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Preliminary Development of a Collagen Membrane for Use in Urological Surgery

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Summary. A series of experiments have been carried out in-vitro in order to assess the possibility of using a collagen membrane in the repair of various sections of the urinary tract following operative surgery such as the removal of a stone from the ureter. The collagen film has been tested for its compatability with urine, its ability to prevent leakage of fluid in a simulated wound in-vitro and for its ability to withstand any degradative effect of liver and kidney homogenates. The material was not significantly degraded by either urine or by tissue homogenates and was able to prevent leakage of fluid under the experimental conditions employed. Although some slight build-up of calcium and some trace elements took place after incubation in urine over a six-day period this was not significant. On the basis of the results obtained it has been decided to proceed to in-vivo trials on rabbits using the collagen membrane. The possibility of using such a material in partial nephrectomy operations is discussed.

Key words: Collagen membrane, Urinary tract surgery.

Introduction

Following the surgical removal of a stone from the ureter considerable leakage of urine can take place through the wound in the ureteric wall. This can prolong the healing process by several days. This fluid normally is removed by a drain until the ureter is sufficiently healed to prevent leakage from the urinary tract, during which time the patient must remain in hospital. Clearly, any factor which would assist in preventing leakage of urine and thus accelerate wound closure would be highly beneficial.

The present work describes a preliminary investigation into the possible use of a collagen film which is designed to be wrapped around the ureter immediately after surgery and can be readily sutured in place, thereby preventing leakage of urine. The collagen film is designed to rapidly biodegrade. In order to obtain some background information as to how the membranes may behave in-vivo, several investigations have been carried out. Collagen as a biomaterial has already found considerable use in the medical field, and indeed has been the subject of many reviews [e.g. 1, 2, 4].

An important feature of a biodegradable film designed for urinary tract repair work is that materials must not be allowed to crystallise upon its surface which would, in future, form the basis of another stone.

Firstly, collagen films have been incubated in urine for periods of up to six days where it was found that although some of the films may have been partly degraded, the membranes still remained intact and serviceable after the incubation period.

After incubation in 24 h urine collections, membranes were examined for any crystalline deposits and analysed for any increase in Calcium, Magnesium, Potassium and Zinc content.

The films have been shown to be effective in preventing the leakage of fluid through a simulated wound in-vitro and the effects of human kidney and liver homogenates on the film have been tested in an attempt to gain some information on the biodegradation of these materials. Clearly, the results of such experiments cannot be extrapolated to the situation in-vivo. Some breakdown on the films was observed after 24 h with both tissue preparations and the collagen membranes were shown to be essentially leakproof under the conditions tested.

Materials

Preparation of Collagen Films

Collagen was prepared from limed bovine hide by a process involving decalcification, buffering to pH 4.6, washing, thorough grinding

and comminution. The pulp so formed was lyophilised and residual lipid removed using either chloroform or petroleum ether.

To 3 g of this collagen preparation was then added 0.05 M – acetic acid (1 l) at 4 °C containing 0.25% glycerol and the whole homogenised in a Waring Blender for 90 s (or until constant turbidity as measured at 440 nm in 1 cm light path is reached). The resulting liquid was then poured into plastic trays (32 x 44 cm) and allowed to dry over a continuous stream of air at room temperature. Films prepared by this method usually had a thickness of 20–25 μ.

Experimental

Portions (3 x 4 cm) of collagen membrane were cut from the sheets prepared as described above.

After measuring the thickness of each individual piece of collagen the films were placed in a closed circulating system maintained at 37 °C for periods of up to six days. During this time a 24 h urine collection was continually pumped across the membrane surface at a flow rate of 1 ml/min. The pH was monitored daily and in control experiments urine was replaced by deionised water.

At the end of each experiment, the films were rinsed in deionised water and analysed for their Ca, Mg, K and Zn contents. The tensile strength of the materials was also measured to assess any degradation of the film which may have taken place. Before washing, the surface of the films were examined microscopically for any crystalline deposits.

