

Tissue Reaction to Implantation of Collagen Film

An Experimental and Clinical Study

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ABSTRACT

A new modified collagen film was investigated to evaluate the tissue reaction and absorption time (a) after implantation in rabbit muscle, and (b) in a human model where collagen was implanted in the prostatic cavity during operation. Clinical follow-up by cystoscopy and biopsy was made one week postoperatively. The animal results showed that for 6 weeks after implantation there was a cellular reaction associated with phagocytosis of the collagen film. Then a scar was formed, similar to that in the controls. The human results showed a similar tissue reaction and no adverse effects from the collagen film.

INTRODUCTION

Collagen is a potent stimulator of platelet aggregation and release reaction (9, 10) and can also activate the intrinsic blood coagulation system (13). Although it is uncertain if collagen initiates haemostatic plug formation in microvessels (4) its topical application to induce haemostasis is a theoretical possibility. Patients with defective collagen may show a slight tendency to bleed. Microcrystalline bovine collagen preparations have been used experimentally for topical haemostasis (1, 6, 8). Application of collagen film to a surgically exposed surface is a new principle and is of potential interest due to its promotion of haemostasis and enhancement of wound healing. This study was undertaken to investigate the local tissue reaction to implantation of bovine collagen film. Initially the tissue reaction and time of absorption were studied in animal experiments. Subsequently local tissue reaction was studied in an experimental model in human beings treated surgically for benign enlargement of the prostatic gland.

MATERIAL AND METHODS

Collagen preparation

The collagen film is developed from native skin collagen manufactured from the deep layers of bovine corium and

sterilized by gamma-irradiation. It consists of pure collagen fibres with an amino-acid composition very similar to that of plain catgut (Arfors & Holtz, personal communication).

Animal experiments

Fifty rabbits of about 3 kg body weight, fed on a standard diet (rabbit pellets, Astra Ewos AB, Södertälje, Sweden) and with free access to water, were used. The rabbits were anaesthetized intravenously with a barbiturate. The skin over the musc. longissimus dorsi was shaved, cleaned and cut through. A deep longitudinal incision was made in the muscle. In 35 rabbits a piece of about 3×3 cm of the collagen film was placed in the muscular incisions after having been folded twice or three times. In 15 control animals the same surgical procedure was performed but nothing was placed in the muscular incisions. The muscle was sutured superficially with plain surgical catgut 00 and the skin was sutured with stainless monofil sutures.

The sites implanted with collagen film were examined after one, 2, 3, 4 and 6 weeks and after 3 and 6 months. Five rabbits were sacrificed for each examination. Five control rabbits were sacrificed after respectively one, 2 and 4 weeks. Sacrifice was made by an overdose of barbiturate. The implantation sites were carefully searched out, dissected free and examined macroscopically.

Human studies

Patients selected were those undergoing transvesical enucleation of prostatic adenomas weighing more than 50 g. A random allocation was made so that half of the patients served as controls and in half of the patients collagen film was used as a haemostatic agent. After enucleation, capsular catgut sutures were used in all patients. The collagen film was then placed in the prostatic cavity and fixed with a tamponade for 3–4 minutes (Fig. 1). 0.5 g tranexamic acid (Cyklokapron®, Kabi, Stockholm) was given intravenously. A three-way catheter à demeure (Couvelaire, Ch 22–24) was used for continuous bladder irrigation. Primary bladder suture was made with catgut. One week after the prostatectomy the patients were investigated with cystoscopy and biopsies were taken with the resectoscope. There were 21 control and 19 collagen-treated patients. From 15 patients (8 control and 7 collagen) further biopsies were taken 2 months after the operation. The pathologist was unaware of the group

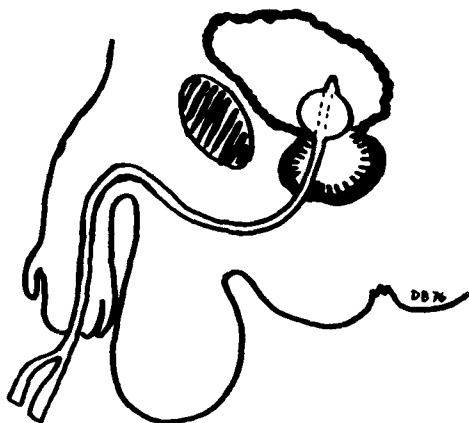


Fig. 1. Schematic drawing of collagen inserted in the prostatic cavity and the position of the balloon of the catheter.

randomization. The clinical progress of 50 patients (25 control and 25 collagen) including the patients in this study will be published in detail elsewhere (5).

