incubation, costars observe under the microscope. Biological reactivity is evaluated in accordance with following table.

Grade	Reaction	Culture media status
0	none	no separate cytoplasm granules, no cell growth decreasing, no cell lysis
1	very little	seldom cell lysis, non circular cell more than 20%, weak bounding and no cytoplasm granule and no changing on morphologic, only little growth inhibition
2	some	Circular cell count is less than 50%, no cytoplasm granule, observable cell inhibition is not more than 50%

3	mediocre	Circular or cell lysis counts are not more than 70%, cell layers are not completely divided
4	harsh	cell layers are completely or almost completely lysed.

5

Results numeric value: If the sample's grade is bigger than 2, it is cytotoxic; if smaller, it is noncytotoxic.

Sensitivity:

TS EN ISO 10993 medical device evaluation, TS EN ISO 10993-1 evaluation and trial, TS EN
10 ISO 10993-5 in-vitro cytotoxicity assay, TS EN ISO 10993-10 Irritation and delayed type
erethism tests.

Study: Negative control (25 x 25 mm four layered textile)

Test procedure: 300 - 500 g, healthy, mature, same race female and male albino rats. Ten
animals for test, five for control test is used. Back hairs are shaved.

15 Induction phase: Direct application of test and control samples on shaved area, wait for 6 h.
and take off. These procedure applied 3 days per a week during 3 weeks.

Challenge phase: On 14. days, test samples all animals of test and control groups applied
only test sample to the shaved area and not applied area and take off after 6 h.

20 Evaluation criteria: After taking off challenge patch, test areas at 24. and 48. hours is
evaluated by **Magnusson and Kligman scale** (TS EN ISO 10993-10; irritation and delayed
type erethism tests).

Magnusson and Kligman Scale Table 7

Test Reaction	Grade
no change visible	0
spread-partly erethism	1
mild and nonspread erethism	2
intense erethism and/or inflation	3

- 5 * Subject group with non-erethism shows us no sensitivity

Skin Irritation:

Working design: 25 x 25 mm four layer textile sample which added negative control, 0.9% saline solution.

- 10 Test procedure: 3 healthy adult rabbit, female and male 2 - 3 kg. One day before back of animals is shaved. Sufficient distance on two side of backbone as test area are selected for application and observation. If test sample is powder, add 0.5 ml 0.9% saline solution and apply two each on two sides. And also negative control is applied two each on two sides. Applied area cover with 25 x 25 mm gauze and with semi-permeable bandage for 4 h. After contact time take off the bandage and mark the applied area.
- 15 Evaluation criteria: After taking off patch, at following 1., 24., 48. and 72. h is observed for erethism and oedema. Irritation grade is average test and negative control for each applied area. Irritation score is obtained by divide three observe grade at 24., 48., 72. to all irritation grade. Primer irritation index calculate to divide primer irritation score to animal count. Results are evaluate correspond to table below.

20 Scoring for Skin Reaction

Reaction	Irritation score
Erethism and scar forms	
no erethism	0
dull erethism (visible hardly)	1
clear limit	2
mild erethism	3
scar form to prevent sharp erethism observation	4
Oedema formation	
no oedema	0
dull oedema (visible hardly)	1
clear limited oedema	2
mild oedema (approx. 1mm)	3
sharp oedema (wider than 1 mm and application area)	4
Most sharper scar for irritation	5

All changes observed will be recorded.

5 Results:

During all test done cytotoxicity' s result are +1; raw **REOXC** is +2 and after H₂O₂ stabilisation of product and after rinsing, result is +1 observed and is determined **non-cytotoxic**. All samples are **non-irritant** observed. On skin irritation tests, primer irritation index of all samples are 0 - 0.28 determined, **skin irritation** is **not** observed.

10 3.9. Measurement of Hemostatic properties

As we mentioned on introduction surgical operation and trauma can cause health risk, massive bleeding. After determine bleeding area, can stop with cauterisation and ligation and similar application, most important point is ongoing haemorrhage.

For surgical application, there are local effective haemostat products in the market. These
 15 are hydrogel, polyglucuronic (PAGA) and/or polyglucosamin derivatives. Coagulant materials, antimicrobial compounds, anti-inflammation, analgesic and antihistaminic have been added. Gelatine, collagen sponch, oxidise cellulose, fibrin binding, natural biological polysaccharide are used frequently as absorbable haemostat (**Horio and ark., 2010^{lit1}, Murakami and ark., 2008^{lit2}, Murakami and ark., 2009^{lit3}**).

20 Production development on this area must have biological compatibility, stop bleeding within the shortest time, preventing the exudation, not cause to stick inter tissue, speed up tissue healing and absorption features (**World Intellectual Property Organisation, 2011^{lit4}**).

During the haemostat production development, rat carotid artery transection and liver laceration studies as in-vivo animal tests are done (**World Intellectual Property
 25 Organisation, 2009, 2011^{Lit5,4}**).

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3.10.1. Application of Haemostat tests

* Ethical committee approval:

15 **Decision no:** Başkent Üniversitesi Tıp ve Sağlık Bilimleri Araştırma Kurulu ve Hayvan Deneyleri Etik Kurulu'nun 01/04/2013 tarih ve 13/24 sayılı kararı

* **Place:** Başkent Üniversitesi Deney Hayvanları Üretim ve Araştırma Merkezi laboratuvarı.

* Starting date of all animal tests: 07.May.2013

All animal tests is acted upon instructions of "Baskent Üniversitesi Deneysel Araştırma İlkeleri".

20 **Animals Material:** 64 ea. S. Dawley/W. Albino, 370 - 450 g male rats supplied by Başkent University, Laboratory Animals Growing and Research Centre. Rats were feeding with standard rat feed and fresh water.

Animal classification:

25 There are two stages. At the first stage 32 prototype product tested on medial and lateral liver lobes laceration of 38 rats for haemostat properties. After tests, 4 prototype product which have appropriate coagulation product, 2 product in the market and 4 control animals are compared. The aim of this stage is determination of most effective powder and spanch prototype product to get the criteria of clotting time.

30 At the second stage, 4 prototype product which determine at the first stage is compressed on the cut of 12 rat medial liver lobe. Histopathologic evaluation was done indelibly at 5. and 12. days. For 7 prototype products which determined at first stage, clotting time measurement is repeated with 6 rats injected heparin. The aim of second stage test are recorded histopathological changes of visceral organ and determination of adhesion forms, biological incompatibility, resorption, morphological changes, internal bleeding after certain
35 time.

5 Anaesthesia and Heparin application Protocol:

All animals studied on, is anaesthetised with intraperitoneal xylazine hydrochloride (6mg/kg) and ketamine hydrochloride (60 mg/kg). After anaesthesia, for 6 animals, lateral tail vein catheterisation is done and injected 500 IU/kg heparin. After 15 min, laparotomy operation is started.

