

Collagenic Surgical Dressing

Collagen has been used in surgery for a long time. It can be used in the form of either sponges or threads to stop bleeding and is suited, after appropriate modification, for speeding wound healing. The normal collagenic surgical dressings are however not sufficient for patients with defective blood coagulation or with widespread bleeding. It has already been tried to bond collagenic material to tissues, whereby one has tried bonds composed of resorcin-formaldehyde, collagen or gelatine. Such bonds are hemostypic, are however unsuited for practical use because of their toxicity. This applies also for acrylate bonds and the combination with collagenic surgical dressings.

It is known, that collagen covalent interlaced with components of connective tissue can shrink thereby interlacing of collagen over Schiff-Basen as well as aldol condensation occurs. It is also known that the tissue firmness of basal membranes is achieved by additional "S-S" bridges of the basal membrane collagens. It is known furthermore how to connect proteins, like albumin with inter-molecular bonds after a mild reduction and subsequent oxidation over "S-S" bridges.

After injuries the blood clots build a primary scar. Aggregate thrombocyte (blood platelets) and fibrin network are responsible for this. It is known that individual fibrin molecules are cross-linked by transglutaminase. Hereby new peptic connections are formed between the glutamin acids and lysin and between neighbouring chains. By the use of the technique of fibrin bonding, in other words through the

use of fibrinogens and thrombines, the end phase of plasmic blood coagulation is simulated.

The fibrin bonding is not sufficient of itself to stop widespread bleeding. This can be achieved only through a combined application of fibrin bonding and resorbable collagenic surgical dressings. However, one must hold three components at the ready, namely, the collagenic surgical dressing, a thrombrin solution with antifibrinolytics and a deep frozen, highly concentrated fibrinogen solution. Since bleeding often begins suddenly and unprogrammed, these three components are not necessarily ready and available in crucial moments, for example the deep frozen fibrin solution must first be thawed. Also the mixing of the solution before application is relatively difficult.

It is the object of this invention to produce a surgical dressing, that will bind with the tissue and which will not show the known disadvantages of the fibrin-bonding in combination with resorbable collagens. Coupled with this object is an improvement of the collagenic surgical dressing for local haemostosis.

A tissue bonding collagenic surgical bond, which is characterized in that it contains collagen in combination with a resorbable biopolymer from the groups fibrinogen, SH-groups-modified-gelatine SH-groups of modified collagen and SH-groups - modified - oxy-cellulose, will accomplish this object.

Collagens and fibrinogens, or SH-groups-modified-gelatines, correspondingly modified collagens or

correspondingly modified oxycellulose can be combined advantageously with each other through freeze dried processing. In the case of the combination of collagens with SH-modified-collagens is however also possible to introduce SH-groups into the collagen.

The invention is described in the following by example of the combination of collagen with fibrinogen, however fibrinogen is used here representatively for the above mentioned resorbable biopolymere, namely SH-groups-modified gelatine, SH-groups-modified collagen or SH groupsmodified oxycellulose.

Problematic with the application of fibrinogens is the occasional appearance of hepatitis. This problem can be naturally totally avoided, if one does not use fibrinogens, rather one introduces other reactive SH-groups, which are contained in biopolymers. Such SH-groups containing biopolymers are SH-group-modified gelatines, SH-group-modified regenerated oxycellulose or SH-groupmodified collagens. The introduction of SH-groups in collagens can occur in a way known per se. For example, one can work according to the regulations of Benesch & Benesch in "Proceedings of the National Academy of USA, Washington" volume 44, 1958, pages 848 to 853.

The introduction of SH-groups into collagens can however, occur thereby, that one applies SH-group-modified gelatines or a correspondingly regenerated oxycellulose to the collagens, or by mixing it in a gradient mixer. Gelatine is produced by chemical or enzymatic splitting of collagen and therefore has the same chemical composition. Consequently, collagenic surgical dressings, which have been treated with

SH-group-modified gelatines, have essentially the same properties as the collagens, together with the advantage, that because of the SH-groups in these materials, cross-linking occurs through oxidation.

The collagenic surgical dressings, according to the invention, can also be used in the conventional way with pharmaceutical active ingredients.

The collagens which are used as surgical dressings are available in the standard form of surgical dressings, as gauze, tissue, sponge etc. Such surgical dressings are known in the state of the art.

The collagens which have been used, show a purity expressed through the amount of carbondioxide: hydroxyprolin < 4 preferably < 3 . Since hydroxiprolin exists only in collagen, it is used as the measure of the purity of the collagen.