Histological investigation

The implantation sites from the rabbits and the biopsies from the patients were fixed in 10% neutral buffered formalin, embedded in Paraplast® and sectioned for light microscopy. Stainings were made with haematoxylin-eosin and with van Gieson's stain. The preparations from the rabbits were scrutinized for the presence of implanted collagen, evidence of absorption and any cellular reaction involved.

The biopsies were particularly examined for signs of tissue irritation such as cellular infiltration in the resected tissue. The degree of cellular infiltration was graded arbitrarily from (+) to +++.

RESULTS

ANIMAL STUDIES

Macroscopic examination

The control incisions had healed with little scar formation and without signs of infection. The amount of scar tissue diminished with time and was hardly discernible after 4 weeks in any of the rabbits. The collagen implantation sites had also healed with scar formation and without signs of infection but these cicatrices were of a larger volume than in the controls and contained a greyish-brown flabby mass in the centre during the first 2 weeks. At 3–6 weeks, small amounts of a brown material were found in the scars. At 3 and 6 months the scar formations were very small and hardly discernible.

Microscopic examination

One week. Controls: a moderate amount of young cell-rich connective tissue with slight infiltration of polymorphs occupied almost the whole wound cavity. At scattered locations small amounts of fibrin were also present. Adjacent to the wound margins there was necrosis of some muscle fibres.

Collagen film group: material from the implanted collagen film could be demonstrated in the centre of all five implantation sites by positive van Gieson staining, a basophil staining with haematoxylin-eosin, and a disorganized pattern of fibres not seen in normally occurring collagen. In contact with the foreign collagen were masses of fibrin and many polymorph-nucleated cells (Fig. 2). Peripherally there was connective tissue similar to that in the controls with a marked proliferation of fibroblasts and necrosis of some muscle fibres.

Two weeks. Controls: in comparison with the one week controls the connective tissue was now smaller in volume and more fibrous. The cellular infiltration was also less and no fibrin was found.

Collagen film group: remains of the implanted collagen film were still present in all 5 rabbits and adjacent to this foreign material there was cellular infiltration indicating ongoing phagocytosis. Besides a decreased number of polymorphs there were many small mononucleated cells and accumulations of swollen macrophages with a brown cytoplasm and also single foreign body giant cells. The surrounding connective tissue was fibrous as in the controls.

Three weeks. Collagen film group: in 2 of the 5 rabbits, small remains of the implanted collagen film were found. The number of infiltrating cells was less than after 2 weeks, but in all implantation sites there were accumulations of swollen macrophages and some foreign body giant cells. In one implantation site, hypermature macrophages had formed the structure of a granuloma around remains of the collagen film. There was a small amount of fibrous connective tissue in the surroundings.

Four weeks. Controls: a small or very small amount of fibrous connective tissue was the only finding.

Collagen film group: as after 3 weeks, two implantation sites contained small remains of the collagen film (Fig. 3). In one the collagen was located in the centre of epithelioid granulomas. The other tissue reactions were essentially the same as after 3 weeks.