10 3.9.2. Surgical operation:

First test stage:

Anaesthetised rats are fixed on cork board. Ventral abdominal section are shave and disinfected. Under the aseptic conditions, made an incision 3 - 4 cm. mid line on thorax xiphoid approx. 2 cm near caudal through the abdominal cavity. Liver lobes is taken out
15 incision line. After filtering the liquid, put the stretch film between liver lobes and visceral and for determination the bleeding, put the filter paper between the lobes. For every rat median and lateral 1 cm length, 0.3 cm depth incision is done separately. 30 s. blood flow for 45 degree angled rats are observed and compressed by prototype products and equivalent product for 5 min. excluding negative control groups. During 5 min. bleeding and blood on
20 filter paper is recorded. Figure- 3

Second test stage:

4 prototype product determined at first stage and the animals injected heparin are tested specified in first stage. Operation incision stitched and kept animals alive. After operation at 5. day after one each group and at 12. day others was done euthanasia and took the
25 samples of necropsy, histopathological.

* Data statistical analysis is evaluated by student -t tests.

Table-4: Haemostat values of 14 textile spanch prototype product and 2 equivalent product is tested.

n= 24 rats, 2 prototype product test for each

30 Hemorrhage= (Weight of filter paper blood absorbed) - (Nature filter paper weight)

Code	Test number	Applied amount (cmxcm)	Hemorrhage (gr)	Bleeding time (s)	-COOH %
REOXC-(1)	6	2x4	0,418	10 - 15	19.80
REOXC-CaNa	2	2x4	0,230	10 - 20	19.20
REOXC-(2)	6	2x4	0,019	10 - 20	19.35

REOXC-CaNaT	3	2x4	0,420	10 - 30	19.25
PA-RB	2	2x4	0,414	within 120	19.58
REOXC-CaT(1)	1	2x4	0,659	30	20.00
REOXC-CaNaT/Zn	2	2x4	0,418	in the range of 90 - 180	18.50
REOXC-CaT(2)	2	2x4	0,332	in the range of 40 - 80	20.00
REOXC-CaNaT/Bi	2	2x4	0,627	<80	19.35
REOXC-CaNa	1	2x4	0,309	80	18.50
REOXC-CaT(3)	6	2x4	0,418	10 - 12	19.35
PA-SB	3	2x4	0,432	90	19.58
Equivalent product 1 (US)	2	2x4	0,272	90 - 150	17.50
Equivalent product 2 (TR)	2	2x4	not work	ineffective	15.20

5

Table-5: Hemostatic values of 8 ea. prototype powder product

n= 14 rats, 2 prototype product test for each

Hemorrhage= (Weight of filter paper blood absorbed) - (Nature filter paper weight)

Cod	Test number	Applied amount (g)	Hemorrhage (g)	Bleeding time (s)	Description
PA-1	2	0,210	0,911	10 - 20	
PA-2	3	0,210			ineffective
PA-3	2	0,210	0,280 - 0,658	55	
PA-4	8	0,210	0,185 - 0,979	10 - 45	
PA-5	7	0,210	0,153 - 0,987	10 - 35	
PA-6	1	0,210	0,268	25	
PA-7	3	0,210		40 - 70	
PA-8	1	0,210		25	
PA-9	2	0,210		6 - 10	
HG-1	2	0,210		45 - 65	
HG-2					ineffective

HG-3				55 - 75	
HG-4				25 - 45	
HG-5				25 - 35	
HG-6				45	
HG-7				45 - 75	
HG-8				25 - 35	
HG-9				13 - 20	

5

Table-6: As a result go first stage tests, hemostatic values after I.V. heparin injection.

n= 6 rats

Code	Test count	applied amount (g or cmxcm)	Bleeding time (s)	Description
PA-4	1	0,400 g	110	
PA-5	1	0,200 g	115	
REOXC S	1	2x4	38	
REOXC-Ca Spanch	1	2x4	33	
REOXC-CaT S	1	2x4	40	
PA-9	1	0,200 g	15	

Table-7: Haemostatic values of 4 prototype products is applied on liver lob incision.

Code no. (n)	applied amount	Bleeding time(s)
PA-4 powder n=3	0,210 g	10 - 20 s
PA-5 powder n=3	0,210 g	10 s
REOXC S n=3	2 x 4 cm	10 - 15 s
REOXIC-CaT S	2 x 4 cm	10 - 15 s

10

*S= spanch

- 5 **Table-8:** Chosen4 prototype after first stage tests applied liver lob incision, necropsy and histopathologic value of following 7. day of alive animals:

Code no (n)	Necropsy indications	Histopathologic indications
PA-4 powder (n=1)	All animals in this group: - alive - internal bleeding negative - powder applied absorbed completely - incision point has whitened tissue appearance	There are connective tissue increasing on glisson capsule and coagulation necrosis at the centre, giant cell, lymphocyte, macrophage around. Outmost tissue is unnatural granulation. Degenerative changes on the centre of lob is make attention. Neutrophile leucocyte is observed on the portal region. All vein and sinusoid is filled with blood.
PA-5 powder (n=1)	All animals in this group: - alive - internal bleeding negative - applied material absorbed completely - incision point has whitened tissue appearance. But less than PA-4.	There are fibrosis and thickening of unnatural granulation tissue. Together with phagocytosed macrophage which get blue colour. Hepatocyte degenerative changes and mononuclear cells are observed. All vein and sinusoid is filled with blood.
REOXC *S (n=1)	All animals in this group: - alive - internal bleeding negative - applied material absorbed completely	Thickening of unnatural granulation tissue and little mono nuclear cell infiltration, fibrosis on glassine capsule are observed. Hepatocyte degenerative necrotic changes and bleeding is noticed. Mono nuclear cells on portal region is observed. All vein and sinusoid is filled with blood.
REOXC - CaT *S (n=1)	All animals in this group: - alive - internal bleeding negative - powder applied absorbed completely	Fibrosis on glisson capsule, little mono nuclear cell infiltration, material phagocytose macrophage and thickening of unnatural granulation tissue is observed. At same time fibrin strings, bleeding and neutrophile leucocyte is available. Hepatocyte degenerative and necrotic changes, pencil and increment on kupffer cell. All vein and sinusoid is filled with blood.

*spanch

- 5 **Table: 9:** Chosen 4 prototype after first stage tests applied liver lob incision, necropsy and histopathologic value of following 15. day of alive animals:

Code no (n)	Necropsy indications	Histopathologic indications
PA-4 powder (n=2)	<ul style="list-style-type: none"> - All animals in this group is alive - Feeding is good - Internal bleeding is negative - Applied material is absorbed completely - Incision is clean and healing - Determine no conglutination of liver tissue and other abdominal organs - Conglutination of omentum tissue to wound is partly determined. - Any pathologic case and liquid on other visceral abdominal organ is not available. 	Fibrosis on glisson capsule, veining, little mono nuclear cell infiltration and thickening of unnatural granulation tissue and material phagocytose macrophage is observed. Hepatocyte degenerative changes are noticed. Mono nuclear cells on portal region is observed. All veins and sinusoids are filled with blood.
PA-5 powder (n=2)	<ul style="list-style-type: none"> - All animals in this group is alive - Feeding is good - Internal bleeding is negative - Applied material is absorbed completely - Incision is clean and healing - Determine no conglutination of liver tissue and other abdominal organs - Conglutination of omentum tissue to wound is partly determined. - Any pathologic case and liquid on other visceral abdominal organ is not available. 	Fibrosis on glisson capsule, veining, little mono nuclear cell infiltration and thickening of unnatural granulation tissue and material phagocytose macrophage is observed. Hepatocyte degenerative changes are noticed. Mono nuclear cells on portal region is observed. All veins and sinusoids are filled with blood.
REOXC *S (n=2)	<ul style="list-style-type: none"> - All animals in this group is alive - Feeding is good - Internal bleeding is negative - Applied material is absorbed completely - Incision is clean and healing - Determine no conglutination of liver tissue and other abdominal organs - Conglutination of omentum tissue to Glisson capsule and liver medial lob is partly determined. - Any pathologic case and liquid on other visceral abdominal organ is not available. 	Fibrosis on glisson capsule, veining, little mono nuclear cell infiltration and thickening of unnatural granulation tissue and material phagocytose macrophage is observed. Hepatocyte degenerative changes, bleeding are noticed. Material phagocytose microphage, mono nuclear cells on adipose tissue and neutrophile leucocyte on portal region is determined. All veins and sinusoids are filled with blood.

