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The In-Vitro Assessment of a Collagen/Vicryl (Polyglactin) Composite Film Together with Candidate Suture Materials for Potential Use in Urinary Tract Surgery

III. Adherence of Bacteria to the Material Surface

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Summary. A Collagen/Vicryl (Polyglactin) composite membrane (developed for use in urinary tract surgery) has been incubated in cultures of radioactively labelled urinary tract pathogens vis *Escherichia coli, Staphylococcus epidermidis,* and *Proteus mirabilis* for up to 1 h. For comparison, collagen film, Vicryl mesh, and a number of absorbable and non-absorbable sutures were similarly tested. Following incubation, samples were also examined by scanning electron microscopy. Under the experimental conditions employed, only minimal adherence of the micro-organisms to the collagen coated Vicryl mesh, its two individual components, as well as Vicryl and nylon sutures was observed. Significantly greater numbers of bacteria, however, ahered to silk and Chromic Catgut. The results suggest that adherence of micro-organisms to the prosthesis even in infected urine is unlikely to develop into a microcolony of bacteria. However, it should be emphasised that great care must be excercised when extrapolating from the in-vitro to the in-vivo situation.

Key words: Collagen Vicryl composite – Urinary tract repair – Urinary tract infection

Introduction

The preparation of a collagen Vicryl prosthesis for potential use in urinary tract surgery has been the subject of an earlier report [3]. Following in-vitro studies [2, 3], the material was subsequently used to repair full thickness defects of the urinary bladder and partial nephrectomy in experimental rabbits [6, 7, 8]. The results showed that the material was biodegradable and readily replaced by host scar tissue. A highly satisfactory repair of the urinary bladder was obtained and was characterised by a normal urothelium and minimum contraction [7, 8]; or, in the case of the kidney, a very good repair was effected, characterised by a thickened renal capsule [6, 8]. These results suggested that in future such a material may be of use to urologists who wish to avoid the use of bowel or omentum in repairing the urinary tract of human subjects.

When considering a new material for surgical use in the urinary tract, it is important for the urologist to have some prior knowledge of any predisposition of those micro-organisms which are commonly associated with urinary tract infections to adhere to any such prosthesis. Clearly, any tendency of bacteria to adhere to the membrane could lead to such complications as continued infection of the urinary tract, a potential nidus for stone formation, or to a more rapid breakdown of the prosthesis. This latter process could, in turn, lead to the additional complication of leakage of infected urine from the operative site.

Using radioactively labelled micro-organisms and by studying the membrane using electron microscopy, we examined the ability of *Escherichia coli, Staphylococcus epidermidis,* and *Proteus mirabilis* to adhere to the collagen/Vicryl mesh. Taking into consideration the fact that an inflammatory response invariably follows bacterial infection of the urinary tract, the in-vitro adherence experiments were carried out in the presence and absence of polymorphonuclear leucocytes (PMNs). In addition, it has been considered relevant to the investigation to test a number of suture materials which could be used to secure the prosthesis. These were Vicryl and Chromic Catgut (absorbable sutures); and for comparison the non-absorbable sutures Mersilk (braided silk), and Ethilon (nylon monofilament).

Materials and Methods

Suture materials were obtained from Ethicon Ltd, Edinburg, Scotland and were all of 3/0 gauge.

Collagen coated Vicryl mesh was prepared by the method described in an earlier report [3]. In addition some samples were presterilised with gamma-irradiation (2.5 megarad) from a ^{60}Co source.

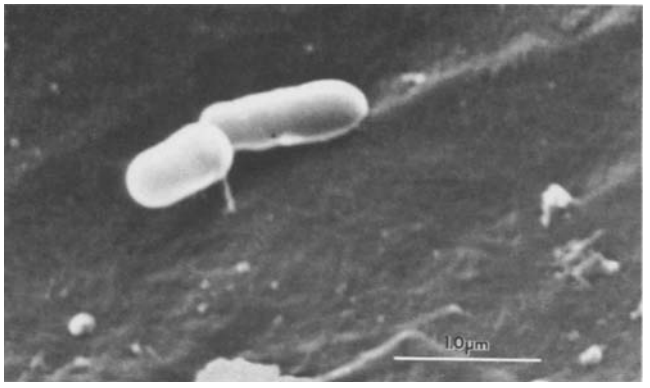

Fig. 1. Scanning electron microscopic appearance of collagen surface after incubation with *E. coli* for 60 min

Measurement of Bacterial Adherence

1 cm squares of the collagen coated Vicryl mesh, collagen film, and Vicryl mesh were immersed for either 30 or 60 minutes in 1 ml of a bacterial suspension containing 1×10^7 cells of either *Escherichia coli, Staphylococcus epidermidis* or *Proteus mirabilis.* The experiments were carried out in either nutrient broth, physiological saline, or urine which had been presterilised by membrane filtration (0.22 μ, Millipore U.K.), and were subsequently repeated in the presence of polymophonuclear leucocytes.