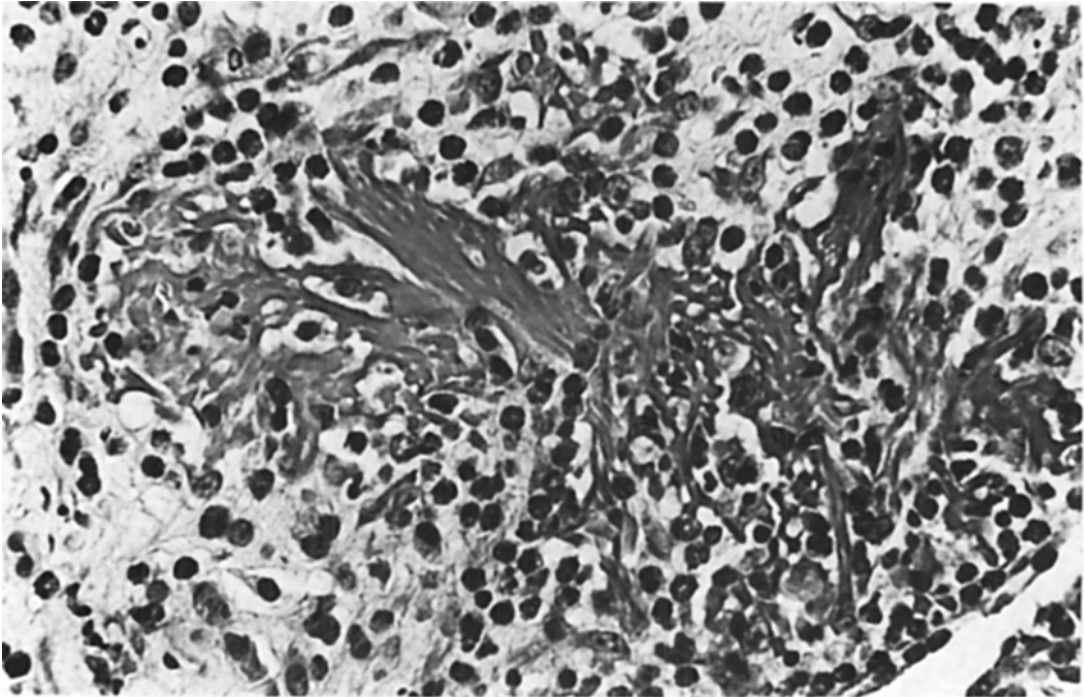


Fig. 2. One week after implantation of the collagen film into a rabbit's skeletal muscle the implanted collagen is seen as a dark, irregular, rather coarse, fibrous material which is infiltrated by polymorphs. H-E, $\times 250$.

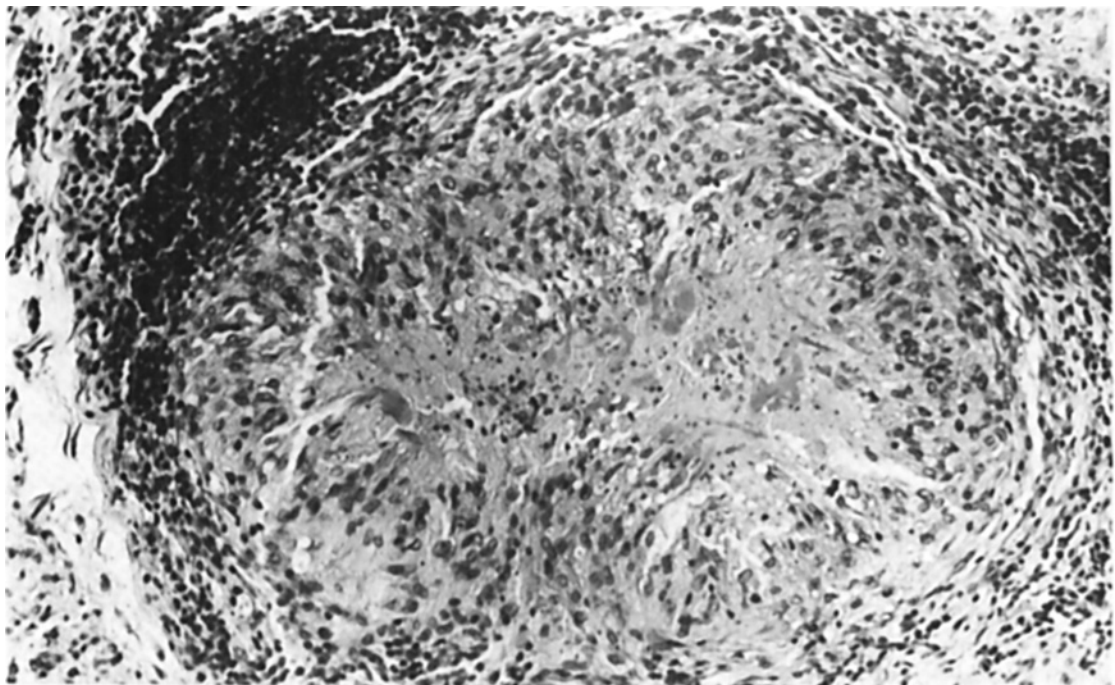


Fig. 3. Four weeks after implantation of the collagen film into a rabbit's skeletal muscle, small amounts of the implanted collagen may be found in the centre of a granuloma. H-E, $\times 100$.