REOXC-CaT *S (n=2)	<ul style="list-style-type: none"> - All animals in this group is alive - Feeding is good - Internal bleeding is negative - Applied material is absorbed completely - Incision is clean and healing - Determine no conglutination of liver tissue and other abdominal organs - Conglutination of omentum tissue to Glisson capsule and other abdominal organs is not available. Available for other samples is not observed. - But conglutination of medial lobe to incision is lightly determined. - Any pathologic case and liquid on other visceral abdominal organ is not available. 	<p>Fibrosis on Glisson capsule, mono nuclear cell infiltration, material phagocytose macrophages and thickening of unnatural granulation tissue formation is observed. Hepatocyte degenerative changes and mono nuclear cells on sinusoids is noticed. All veins and sinusoids are filled with blood.</p>
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5

Result: 4 prototype products (**PA-4, PA-5, REOXC S, REOXC-CaT S**) choose on preliminary test, are effective haemostat as a result of animal test and applications. Although all materials have acceptable limits for inflammation, fibrosis and necrosis, **REOXC-CaT S** and **PA-5** powder products have better results for tissue reaction.

10

3.10. Antimicrobial tests

Bacteria culture preparation:

On the study, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* DSM 20044, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* DSM 30007 and *Pseudomonas aeruginosa* ATCC 27853 is used. ATCC strain is supplied by American Type of Culture Collection (Wesel, Germany), DSM strain is supplied by Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

15

Bacteria lyophilised, is diluted according the instruction of manufacturer. Sheep blood agar is incubated at 37° C for 24 h. (Salubris A.S. Turkey). Two passages are done. After than Brain Heart Infusion (BHI) liquid medium (BBL, BD Diagnostics, USA) 0.5 McFarland turbidity is adjusted 10⁸ cfu (colony units/ml) and serial dilution inoculum amount is adjusted 10⁶ cfu/ml.

20

25

5 Determination of minimal inhibitor concentration (MIK) of powder products:

200 mg of every product is diluted in 10 ml BHI liquid medium (20 mg/ml). 5 tubes contained 4.5 ml BHI liquid medium is prepared. One of them put 0.5 ml diluted product and serial dilution is done. 5 dilution tests between 0.2 mg/ml and 2×10^{-4} mg/ml concentration is studied. 0.5 ml 10^6 mg/ml bacteria solution is added. Last bacteria concentration in tube is 10^5 cfu/ml. After incubation at 37°C , for 24 h, no growth is defined as MIK.

Determination of powder products antimicrobial activities:

200 mg of every product is diluted in 10 ml BHI liquid medium (20 mg/ml). 5 tubes contained 4.5 ml BHI liquid medium is prepared. One of them put 0.5 ml diluted product and serial dilution is done. 0.5 ml 10^6 mg/ml bacteria suspension for every tube is added. After incubation at 0.h and 24. h, take 0.1 ml (10^4 cfu/ml) from every tube, add to Nutrient Agar (Salubris A.S. Turkey). After incubation at 37°C for 24 h, colony count. Positive control tube has no product but have 10^4 cfu/ml bacteria, also incubated at the same condition and colony count. Antibacterial results are listed on Table-10.

Table-10: MIK* results of ten different products

	S. aureus ATCC 29213 (mg/ml)	S. epidermidis DSM 20044 (mg/ml)	E. coli ATCC 25922 (mg/ml)	P. aeruginosa ATCC 27853 (mg/ml)	A. baumannii DSM 30007 (mg/ml)
PA-1	>2	>2	>2	2	2
PA-2	>2	>2	>2	>2	>2
PA-3	>2	>2	>2	>2	>2
PA-4	>2	>2	>2	>2	>2
PA-5	>2	>2	>2	>2	>2
PA-6	>2	>2	>2	>2	>2
PA-7	$<2 \times 10^{-3}$	2×10^{-2}	$<2 \times 10^{-4}$	$<2 \times 10^{-3}$	2×10^{-2}
PA-8	$<2 \times 10^{-4}$	$<2 \times 10^{-4}$	2×10^{-1}	2×10^{-1}	2
PA-9	2×10^{-1}	2×10^{-1}	2×10^{-1}	2×10^{-3}	>2
PA-10	>2	>2	>2	>2	>2

* Minimal inhibitor concentration (MIK) is the concentration of no bacteria, no colony count. This table show us PA-1 partly, but PA-7, PA-8, PA-9 is highly antibacterial.

5 Antimicrobial Activity Determination of Textile material:

Bacteria medium preparation:

In the study for quantitative method, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 medium is used. Medium is tested for vitality and purity control, to passage blood agar.

10 Qualitative method:

For this method, AATCC 147 standards is used. Test bacteria, 5 different textile and antibacterial effect of unprocessed textile as control by diffusion method is studied.

Every strain Mac Farland 10^8 cfu/ml is prepared by the explained method above. After that spread plate technique is applied on Nutrient Agar (**Salubris A.S. Turkey**) by sterile swab and narrow lines. After spreading, put 5 textiles (2x2 cm, sterilised by ETO) and unprocessed textile as negative control at middle of plates. All plates incubated at 37° C. The plates is evaluated from the point of inhibition zone and bacteria growth. If there is inhibition zone, measure the zone as mm (**Vytrasova et al. J Ind Microbial Biotechnol 2008; 35: 1247, Pinho E et al. Ann Microbiol 61:493-498**).

20 **Table-11:** Five different products results after 24 h incubation in *S. aureus* ATCC 6538 and *E. coli* ATCC 25922 strains.

Products	<i>S. aureus</i> ATCC 6538 Zone (mm)	<i>E. coli</i> ATCC 25922 Zone (mm)
PA-RB	53	31
PA-SB	27	28
REOXC-CaNaT	31	24
REOXC (1)	27	NZ
REOXC (2)	31	22

NZ: No inhibition zone

25 These values show us zone (mm) has no growth. Measurement is done at the middle of edge of square inhibition zone which is composed peripheral of square textile.

This table show us, PA-RB is most efficient against *S. aureus* and *E. coli*. One can see effective zones at Figure-3.