The bacteria had been grown previously in Mueller Hinton broth (Oxoid Ltd, Basingstoke, Hants, U.K.) for 24 h at 37 °C containing 10 μl ^{3}H-adenine solution (27 Ci/mmol specific activity, Amersham International, U.K.) and subsequently washed threee times in sterile saline. Following immersion, the squares of test materials were either simply drained, or drained and washed in sterile saline before analysis. Adherent bacteria were determined by adding 3 ml of scintillation fluid (Packard Instruments, Caversham U.K.) followed by measurement of labelled organisms in a Beta counter (LKB) Instruments, Bromma, Sweden).

Alternatively, 2.5 cm length of Chromic Catgut, Vicryl, Mersilk, and Ethilon were similary immersed in the baterial suspensions for 30 and 60 min periods and subsequently treated as described above.

In all cases, bacterial adherence was measured by comparing the amount of radioactivity associated with the various materials so that initially present in the bacterial suspension. Results are expressed in terms of the numbers of bacteria adhering per sq mm under test conditions. Materials were also examined by scanning electron microscopy in order to obtain a visual assessment of the adherent micro-organisms.

Preparation of Polymorphonuclear Leucocytes (PMNs)

PMNs were isolated from fresh heparinised human blood donations using a modification of the method of Böyum [1]. The number of PMNs used in each experiment was 1×10^6/ml.

Results

Initial experiments were directed to the possibility that bacteria were able to adhere to the collagen/Vicryl composite thus making a focus of infection a problem during the in vivo use of such a material in urinary tract repairs.

Scanning electron microscopy revealed that no bacteria adhered to the Vicryl support matrix and only a small number adhered to the collagen layer. Figure 1 illustrates the typical appearance of the collagen surface after contact with *E. coli* for 1 hour.

In order to quantify this degree of adherence it was necessary to analyse the level of surface radioactivity attributable to the presence of bacterial cells. Incubation of the collagen/Vicryl sheets for 30–60 minutes revealed that low levels of adherence as indicated by radioactive counts was apparent on draining of the sheets and this was considerably reduced by gently washing. Typical results are presented in Table 1 which demonstrates that the presence of polymorponuclear leucocytes did little to alter the adherence of any of the bacteria tested.

Gamma-Irradiation of the collagen-vicryl was used as a sterilisation procedure and tests were performed to determine whether the surface characteristics vis à vis bacterial adherence were altered in the process. Slightly lower levels of bacterial adherence were observed (see Table 2).

In the initial experiments bacteria were suspended in nutrient broth for the adherence assay. However bearing in mind that this is not a physiological environment, the experiments were repeated using the bacteria suspended in sterile urine. The same bacteria suspended in saline were used as a control. In neither case was there any change in the degree of adherence (Table 3) and the presence or absence of polymorphonuclear leucocytes did not alter the number of organisms. These experiments support the results of the electron microscopic study and provide evidence that

Table 1. Bacterial adherence to collagen/vicryl sheets after 60 minutes incubation

Conditions of test	Presence/ absence of PMN	Numbers of bacteria adhering/sq mm:		
		E. coli	*P. mirabilis*	*S. epidermidis*
with draining	–	1,040	560	1,400
with washing	–	270	230	720
with draining	+	910	540	1,050
with washing	+	250	160	650

mean of three separate experiments for each bacterium; – = absence of PMNs; + = presence of PMNs

Table 2. Adherence of *S. epidermidis* and *E. coli* to collagen/vicryl sheets with and without Gamma-irradiation after 60 minutes incubation

	Numbers of bacteria adhering/sq mm	
	S. epidermidis	*E. coli*
no Gamma-irradiation	600	430
with Gamma-irradiation	480	170

surgery to secure the collagen/Vicryl patches. None of the suture materials tested were found to permit any significant adherence of bacteria (see Table 4) although Mersilk and Chromic Catgut consistently gave significantly higher readings than the other two materials. Little difference were seen between *Escherichia coli, Proteus mirabilis* or *Staphylococcus epidermidis,* although *Proteus mirabilis* appears to show less of a tendency to adhere to collagen/Vicryl. In vitro it is likely that rapid adhesion (i.e. within 1 hour) of bacteria does not occur when collagen is layered onto a Vicryl matrix thereby minimising the risk of significant colonisation.

Table 3. Bacterial adherence to collagen/vicryl sheets (*Proteus mirabilis* was used in these experiments)

Treatment	Numbers of bacteria adhering/sq mm in			
	(a) Saline		(b) Urine	
	with PMN	without PMN	with PMN	without PMN
30 min, draining only	600	800	600	800
30 min, plus washing	120	150	100	130
60 min, draining only	540	550	550	550
60 min, plus washing	160	250	150	230

Table 4a. Adherence of *E. coli* to different suture materials

Treatment	Numbers of bacteria adhering sq/mm			
	Vicryl	Chromic Catgut	Ethilon	Mersilk
30 min, drained	200	1,310	610	920
30 min, washed	260	1,100	460	920
60 min, drained	180	1,580	590	940
60 min, washed	1,100	810	180	780

Table 4b. Adherence of *Proteus mirabilis* to different suture materials

Treatment	Numbers of bacteria adhering/sq mm			
	Vicryl	Chromic Catgut	Ethilon	Mersilk
30 min, drained	740	1,110	230	550
30 min, washed	720	810	380	470
60 min, drained	460	740	180	790
60 min, washed	450	770	230	810

the collagen/Vicryl composite does not provide a suitable substrate for bacterial adherence.