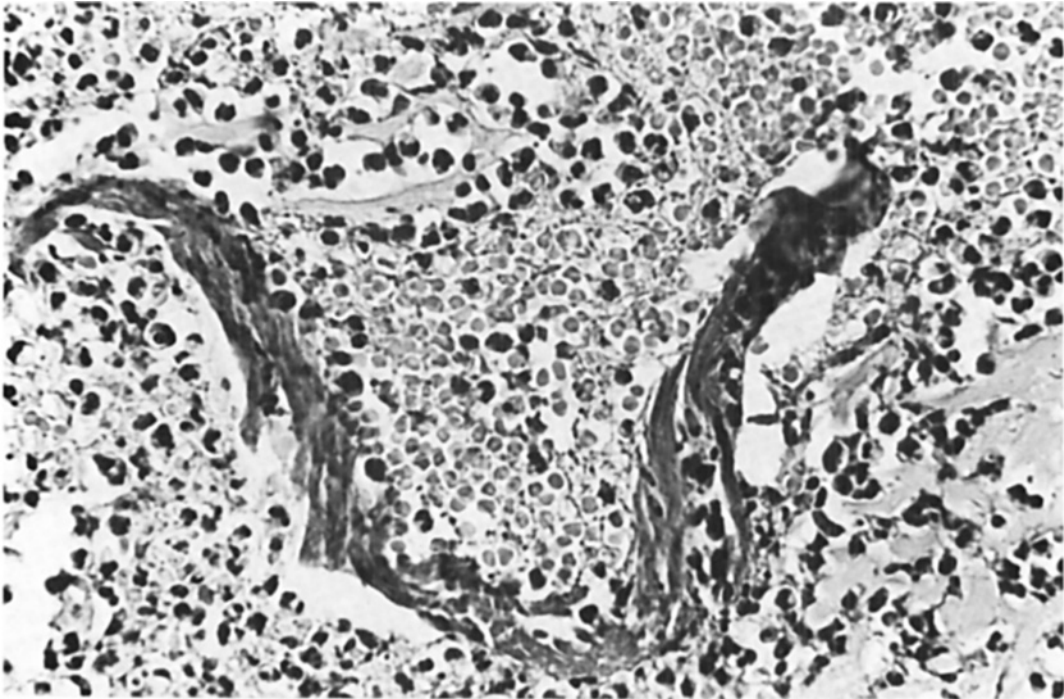


Fig. 4. In this sample from a patient, implanted collagen is seen as a dark-stained curl. The infiltrating cells are predominantly polymorphs. v. Gieson, $\times 250$.

Six weeks. Collagen film group: minute remains of the collagen film were found in one rabbit only, located in the centre of a granuloma. Swollen macrophages with brown cytoplasm were found in all rabbits but to a reduced extent in comparison with shorter implantation times. The macrophages were often situated in adipose tissue. Small amounts of fibrous connective tissue were also found in the surroundings.

Three months. Collagen film group: the implanted collagen film could not be demonstrated in any of the 5 rabbits. A few swollen macrophages could be found in adipose tissue, occupying the previous wound cavity in 3 rabbits. The amount of connective tissue in the surroundings was very small.

Six months. Collagen film group: findings were similar to those after 3 months, but the histological picture was even more normalized.

Adverse reactions

In this experimental study the collagen film caused no other tissue reaction than a reversible cellular reaction associated with phagocytosis of the collagen film, leading to a retarded healing of the muscle incision in comparison with the control group.

PATIENT STUDIES

Macroscopic examination

At cystoscopy one week after operation a structure that was judged to be a remnant of the collagen film was seen in 9 of 19 cases. There were no other changes which could be attributed to the implantation of collagen and the cystoscopic findings were the same as in the control patients.

Microscopic examination

At microscopic examination of the removed prostates all patients were found to have had a benign, often fibromuscular, hypertrophy of the prostate and there was no case of prostatitis.

BIOPSIES ONE WEEK AFTER OPERATION

In the resected tissue samples there were focal or sometimes widespread slight to moderate infiltrations of inflammatory cells, mainly polymorphonuclear leukocytes. In many samples hyperemia and oedema were also evident. In a few cases large parts of the resected tissues were necrotic and degenerated polymorphs were found. On grading the degree of cellular infiltration the collagen-group scored a mean value of 1.8 ± 0.6

points and the control group scored a mean value of 2.1 ± 0.8 ($p=0.351$). The grading was difficult in some cases, due to very small tissue samples.