4. Experimental procedures and samples

5 4.1 Basic matrix samples

Example-1. Regenerated oxidise cellulose powder (liquid phase oxidation)

REOXC Pudr: **REOXC-P** (P=pudr)

Put the mixture of 50 ml H_3PO_4 85% and 75 ml HNO_3 65%, 8 g viscon fiber or 5x5 cm 28DN woven viscon in the two necked flask. To keep temperature of reaction at 38 - 40 °C, aerate from one neck of flask. Put cold demineralised water 6 times of flask volume and mix strongly. Filter with small pored gauze. Rinse with 200 ml water 3 - 5 times. Keep rinsing till pH of flushing water is 2.8 - 3.1. After then rinse with 98% ethanol and dry at room temperature. Then dry with P_2O_5 in vacuum oven at 50 °C for 2 h. Drying loss is 4 - 5 %, -COOH yield is between 20.0 - 21.8 %; aldehyde yield is 0.3 - 0.5%, bound nitrogen is 0.1 - 0.25%. If change the conditions of rinsing (30% H_2O_2 and 98% ethanol), -COOH yield is 21 - 22.2%, nitrogen is <0.05%, aldehyde yield is <0.1%.

Example-2. Regenerated oxidise cellulose cotton and textile (gas phase oxidation) "REOXC"

Glass reactor at Figure-1, Put 1000 ml 85% H_3PO_4 and 1500 ml 65% HNO_3 on 5 l flask. Add 120 g of 150 g/m² raw woven viscon to this mixture. 33 g NaNO_2 is added slowly within 45 min. 400 g viscon cotton dried with P_2O_5 at 50 - 60 °C for 2 h (30DN woven, 30DN textile or 28 DN woven and 28 DN textile) is put 20 l N_2O_4 gas obtained outlet of Flask **A** pass through to Flask **B** for 150 - 170 h. Flask **A** is heated at 45 - 50 °C for 1.5 - 2.5 h. Flask **B** is heated from gas outlet by 0.5 l/h air. Whenever colour of oxidation material on flask B is red-brown, it means N_2O_4 saturated. Cellulose material:gas rates must be (<2:0.6 - 2:1).

At the end of the reaction 2 no. tap on Flask **B** close and aerate from 3 no. tap and take off system. Gas is aspirated. Evolved gas is hold with wet CaO granule holder or by passing through to water. Put the oxidised textile or woven material on 10 l flask. Rinse with demineralised water (1:4) three times. Measure the rinse water. If pH 2.8 - 3.2, add on 150 ml water, 4 ml 30% H_2O_2 and wait for 1 h and filtrate. Rinse with 1.5 l water and once 1.5 l 98% alcohol and dry at room temperature. Put in vacuum oven with P_2O_5 at 50 °C for 2.5 h.

Product ready to pack:

Dry loss	2.4%
-COOH	19.32 - 20.1%
Aldehyde	0.2%
N	0.11%

5 After aspiration, amount of nitrogen is 0.6 - 1.1%, but for final product it is 0.05 - 0.1% after rinsing. Before H_2O_2 reaction, aldehyde yield is 2.2- 3.1% but this yield is almost 0.2 - 0.4% for final product. However **Brisk T.S., Beverly H., Remanick A.H., Pasadena C. DP2061796 (1970)** is recorded that the stabilisation of **REOXC** is done by NaBH_4 ; we prefer Hydrogen peroxide stabilisation. This is advantage reflection for hemostatic feature (See Table.
 10 2). And also -COOH yield is increasing in the range of 0.2 - 0.5%. In general -COOH value of the final product is 18.75 - 20.15%. This value is sufficient hemostatic feature for qualified textile according to USP. It is recommended that the temperature of **B** flask keep $<19 - 26^\circ\text{C}$ during the reaction. N_2O_3 gas obtained after oxidation, at this temperature, is going to cooler with keeping gas phase. With 0.5 l/h air oxygen, it re-convert into N_2O_4 and feature in
 15 oxidisation.

On this invention we design the reactor (**Figure.2**) for industrial production. -COOH oxidisation is controlled kinetically by using certain amount of air oxygen. We determined during the validation studies that 70 - 100 g/m^2 textile woven (DN=32,30,28), DN number of viscon cotton has important role; as a result -COOH yield of **REOXC** for DN=28 is 18.6%, for
 20 DN=30 is 19.35%, for DN=32 is 20.30%. Preferably DN=30 - 32 viscon woven textile is predicted to use. If DN=28 will use, woven's density must be 75-80 g/m^2 . There is proportional rates between reaction time in the range of 130 - 168 h and -COOH amount in the range of 17.9 - 20.15%. Process time is adjusted with -COOH amount determination in every 24 h. Production of NO_2 gas in 5 l reactor and 45 - 55 $^\circ\text{C}$ process temperature must be controlled.
 25 Till the colour becoming deep brown, this heated process keep on going and stop to heat. whenever the colour become light brown, heating must be started at 45 - 55 $^\circ\text{C}$. This is the kinetically control production.

Example-3. Adding Calcium and Sodium to powder oxidise cellulose "**PA-3**"

a. 16.0 g REOXC pudr containing 21.1% -COOH is solved in solution of 3.6 g NaOH in
 30 175 ml demineralised water. Put 3.51 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ compound with 25 ml demineralised water in solution of first step. After adding 4 ml 30% H_2O_2 , put mechanical stirrer for 60 min. pH is adjusted with acetic acid for 5.3 - 5.4. After adding 175 ml. 98% ethanol, suspension is mixed for 20 min. For separation centrifuge at 4000 rpm for 8 - 10 min. Rinse with 60% ethanol three times and centrifuge. Take the sediment and put petri dishes and freeze at -40°C and
 35 lyophilise for 24 h.

5 Product:

-COOH 21.15%

Ca 2.0-2.1%

Na 1.6 - 1.9%

N 0.11%

10 Aldehyde 0.2 - 0.3%

b. If it is preferred that Na rates is smaller than 1.8%, pH will be adjusted 4.3 - 4.5 and obtain that Na yield is 0.9 - 1.1% and so hemostatic features increasing. At the same time bioabsorbability is increasing and optimising in good conditions. See hemostatic studies "**PA-**

15 **3"**

Product:

-COOH 21.15%

Ca 2.0 - 2.1%

Na 0.9 - 1.1%

20 N 0.11%

Aldehyde 0.2 - 0.25%

Example-4. Preparation of Na-2-Ethyl hexanoat

14.6 g 2-Ethyl hexanoat is solved in 100 ml methanol. 4.0 g NaOH is solved in 100 ml methanol. These two solution is mixed and fill 500 ml up methanol which must be fresh
25 distilled on KOH. And then Na-2-ethyl hexanoat solution in Methanol is prepared.

Example-5. Calcium and gelatine impregnated powder oxidise cellulose "**PA-CG"**

Powder or textile oxidise cellulose which contain 15 g 20.1% -COOH, is mixed with solution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1250 ml demineralised water, and is added 12 g 20% Na_2CO_3 and centrifuge 200 rpm for 30 min at 95 ° C. The mixture is cooled down 60 ° C and is mixed with 4 ml 30%
30 H_2O_2 at room temperature, adjust pH 5.3 with 20% acetic acid. 3 g gelatine is solved in 25 ml water and stand over night to provide the gelling and is added to main solution which must be 50 ° C and is mixed strongly and slowly (300 - 500 rpm). Residue is cooled down 30 ° C and add 150 ml 98% alcohol and separation is done by centrifuge 4000 rpm for 15 min. Rinse with

- 5 60% ethanol three times and take in petri dishes and is frost at -40°C , is lyophilised 18 h and then is dried at 50°C on P_2O_5 in vacuum oven.