As an integral part of the present study, it was considered essential to assess whether the collagen membranes would indeed prevent leakage of fluid through a simulated wound system. Slits approximately 1 cm long were cut longitudinally into lengths of plastic tubing (3 mm external diameter) around which pieces of the collagen film were wrapped. Half of the films were further secured in place by tying with cotton thread. The tubing was then immersed in phosphate buffered saline (PBS) solution (250 ml), and a 0.5% w/v solution of erythrocene dye in PBS pumped through the tube at a flow rate of 1 ml/min for three days.

Human liver and kidney homogenates were prepared from thawed deep frozen tissue in a Waring Blender at 4 °C in 0.01 M – sodium phosphate buffer at both and pH 6 and at pH 7. The final protein concentration in all preparations was adjusted to 25 mg/ml. Sodium azide (0.02%) was included as a bacteriocide.

Portions (1.25 x 8 cm) of the collagen film were incubated at 37 °C in the homogenates for 24 h, thoroughly washed in deionised water, and any change in tensile strength resulting from degradation of the film, measured using the Instron system [5]. In control experiments, the films were incubated over the same period in the corresponding buffer solution.

Protein Estimation

Protein was determined by UV absorption using the following relationship

$$C = 1.45\ A280 - 0.74\ A260$$

where A280 and A260 are the respective absorbances at 280 and 260 mm. This method will correct for any interfering absorbance due to nucleic acid present in the solution [3].

Instron Testing

The collagen films were tested on an Instron 1122 tensile testing system for any breakdown which may have taken place [5].

The membranes were streched by 16% or by 50% of their original length and the strain on the sample maintained. The actual load on the film was then measured when the final constant load value after relaxation was obtained. The determinations were carried out at room temperature and the films were kept permanently moist using an aerosol spray.

Fig. 1. Crystals found on collagen surface after 6 days incubation in urine (magnification x 200)

Results

The results of a six day incubation of the collagen films with urine are given in Table 1. The figures quoted are the mean of eight determinations and show that, in all but one case, the films were still relatively undegraded. In this one instance the considerable breakdown observed may be the result of bacterial action rather than any proteolytic degradation of the collagen by the urine itself.

On examination of the membranes immediately after the incubation period for any crystalline deposits, light microscopy revealed the presence of some crystals of the type shown in Fig. 1 in all cases. The crystals are of the shape normally associated with phosphate or oxalate deposits. However, during the course of the experiments, the pH of the urine rose considerably as shown in Table 1, a phenomenon which in itself could well cause crystallisation of these anions. Such deposits might not occur, in-vivo, therefore, when the pH would normally remain at a value of less than pH 7.

Analysis of the films after six days incubation and subsequent washing did show increased levels of calcium, magnesium, potassium and zinc ions which presumably had absorbed onto the collagen surface (Table 2). However, in another series of experiments in which the films were incubated in 24 h urine collections for three days only, almost no build up of ions was observed (Table 3). Some of these experiments were continued for a further three days (Table 3) again with no adverse increase in metal ions.

From eight experiments, a leakage of fluid was observed in one case only, corresponding to less than 0.35 ml in 72 h.

Table 1. 6 day incubation of collagen films with 24 h urine collections Films were streched to 50% of their original length using a cross-head speed of 1 cm/min in the Instron System. An effective sample length of 2 cm (i.e. 2 cm gap between the jaws) was used in all cases. Each value represents the mean of eight determinations

Expt.	Blank[a]	Incubation[a]	pH at start	Final pH
1.	124.85 g strain	103.35 strain	6.1	8.7
2.	124.85	78.42	5.5	8.8
3.	120.83	115.94	5.7	8.8
4.	120.83	87.06	5.8	8.7
5.	125.18	86.20	5.2	8.7
6.	125.18	40.43	6.2	8.6
7.	205.73	250	6.3	8.8
8.	205.73	292.9	6.5	8.7
9.	380.43	too low to measure	6.0	7.5
10.	304.3	136.5	6.3	8.7

[a] Values at 50% strain. Corrected for 25 μ thickness

Table 2. Analytical results of collagen films for trace elements after 6 day incubation period