The resorbable biopolymer is contained in the tissue bonding collagenic surgical dressing in an amount of 0,5 to 10 mg/cm² preferably 4 to 6 mg/cm². The number of SH-groups per molecule of the resorbable biopolymer can fluctuate in a wide range. It lies by gelatines with an average molecular weight from about 40 000 at approximately 2 to 7 and in average approximately at 5, with similar resorbable biopolymers in the same range.

It is known that collagens are suitable as carriers for antibiotics such as gentamycin. Also tetracyclin or other antibiotics or chemotherapeutics can be worked into SH-groups-modified collagens. This is an additional effect, which can be reached with the surgical dressing according to the invention.

The production of collagens:

Fresh beef sinew freed from all layers of pigment and muscle rests are homogenized and an amount that equals 100 g dried weight was extracted in 3 L 0,05 M citrate buffer (pH 3,7) for 24 hours. Afterwards they are subjected to dialysis for 12 hours in 1% acetic acid.

The tissue which had been suspended in 3 L of 1% acetic acid, is then incubated at 15°C under constant stirring with pepsin in a relation collagen: pepsin = 50:1.

The composition with the 1% acetic acid is then thinned to 5 liters and freed from the undissolved sinew fragments by centrifuge.

The viscous collagen solution is then dialysiert against alkalized tap water (pH 8.0) and then centrifuged sharply. The residue is again then dissolved in 5 liters of 1% acetic acid and dialysed. This procedure is repeated until the factor carbondioxide to hydroprolin < 3 is reached. After the final dialysation a 1,5% collagenic solution is produced using 0,05% acetic acid, which is then used for the final tests.

The production of SH-modified gelatines, SH-collagens or SH-regenerated oxycellulose:

1000 ml of a gelatine solution (this requirement applies analogously for 1,5% collagenic solutions or a suspension of 50 g collagen or regenerated oxycellulose) are added at pH 7,0 to 318 mg acetylhomocysteine acidthiolactone, then 340 mg AgNO_3 are

added, whereby the solution is held at pH 7,0 through the addition of NaOH.

After two hours the solution is brought with $\ln$ HCl to pH 2,5 and thiourea is added in excess. The silverions are removed by cation exchanger and the solution is dialysed under carbondioxide. From the resulting solution one then produces the modified SH-gelatines as well as the modified SH-collagens in a concentration of one percent for the following experiments.

The regenerated oxycellulose is dried by lyophilization.

The production of fibrinogen solutions:

Commercial sterile figrinogen bulk product is dissolved in sterile Acqua dest. so that a solution of 50 mg fibrinogen/ml solution results, which is then used for the following experiments.

Example 1

The production of collagen-fibrinogenic surgical dressings.

Approximately 10 ml from a 1,5% collagenic solution, which has then been sterilized by radiation, is placed under antiseptic conditions in a sterile bottle with septum and is deep frozen in a cold bath (dry ice - ethanol) while being stirred slightly. After approximately 2/3 of the solution has been frozen, 5 ml of the collagenic-fibrinogen solution (collagen: fibrinogen = 1:1) is added and again deep frozen until 2/3 of this solution is deep frozen. Then 5 ml of the fibrinogenic solution is added, deep frozen and lyophilized.

Example 2:

Production of a surgical dressing, which contains collagens and modified SH-gelatines:

10 ml of a collagenic solution (1,5%) is placed in a bottle with septum thereafter in cold bath (dry ice/ethanol) and frozen, while being lightly stirred. After approximately 2/3 of the solution is frozen, 10 ml of modified SH-gelatine solution is added, deep frozen and lyophilized. The sponge is then sterilized by radiation.

Example 3:

The production of surgical dressing containing gentamycin:

To produce surgical dressings containing gentamycin, 100 mg gentamycin is added to 100 ml of a 1% collagenic solution. This solution is then used to produce the collagen fibrinogenic surgical dressings as well as for the production of collagenic SH-gelatine surgical dressings.

Example 4:

The production of surgical dressings, which contain collagens and fibrinogens:

100 ml of a 0,5 - 1% collagenic solution is placed in a metal form measuring 10 x 10 cm, it is then lyophilized according to the usual techniques and the resulting sponge is then sterilized. Under anti-septic conditions the sterilized collagenic sponge is then sprayed with a fibrinogenic solution, whereby 0,5 to 10 mg of fibrinogen is applied per square centimetre of collagenic surface. These are then again frozen and packed under sterile conditions.

Through application of the modified tissue bonding collagenic surgical dressings according to the invention, resorption occurs in practice in a clinically reasonable time. By means of this invention large wounds, in particular those in the stomach area can be treated.