Dry loss	3.2%
-COOH	19.6 - 20.05%
Ca	2.0%
10 N	2.8%
Na	1.2%
Aldehyde	0.14%

4.2. Hemostatic and antiseptic samples

- 15 On **WO2000/04939 A1** studies is determined that Zn^{+2} , Ag^{+1} , Bi^{+3} , Na^{+1} ions are impregnated oxidise cellulose (linter cotton) contain 16.2% -COOH; these compound can be used for gastrointestinal antiseptic. On our studies gelatine and chitosan is also used. Same process on **WO2010/086616A1** studies is investigated that especially helicobacter pylori effect of product' s anti microbial spectrum.
- 20 On present invention it is tested separately impregnated all five ions (Ca^{+2} , Zn^{+2} , Ag^{+1} , Bi^{+3} , Na^{+1}) and they are standardised. It is important invention determined that the sampled called HA-9 (REOXC-Bi) impregnated with Zn^{+2} , Ag^{+1} , is quite good hemostatic, antiseptic properties with chosen antibiotics and additionally have synergic effect altogether.

Example-6: Oxidise cellulose-Bi compound "REOXC-Bi" "HA-9"

- 25 On this invention different techniques are used than **WO 00/04939A1** which is applied for amine-dithiocarbarnates.

The mixture of 9.52 g oxidise cellulose (contain 19.35 - 20.05% -COOH) is solved in 1.9 g NaOH by mixing (Solution I). 9.7 g $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ compound is solved in 20 ml 2% HNO_3 and 80 ml water (solution II). Firstly mix Solution I strongly and add in Solution II drop by drop

30 within 3.5 h. This mixture centrifuge over night at 250 - 300 rpm. Add on 200 ml 98% ethanol and put $+4^{\circ}\text{C}$ for 2 h. The dispersion is separated to centrifuge 4000 rpm for 10 min. Rinse with 80% ethanol two times and with 75% acetone and with 98% ethanol. Residue separated by centrifuge, is taken petri dishes and frost at -40°C and is lyophilised for 12 h and then is dried at 50°C in vacuum oven on P_2O_5 .

5	Particle size	2 - 5 um
	Dry loss	2.4%
	-COOH	19.65%
	Bi	16.5%
	N	3.5%
10	pH	3.35 (1% suspension)

Example-7: Oxidise cellulose-Zn compound "REOXC-Zn/Na"

Solution of 1.8 g NaOH in 60 ml water is added oxidise cellulose which contains 10 g 19.35 - 20.05% -COOH. 3.31 g ZnCl₂ is solved in 20 ml demineralised water and add the solution drop by drop within 45 min. and mix at 250 rpm. Add 3 ml 30% H₂O₂ and mix 30 min at 300 rpm. pH 5.5 is adjusted with 20% acetic acid. 80 ml 98% ethanol is added and mix 10 min at 300 rpm and then centrifuge 10 min. at 4000 rpm. Rinse with 70% alcohol two times and disperse sediment is separated and frost -40 ° C and lyophilised for 18 h and then is dried at 50 ° C in vacuum oven on P₂O₅.

20	Particle size	3-5 um
	Dry loss	3.8%
	-COOH	19.6%
	N	0.05%
	Na	2.5%
25	Zn	8.2%

Example-8: Oxidise cellulose-Ag compound "REOXC-Ag" "PA-10"

Method determined **WO2010/086616A1** is modified.

- a. Solution of 1.8 g NaOH in 60 ml water is added oxidise cellulose which contains 10 g 19.35 - 20.05% -COOH. Add 3 ml 30% H₂O₂ and mix 30 min at 250 rpm. 8 g AgNO₃ is solved in water. Add slowly to oxidise cellulose solution within 30 min. and mix at 300 rpm for 2 h. Residue is separated by centrifuge. Rinse with 2 ml 30% H₂O₂ in 50 ml water. Rinse with 80% ethanol two times and with isopropanol once. Dry at room temperature at first step and then dry at 50 ° C in vacuum oven.

5

Particle size	5-8 um
Dry loss	3.7%
-COOH	19.7%
N	0.6%
Na	0.4%
Ag	18.2%
pH	4.7 (1% suspension)

10

- b. Solution of 1.8 g NaOH in 60 ml water is added oxidise cellulose which contains 10 g 19.35 - 20.05% -COOH. Add 4 ml 30% H₂O₂ and mix 30 min. 6 g AgNO₃ is solved in 20 ml water. Add slowly to oxidise cellulose-Na solution and mix for 4 h. Residue coloured light brown is separated by centrifuge at 4000 rpm. Rinse with 2 ml 30% H₂O₂ in 50 ml water. Rinse with 80% ethanol two times and with 100 ml isopropanol once. Dry at room temperature at first step and then dry at 50 ° C in vacuum oven for 3 h.

15

20

Particle size	4-7 um
Dry loss	2.7%
-COOH	19.7%%
N	8%
Na	0.9%
Ag	16.2%
pH	15.7% (1% suspension)

25

4.3 Hemostatic textiles

Example-9. Ca⁺² impregnated to REOXC "REOXC-Ca"

- 30 200 ml demineralised water mix with 200 ml isopropanol and 1.171 g Ca(CH₃COO)₂·2H₂O is solved in this solvent. Add 10 g REOXC woven which contains 19.6 - 20.01% -COOH and then is evaporated at 25 ° C for 1 h. Rinse with 50 ml isopropanol twice and dry at room temperature. Dry in vacuum oven at 50 ° C on P₂O₅ for 2 h.

5

Dry loss	2.5%
-COOH	19.7 - 20.01%
Ca	2.2 - 2.5%
N	0.10%

10 **Example-10.** Ca^{+2} and Na^{+1} impregnated to REOXC "**REOXC-Ca, Na**"

a. 170 ml demineralised water is mixed with 170 ml isopropanol and 0.984 g $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ and 1.32 g Na-acetat trihydrate is solved in this solvent. Add 8.4 g REOXC which contains 19.5 - 20.05% -COOH and then is evaporated at 25 ° C for 1.5 h. Rinse with 50 ml isopropanol twice. Dry at room temperature and at 50 ° C on P_2O_5 in vacuum oven for 2 h.

15

Dry loss	2.9%
-COOH	19.6 - 20.0%
Ca	2.1 - 2.2%
Na	1.8 - 2.1%
N	0.12%

20

b. 10.0 g REOXC-Ca (sample-9) is weighted and add the mixture of 20 ml demineralised water and 80 ml isopropanol. Add on 9 ml of sodium-2-ethylhexanoat in 16.8% methanol and 15 ml isopropanol. Mix them on evaporator for 10 min. Obtained product is rinsed with 50 ml isopropanol twice. Dry at room temperature and at 50 ° C on P_2O_5 in vacuum oven for 2 h.

25

Dry loss	3.1%
-COOH	19.8 - 20.0%
Ca	2.1 - 2.2%
Na	1.4 - 1.5%
N	0.11%

30

Note: Effective hemostatic, biocompatible and bio-absorbable product must have less than 1.1% sodium yield. As quantitative sodium impregnation is proper method, it is found that 4 - 6 ml 16.8% Na-2-ethylhexanoat solution is correct for 10 g REOXC.