In many biopsies there were large masses of fibrin adhering to the tissue surface. Some areas of the fibrin network contained numerous polymorphs and red cell debris. In a few samples small remnants of the collagen film were also found within the fibrin clots. The collagenous material was surrounded by numerous polymorphonuclear leukocytes (neutrophilic granulocytes). The cytoplasm of some polymorphs stained positively with van Gieson's stain, indicating that phagocytosis of collagen was proceeding (Fig. 4).

BIOPSIES ABOUT TWO MONTHS AFTER OPERATION

Of the fifteen "two-month biopsies", eleven had less cellular infiltration than the previous biopsy from the same patient. Two were judged to be similar and in two others the degree of cellular infiltration was greater than before. Arbitrary grading of the cellular infiltration was 1.3 ± 0.5 in both groups. The cell populations at 2 months contained characteristically more chronic inflammatory cells such as lymphocytes and plasma cells. No remains of the collagen film were found at this time. The groups showed the same histological picture.

Adverse reactions

No adverse reactions were seen which could be ascribed to the use of collagen film. For an analysis of complications, see Bergqvist & Ståhl (5).

DISCUSSION

This study was undertaken to investigate the tissue reaction caused by collagen film, a new substance with an interesting potential as a haemostatic and wound healing agent. The minor tissue reactions in implantation studies in animals motivated further investigation in an experimental human model, where the results could be followed up. We chose transvesical prostatectomy as it caused some bleeding and as follow-up can be made with cystoscopy and biopsy. This is the first study where internal application of collagen film has been investigated. No adverse reaction could be seen (5). Furthermore, no untoward reactions have been reported in connection with topical application to human skin wounds (14).

The histological tissue picture is very similar in both animal and human studies with much fibrin and many polymorphonuclear cells around the collagen film. Polymorphonuclear leukocytes (granulocytes) contain collagenase capable of degrading collagen at normal pH (11). After degradation *in vivo*, phagocytosis takes place, as was seen in this study. When phagocytosis is prolonged, the histological picture becomes dominated by a differentiation of mononuclear phagocytes from monocytes over macrophages to epithelioid cells (3). The epithelioid cells may form structures recognized as granulomas. Such granulomas were found in 3 rabbits 3–6 weeks after implantation of the collagen film. As the granuloma-provoking agent is destroyed, the highly differentiated mononuclear phagocytes change into less mature forms (3).

In rabbits the absorption time for the collagen film is somewhere between 6 weeks and 3 months, which correlates with findings in the human biopsies where no collagen remains were seen at 2 months. After absorption of the collagen film in the rabbits, the tissues were normalized and a scar was formed similar to that in the controls. Plain catgut has an amino-acid composition similar to that of the collagen film (Arfors & Holtz, personal communication) but the structure of catgut is more solid than collagen film. In rare cases, catgut sutures can remain unabsorbed for several years in humans (15).

The scores after arbitrary grading of cellular reaction and infiltration did not differ significantly between control and collagen patients.

As collagen film is essentially a new preparation, no comparative studies can be reported. However, some investigations have been made with microcrystalline collagen. Thus, Hait et al. (8), Abbott & Austen (1) and Cochran & Hait (7) carried out microscopic studies and also found a very moderate tissue reaction, which differed minimally from control specimens. The collagen film used in this study has been shown to be non-immunogenic in rabbits (Richter, 1975, personal communication).

As to the haemostatic action of collagen film, no animal experiments have yet been made. To judge from the clinical part of our study there is a slight but definite haemostatic effect after prostatectomy (5). Again, microcrystalline collagen has been shown to induce topical haemostasis in different animal experiments (1, 2, 6, 8), collagen being more effective than Surgicel® or thrombin. Vistness et al.

(16) used microcrystalline bovine collagen externally on human skin donor sites with good haemostatic effect. The haemostatic mechanism is undoubtedly rather complex (12, 17).

On the basis of this study it can be concluded that collagen film is rapidly absorbed and it is also well tolerated by rabbit and human tissues. Implantation in man would be favourable in selected situations. The haemostatic effect needs to be further evaluated but we have used it with success on raw surfaces with capillary bleeding.

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