In a similar way experiments were conducted using the various suture material which might be used in urological

Table 4c. Adherence of *S. epidermidis* to different suture materials

Treatment	Numbers of bacteria adhering/sq mm			
	Vicryl	Chromic Catgut	Ethilon	Mersilk
30 min, drained	260	1,080	190	1,320
30 min, washed	220	1,090	520	870
60 min, drained	280	1,250	250	1,370
60 min, washed	170	1,060	520	920

Discussion

The real and potential effects of infection associated with the use of a number of suture materials when applied to the urinary tract have already been reported in the literature, and such results may well also be relevant to the collagen Vicryl prosthesis examined in this study. Holbrook [4] incubated polyglycolic acid (a material chemically similar to Vicryl) at 37 °C in presterilised urine infected with *Escherichia coli, Streptococcus faecalis, Proteus mirabilis* and *Pseudomonas aeruginosa.* The polyglycolate was destroyed after 3 days by the proteus-infected urine but, over the same time period, was unaffected by the other organisms. The author concluded that this type of suture should be avoided for closing urothelium in patients with a proteus infection.

Sebeseri et al. [9] reported the effects of *Escherichia coli* infected urine on the strength of polyglycolic acid and cat-

gut sutures. After 6 days incubation in sterile urine the polyglycolate had dissolved but the catgut remained unaffected. However in the infected urine, the polyglycolate had dissolved after 3 days and the catgut after 8 days. In contrast, Williams [11] reported a slower rate of hydrolysis both in vivo and in vitro of polyglycolate sutures in the presence of *Escherichia coli, Streptococcus mites* and *Staphylococcus albus* as compared with sterile broth. Approximately equal rates of degeneration were reported for plain catgut in the presence and absence of micro-organisms in vitro, but in vivo, in infected tissues with a high bacterial count, the material degraded more rapidly.

The possible effect of infection on stone formation was investigated by Yudofsky and Scott [12] where enhanced lithiasis was observed in dogs with a urinary Proteus infection presumably due to an elevation in pH caused by the release of ammonia by this urea-splitting organism. This latter phenomenon would also certainly lead to an increased hydrolysis of absorbable materials such as vicryl and polyglycolate. We have shown that Vicryl hydrolyses more rapidly urines with the higher pH values [3].

The binding of some micro-organisms to collagen itself has been the subject of some recent investigation. Holderbaum et al. [5] showed that ^{125}I-labelled soluble calf-skin collagen would bind readily and reversibly to *Staphylococcus aureus* Cowan 1. Four strains of staphylococcus were investigated where it was found that Cowan 1 type *Staphylococcus aureus* and *Staphylococcus aureus* ATCC 25923 showed saturable specific binding to collagen while strains Wood 46 and S4 showed a complete lack of binding. The results suggested that only some strains of *Staphylococcus aureus* contain the necessary high-affinity binding sites for collagen. Speziale et al. [10] showed that there is a receptor protein on the surface of *Staphylococcus aureus* Cowan 1 which is capable of binding reversibly to type II collagen.

The results of our own experiments have shown that neither of the three strains of bacteria tested i.e. *Escherichia coli, Staphylococcus epidermidis* and *Proteus mirabilis* adhered to the collagen/Vicryl composite membrane nor to its two individual components, and indicate that this material fails to act as a good substrate for these particular micro-organisms.

Irradiation also appeared to reduce the number of *E. coli* able to adhere to the prosthesis.

When the absorbable suture materials are compared with respect to the number of organisms adhering per sq mm material, considerably higher values were obtained for Chromic Catgut than for Vicryl (Table 4). Comparing the non-absorbable sutures, the silk suture (Mersilk) also gave consistently higher values than the other non-absorbable material (Ethilon). When the percentage of bacteria adhering per square millimeter of collagen/Vicryl is compared to the sutures, there is little difference between the values obtained with Vicryl and Ethilon. The findings in this report shows that of those materials likely to be used to secure the membrane in place, Vicryl would appear to be the most favourable candidate because of its low adherence propensities.

In conclusion, the results suggest that adherence of micro-organisms to the prosthesis in potentially infected urine is unlikely to lead to the formation of a localised and rapidly multiplying colony of bacteria. The results are particularly significant in the case of *Proteus mirabilis* where the lowest values for adherence were obtained. This is a urea-splitting organism which could cause a localised rise in pH due to the release of ammonia, and hence cause a far more rapid breakdown of the collagen/Vicryl membrane [3, 4]. However, the increased tendency of micro-organisms to adhere to chromic catgut and silk may suggest that these sutures are less suitable for use in the infected urinary tract than the other materials tested.

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