Expt. No.	Ca		K		Mg		Zn	
	μg	μmole	μg	μmole	μg	μmole	μg	μmole
2 (iii)	103	2.6	134	3.4	12.3	0.51	7.0	0.11
2 (iv)	64.5	1.6	21.5	0.55	21.5	0.90	4.8	0.07
Controls	13.3	0.33	6.3	0.16	3.3	0.14	3.8	0.06
2 (v)	206	5.2	48.5	12.4	14.3	0.60	7.8	0.12
2 (vi)	30.3	0.76	16	0.41	11	0.46	1.8	0.03
Controls	50	1.25	29	0.74	7.3	0.19	2.3	0.04
2 (vii)	75	1.75	30	0.77	17.5	0.73	7.5	0.12
2 (viii)	17.5	0.44	12.5	0.32	12.5	0.52	5.0	0.08
Controls	17.5	0.44	17.5	0.45	5.0	0.21	5.0	0.08
2 (ix)[a]	630	15.75	484	12.4	145	6.04	97	1.49
2 (x)[a]	297	7.43	173	4.4	99	4.1	74	1.14
Controls[a]	1,053	26.3	333	8.5	53	2.2	17.5	0.26

[a] Results quoted on dry weight basis per gramme sample

In the absence of any collagen film covering the simulated wound areas, a leakage rate of 0.88 ml/min. was observed showing the effectiveness of the membranes in sealing the plastic tubing under the conditions of the experiment.

After incubation with kidney or liver homogenates, the samples were examined for any breakdown on the films utilising the Instron testing system as described. The results are shown in Table 4. Although the values obtained exhibit considerable variation, they nevertheless show that the collagen film can be degraded by homogenates of these tissues under the experimental conditions employed. (With kidney, the films retained 37.8%–61.9%, and 31.5%–68% of their original strength at pH values 6 and 7 respectively, whereas the liver varied from 44.2%–73.3% and 23.4%–35.3% respectively.) The variations obtained may well reflect the state of the particular organ after removal, or the time between the patients death and the post-mortem examination.

Discussion

In order to assess the potential suitability of collagen film as a material for the repair of the urinary tract following the removal of a stone from the ureter, it was deemed essential to carry out a series of investigations in-vitro before any experimental work in-vivo would be attempted. The behaviour of the collagen in urine, an assessment of its biodegradability using tissue homogenates, and the material's ability to prevent leakage of fluid, were regarded as being of particular importance.

When collagen film was incubated in 24 h urine collections for six day periods, it was found that, in most cases, some breakdown on the collagen film was observed, except in one instance where considerable degradation took place. Significantly in this one case, the pH of the urine had not increased as much as with the other experiments. It is not

Table 3a. Analytical results of collagen films for trace elements after 72 h urine incubation

	Expt. No.	μmole Ca	μmole K	μmole Mg	μmole Zn
(i)	Incubation	1.22	2.64	0.627	0.058
	Control	0.43	0.37	0.115	0.018
(ii)	Incubation	5.645	7.365	4.49	0.10
	Control	0.34	0.25	0.16	0.004
(iii)	Incubation	0.75	0.15	0.30	0.03
	Control	0.61	0.13	0.17	0.03
(iv)	Incubation	0.40	0.33	0.11	0.06
	Control	0.34	0.28	0.16	0.05
(v)	Incubation	0.64	0.49	0.16	0.14
	Control	0.55	0.35	0.17	0.08
(vi)	Incubation	0.73	1.2	0.24	0.058
	Control	0.38	0.56	0.2	0.045
(vii)	Incubation	1.45	0.443	0.407	0.042
	Control	0.35	0.193	0.153	0.045
(viii)	Incubation	0.44	0.634	0.160	0.045
	Control	0.51	0.387	0.125	0.053
(ix)	Incubation	1.107	0.485	0.203	0.057
	Control	1.232	0.50	0.123	0.068

Table 3b. Analytical results of collagen films for trace elements after 3 day and 6 day incubation