5 **Example-11.** Impregnation of Anti-plasmin "6-aminocaproic acid or tranexamic acid to
"REOXC-Ca,A,and REOXC-Ca.T"

Take 10 g REOXC-Ca (Sample-9) and add on 0.5 g tranexamic acid or 6-aminocaproic acid is
 solved in 20 ml demineralised water and then evaporate with 30 ml isopropanol for 15 min.
 Stand at room temperature for 5 min. Obtained product is rinsed with 75 ml isopropanol twice.
 10 Dry at room temperature over night and then at 50 ° C on P₂O₅ in vacuum oven for 2 h.

a. REOXC-Ca,T (T=Tranexamic acid)

	Dry loss	2.4%
	-COOH	19.6%
	Ca	2.10%
15	Tranexamic acid	0.8%
	N	0.91%

b. REOXC-Ca, A (A=6-aminocaproic acid)

	Dry loss	2.5%
	-COOH	19.6%
20	Ca	2.11%
	6-aminocaproic acid	0.7%
	N	1.01%

Example-12. Impregnation of calcium, sodium, tranexamic acid and zinc to woven oxidel
"REOXC-Ca,Na,T/Zn"

25 0.5 g Ca(CH₃COO)₂.2H₂O and 0.5 g Zn(CH₃COO)₂.2H₂O is solved in mixture of 170 ml
 demineralised water and 170 ml isopropanol. Add on 10 g oxidel contain 20% -COOH and
 percolate for 2 h at room temperature. Rinse with isopropanol and Dry at room temperature.
 Obtained product is add on 50 ml isopropanol and 5 ml 16.8% sodium-2-ethylhexanoat for 30
 min. Take the product and rinse with isopropanol. Dry at room temperature. 0.5 g tranexamic
 30 acid is impregnated with as sample-11 techniques. Dry at room temperature and at 50 ° C on
 P₂O₅ in vacuum oven for 2 h.

	Dry loss	2.5%
	Ca	1.95%
	Na	1.4%

5	Zn	2.05%
	N	0.82%
	Tranexamic acid	0.72%

4.4. Preparation of powder samples

10 **Example-13.** Regenerated oxidise cellulose (REOXC-P) "PA-4"

Take Example-1 as 20 g liquid phase oxcel and is solved in 3.75 g NaOH (in 100 ml demineralised water). Add on 4 ml 30% H₂O₂ and mix 30 min and mix for 30 min. pH= 2.95 - 3.1 is adjusted with 20% acetic acid. Sediment product centrifuge at 4000 rpm. Rinse with 50 ml demineralised water and 50 ml 75% ethanol. Wet pat is put petri dishes. Keep at room
 15 temperature for 5 h. Frost at -40 ° C and lyophilise for 18 h. Dry at 50 ° C on P₂O₅ for 1.5 h in vacuum oven.

	Particle size	3 - 7 um
	Dry loss	2.8%
	-COOH	20.05%
20	Na	0.14%
	N	0.1%
	Aldehyde	0.11%

25 **Example-14.** Powder sample contain sodium, calcium, tranexamic acid and gelatine (REOXC-Na,Ca,T,P) "PA-1"

10.0 g PA-4 mix with 10.0 g REOXC-Ca, Na and 0.35 g Tranexamic acid, 0.65 g gelatine, 120 ml 50% ethanol for 2.5 h. Add on 100 ml isopropanol and mix for 1 h. Centrifuge at 4000 rpm for separation. Wet product put petri dishes. Keep at room temperature for 3 h. Frost -40 ° C and lyophilise for 18 h. Obtained product is dried at 50 ° C on P₂O₅ in vacuum oven for 1.5 h.

30	Particle size	4 - 7 um
	Dry Loss	2.2%
	-COOH	19.61%
	Ca	1.7%

5	Na	1.2%
	Tranexamic acid	1.4%
	Gelatin	0.32%
	N	0.11%
	Aldehyde	0.11%

10

Example-15. Powder sample with Sodium, Calcium, Tranexamic acid, Gelatine and Zinc (REOXC-Na, Ca, R, T, G/Zn) "PA-2"

10.0 g HA-4 mix with REOXC-Ca, Na and 0.35 g Tranexamic acid, 0.65 g gelatine, 0.5 g REOXC-Zn, 120 ml 50% ethanol for 2.5 h. Add on 100 ml isopropanol and mix at 3000 rpm for 15 1.5 h. For separation, centrifuge at 4000 rpm. Obtained product put petri dishes and keep at room temperature for 3 h. Frost at -40 ° C and lyophilise for 14 h. Dry at 50 ° C on P₂O₅ in vacuum oven for 1.5 h.

	Particle size	3 - 6 um
	Dry loss	2.1%
20	-COOH	19.56%
	Ca	1.6%
	Na	1.1%
	Tranexamic acid	1.5%
	Gelatine	0.32%
25	Zn	0.25%
	N	0.11%
	Aldehyde	0.11%

Example-16. Powder product with sodium, calcium, tranexamic acid, bismuth and gelatin 30 (REOXC-Na,Ca,T,G,Bi) "PA-5"

12.0 g REOXC-Ca,Na mix with 0.42 g Tranexamic acid, 0.75 g gelatine, 1.2 g REOXC-Bi (PA-9), 120 ml 50% ethanol for 3 h (mechanically 250 rpm). Add on 100 ml isopropanol and mix for

- 5 1.5 h. Centrifuge at 4000 rpm. Gel product put petri dishes. Keep at room temperature for 3 h. Frost at -40°C and lyophilise for 15 h. Dry at 50°C on P_2O_5 in vacuum oven for 1.5 h.

	Particle size	3 - 5 μm
	Dry loss	2.1%
	-COOH	19.62%
10	Ca	1.5%
	Bi	2.2%
	Na	1.2%
	Tranexamic acid	1.5%
	Gelatine	0.32%
15	N	0.11%
	Aldehyde	0.11%

Example-17. Powder sample with calcium (REOXC-Ca) "PA-6"

- 16.0 g powder REOXC contains 28% -COOH, is solved in 3.5 g NaOH in 150 ml demineralised water (solution I). 3.50 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ is solved in 20 ml demineralised water and add on
 20 solution I. Before mix mechanically for 60 min, add on 4 ml 30% H_2O_2 . pH=3.8 - 4.0 is adjusted by 20% acetic acid. After adding on 170 ml 98% ethanol, mix at 300 rpm for 30 min. Centrifuge at 4000 rpm for 8 - 10 min. After rinsing with 60% ethanol three times, mix at 300 rpm for 30 min. Centrifuge at 4000 rpm for 8 - 10 min. Rinse with 60% ethanol three times and re-centrifuge. Take gel sediment and put petri dishes and frost -45°C and lyophilise for
 25 24 h. Powder final product is dried at 50°C on P_2O_5 in vacuum oven.

	Particle size	3 - 5 μm
	Dry loss	2.1%
	-COOH	20.3%
	Ca	2.3%
30	Na	0.58%
	N	0.10%
	Aldehyde	0.10%

5 **Example-18.** Oxidise cellulose with antibiotic and antiseptic (A-REOXC) **"PA-7"**

10.0 g powder REOXC (20.5 - 21.5% -COOH) mix with 5 g REOXC-Ca,Na and 70 g Tranexamic acid, 0.6 g antibiotic (rifampicin, gatifloxacin, doxycycline, levofloxacin, lincomycin, clindamycin, ciprofloxacin), 0.80 g REOXC-Bi (PA-9), 120 ml 50% ethanol for 3 h. Add on 120 ml isopropanol and mix for 1 h. Centrifuge at 4000 rpm for 10 min. Gel product is put petri
10 dishes and keep at room temperature for 3 h. Frost -40 ° C and lyophilise for 18 h. Dry at 50 ° C on P₂O₅ in vacuum oven for 1.5 h.

	Particle size	3 - 5 um
	Dry loss	2.1%
	-COOH	19.80%
15	Ca	1.8%
	Bi	1.2%
	Na	1.2%
	Antibiotic	3.8%
	Tranexamic acid	1.7%
20	Aldehyde	0.10%

Example-19. Hemostatic and anti-septic powder with rifocine as antibiotic (R-REOXC) **"PA-8"**

8 g REOXC-Ca,Na mix with tranexamic acid, 0.5 g Rifampicin (2 ampoule rifocine), 0.40 g
25 REOXC-Bi (PA-9), 100 ml 50% ethanol at 300 rpm for 3 h. Add on 120 ml isopropanol and mix for 1.5 h. Centrifuge at 4000 rpm for 10 min. Gel product is put petri dishes and keep at room temperature for 3 h. Frost -40 ° C and lyophilise for 18 h. Dry at 50 ° C on P₂O₅ in vacuum oven for 1.5 h.

	Particle size	4 - 6 um
30	Dry loss	2.4%
	-COOH	19.62%
	Ca	1.8%
	Bi	1.2%

5	Na	0.88%
	N	1.11%
	Rifampicin	4.5%
	Tranexamic acid	1.7%
	Aldehyde	0.05%

10

5. Impregnation of antibiotics (Rifampicin, Gatifloxacin, Doxycycline, Levofloxacin, Lincomycin, Clindamycin, Ciprofloxacin) to textile material

Example-20. Hemostatic, antiseptic band with Rifocine as antibiotic (PA-RB1)

0.40 g tranexamic acid mix 0.5 g rifampicin (2 ampoule rifocine), 0.40 g REOXC-Bi (PA-9), 50 ml 50% ethanol at 300 rpm for 3 h. Obtained disperse suspension is impregnated with 10 g REOXC-CaNa (Sample-10). Rinse with 50 ml isopropanol. Use silicone-plastic roller. Wet textile product is put petri dishes and stand at room temperature for 3 h. Frost -45 ° C and lyophilise. Dry at 50 ° C on P₂O₅ in vacuum oven for 1.5 h. Obtained band is cut and piece together with self adhesive band. Red woven textile is gained. Same process is repeated for other antibiotics (PA-RB, -GB, -DB, -LB, -LIB, -CB, CyB)

	Dry loss	2.4%
	-COOH	19.58%
	Ca	1.8%
	Bi	1.12%
25	Na	0.85%
	N	1.11%
	Rifampicin	4.1%
	Tranexamic acid	1.0%
	Aldehyde	0.04%

30

4.6. Preparation of hemostatic gel (GA1-GA9)

Example-21. 10 g powder (PA1-PA9) is mixed 1 g Chitosan (Deacetyl grade >85%), 1 ml 2% acetic acid, 100 ml 90% ethanol with mechanic stirrer on 350 rpm over night. Disperse

- 5 mixture centrifuge at 4000 rpm for 10 min. Gel pour petri dishes. 1/4 of amount is taken by weighing and frost -45 ° C. Lyophilise for 15 h. Dry at 50 ° C on P₂O₅ for 1.5 h.

	Dry loss	11.2%
	-COOH	18.23%
	Ca	1.62%
10	Na	0.65%
	Bi	0.92%
	N	1.25%
	Tranexamic acid	0.82%
	Aldehyde	0.03%

- 15 * Gel is prepared with 2 - 10 unit weight water according to desired viscosity

CLAIMS

- 5 **1.** Hemostatic powder characterized in comprising 18.6 - 20.2% -COOH, 0.2 - 0.4% aldehyde, 0.1-0.25% nitrogen containing regenerated oxidise cellulose powder impregnated with antifibrinolytic tranexamic acid and 6-aminocaproic acid.
- 2.** Powder according to Claim 1, characterized in containing 0 - 2.2% Ca^{+2} and 0 - 1.1% Na^{+1} ion.
- 10 **3.** Powder according to Claim 2, characterized in containing 1.5 - 2.1% Ca^{+2} and 0.8 - 1.1% Na^{+1} ion.
- 4.** Powder according to Claim 1 characterized in containing 0.1 - 0.8% antifibrinolytic tranexamic acid or 6-aminocaproic acid.
- 5.** Powder according to Claim 1 or 2 characterized in containing antimicrobial active
15 substance.
- 6.** Powder according to claim 5, characterized in containing antimicrobial active substance which is selected from Rifampicin, Gatifloxacin, Doxycycline, Levofloxacin, Lincomycin, Clindamycin, Ciprofloxacin groups.
- 7.** Powder according to Claim 5 wherein antimicrobial active substance is impregnated in the
20 range of 2.3 - 2.9 %.
- 8.** Powder according to Claim 1 or 2 characterized in containing antimicrobial Bismuth complex.
- 9.** Powder according to Claim 1 or 2 characterized in containing antimicrobial active substance.
- 25 **10.** Powder according to Claim 9 wherein antimicrobial active agent is Rifampicin and doxycycline.
- 11.** Powder according to Claim 10 wherein doxycycline is impregnated in range of 1.8 - 3.5%.
- 12.** Haemostatic material characterised in regenerated oxidised cellulose cotton or cloth containing -COOH between 18.6-20.1%, at most 0.2% aldehyde and at most 0.1%
30 relative nitrogen has a loss on drying rate of 2.3% and is impregnated with antifibrinolytic tranexamic acid and 6-aminocaproic acid with hemostatic time of 7 - 12 s.
- 13.** Material according to Claim 12, characterised by containing Ca^{+2} ions between 0-2.2% and Na^{+1} ion between 0-2.1%.