	Expt. No.		μmole Ca	μmole K	μmole Mg	μmole Zn
(x)	3 Day	Incubation	0.719	0.555	0.452	0.076
		Blank	0.657	0.435	0.165	0.061
	6 Day	Incubation	0.500	0.223	0.122	0.045
		Blank	0.657	0.490	0.125	0.058
(xi)	3 Day	Incubation	1.423	0.632	0.164	0.067
		Blank	0.657	0.435	0.165	0.061
	6 Day	Incubation	0.547	0.346	0.135	0.044
		Blank	0.657	0.490	0.122	0.058
(xii)	3 Day	Incubation	1.125	0.375	0.240	0.092
		Blank	1.43	1.192	0.354	0.096
	6 Day	Incubation	1.125	0.501	0.141	0.052
		Blank	1.125	0.385	0.169	0.044
(xiii)	3 Day	Incubation	0.377	0.587	0.132	0.057
		Blank	0.981	0.713	0.198	0.10
	6 Day	Incubation	4.375	0.89	0.958	0.149
		Blank	1.125	0.55	0.25	0.085
(xiv)	3 Day	Incubation	1.274	0.771	0.562	0.046
		Blank	0.981	0.713	0.198	0.10
	6 Day	Incubation	1.625	1.05	0.313	0.08
		Blank	1.125	0.55	0.25	0.085

certain whether any break-down of the film was due to proteolytic action of any components of the urine (which does contain proteases, the levels of which can become elevated in certain pathological conditions), or bacteria should the urine have either been infected initially or have become contaminated with micro-organisms during the incubation period.

The crystal deposits found at 6 days were of the shape normally associated with phosphate or oxalate and their presence could be interpreted as either:

(a) Substances which had crystallised out and merely lodged on the film as a result of increasing alkalinity in the

Table 4. Instron testing of collagen films after incubation with liver and kidney homogenates for 24 h at 37 °C

Expt. No.	Control		Liver homogenate		Kidney homogenate	
	pH 6	pH 7	pH 6	pH 7	pH 6	pH 7
1.	9.0	11.1	6.6	2.6	3.4	3.5
2.	9.4	9.0			6.5	8.3
3.		11.9		4.6		
4.	16.0	17.5	11.4	15.7	9.9	11.9
5.	15.6	23.5	6.9	8.3		

Effective sample length = 5 cm (distance between jaws). Films strained by 16% of their length and allowed to relax. Cross-head speed = 1 cm/min. Strain values are quoted in grams after the specimen had relaxed to a constant value. Film thickness = 25 μ

urine, the pH of which rose during the course of the experiments. Or,

(b) Compounds which crystallised on the membrane surface following removal of the collagen film from the incubation medium before examination under the optical microscope. This could be caused by either a drop in temperature from 37 °C to room temperature or by an increase in concentration due to evaporation of water from the surface. Or,

(c) A genuine crystalline deposit on the collagen membrane, which, in the in-vivo situation, may eventually be sufficient to cause a fresh stone.

The first two possibilities would clearly not present any problem in the in-vivo situation whereas the last cause would have undesirable consequences following surgery. Animal trials will be essential to determine whether this could indeed present any difficulties.

Experiments using erythrocene dye have shown that the films are essentially leakproof over a 72 h period and that a maximum of 76% loss of strength took place after 24 h incubation in the liver homogenate. In kidney, a maximum drop of 68.5% was observed in the strain value after incubation although, as with liver, the values were usually less. This is almost certainly within acceptable limits, as with the film in place, the ureter should heal within 3–4 days.

Because of the leakproof nature of the membranes, and the fact that collagen acts as a haemostat [1, 2, 4] there is a strong possibility that such films may be useful in preventing both leakage of blood from the operation site and bleeding following surgery on soft organs such as liver, spleen and kidney. In order to carry out some initial investigations into such possible uses of the collagen preparation, it is intended to test the effectiveness of the material in rabbits by creating a ureterotomy, or after partial nephrectomy.

It must be stressed at all times that this work represents a preliminary survey of the possible use of collagen film in the areas described, and that extreme caution must be exercised in attempting to extrapolate any results obtained in-vitro to the in-vivo situation.

If the system proves to be viable in-vivo with experimental animals, then such membranes may prove to be of value in reducing urinary leakage postoperatively in open operations on the urinary tract in human subjects.

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