- 5 **14.** Material according to Claim 12, characterised by containing Ca^{+2} ion between 2.1-2.2% and Na^{+1} ion between 1.8-2.1%.
- 15.** Material according to Claim 12, characterised by being impregnated with antifibrinolytic tranexamic acid or 6-Aminocaproic acid at a rate of 0.05-0.1%.
- 10 **16.** Material according to Claim 12, characterised by comprising antimicrobial active substance.
- 17.** Material according to Claim 12, characterised in that the antimicrobial active substance is selected from a group comprising Chlorhexidine, Ciprofloxacin, Gatifloxacin, Levofloxacin, Rifampin, Lincomycin, Clindamycin, and Doxycycline.
- 15 **18.** Material according to Claim 12, characterised in that the antimicrobial active substance is impregnated at a rate of 2.4 - 3.5%.
- 19.** Material according to Claim 12, characterised by comprising a Bismuth complex.
- 20.** Material according to Claim 12, characterised by comprising antibacterial active substance.
- 20 **21.** Material according to Claim 12, characterised in that the antimicrobial active substance is Rifampicin or Ciprofloxacin.
- 22.** Material according to Claim 12, characterised in that Rifampicin and Doxycycline is impregnated at a rate of 2.8-4.5%.
- 23.** Method of production of powder according to claim 1 characterized in comprising steps of,
- Putting 28 - 30DN viscon string woven or viscon fiber on the mixture of H_3PO_4 and HNO_3 in two necked flask.
 - Adding on NaNO_2 as powder to mixture
 - Keeping the reaction temperature at room temperature for 38 - 40 h via aeration from one inlet of flask.
 - Adding on demineralised water 6 times of volume in flask content and mix strongly.
 - 30 • Filtration with small pored gauze
 - Rinse with 200 ml water every time
 - Keeping on rinsing till pH of flushing water is 2.8 - 3.1.
 - Washing with 100 ml 98% ethanol and dry at room temperature
 - Drying at 50 ° C on P_2O_5 in vacuum oven for 2 h.
- 35 **24.** Method of production of powder according to claim 12 characterized in comprising following steps:

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- Raw viscon woven is added on H_3PO_4 and HNO_3 mixture in the range of 1/15 on flask **A** of glass reactor

- Add on NaNO_2 and acid rates must be 12-15/1000.

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- The gas obtained flask **A** pass through for a period of 120 - 170 h flask **B** which contains the material dried at 50°C on P_2O_5 for 2 h.

- Heating of Flask **B** is provided with 0.5l/h air on outlet of gas

- Saturation with NO_2 gas is reached, material colour in flask **B** becomes red-brown.

- Provide the material:gas rates in the range of <2:07 - 2:1.05>

- Gas is in the clear by aspiration during closing inlet valves and opening upper valve (**E**).

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- Take oxidise textile or woven viscon on separate plate and wash with demineralised water (1:4 rated) three times.

- While pH of flushing water is 2.8 - 3.2, add on 150 ml water and 4 ml 30% H_2O_2 for 40 g. After keep for 1 h, filter.

- Wash 150 ml water and 150 ml 98% alcohol once and dry at room temperature.

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- Dry at 50°C on P_2O_5 in vacuum oven for 2 h.

25. Method for gellation of powder as claimed in any of claims 1 to 9 wherein 5 - 10%chitosan is used.

26. Method according to claim 25 wherein chitosan rate to deacetyl is more than 85%.

27. Method as claimed in claim 26 wherein gel is added on water in the range of 10 - 50% and mixed at 300 rpm.

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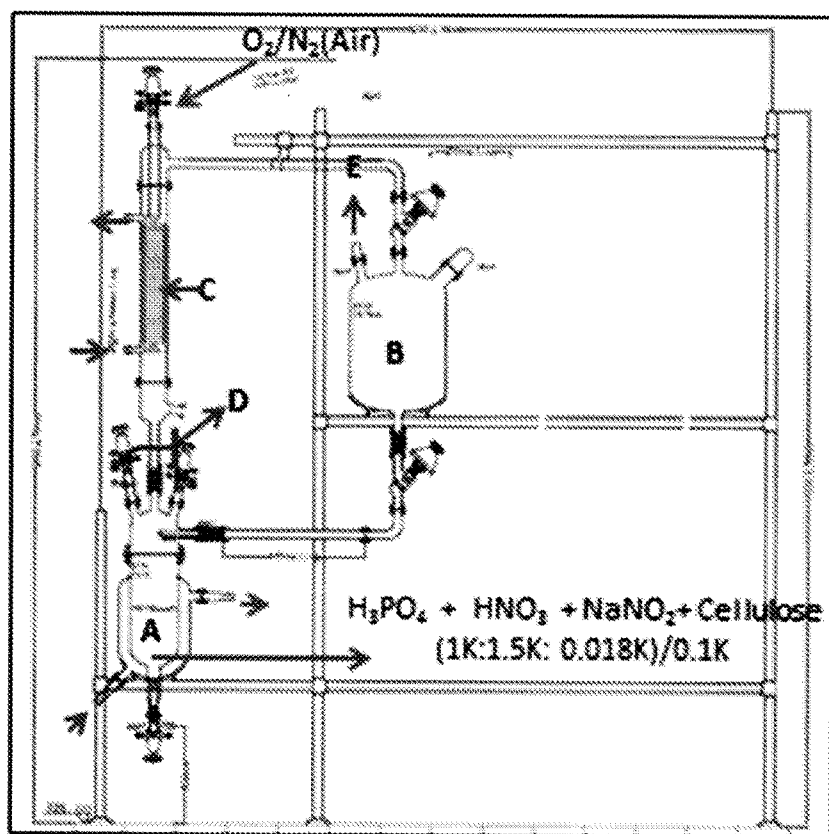
Figure-1:

Figure-2a:



Figure-2b:

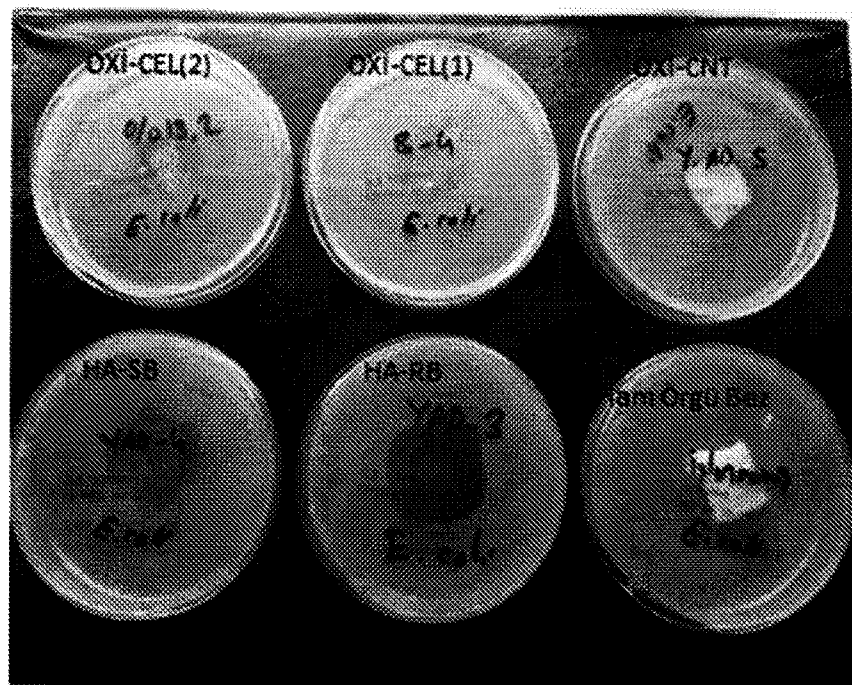


Figure-3:



INTERNATIONAL SEARCH REPORT

International application No

PCT/TR2015/000166

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61L15/28 A61L15/42 A61L15/44 A61L26/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	column 4, line 24 - line 27 column 4, line 66 - column 6, line 14 ----- -/--	1-22



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

10 December 2015

Date of mailing of the international search report

17/12/2015

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Fort, Marianne

INTERNATIONAL SEARCH REPORT

International application No

PCT/TR2015/000166

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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