In vivo evaluation and comparison of collagen, acetylated collagen and collagen/glycosaminoglycan composite films and sponges as candidate biomaterials

S. Srivastava and S.D. Gorham
Devro Ltd, Moodiesburn, Chryston, Glasgow G69 0JE, UK

D.A French and A.A. Shivas
Ethicon Ltd, Bankhead Avenue, Sighthill Industrial Estate, Edinburgh EH11 4HE, UK

J.M. Courtney
Bioengineering Unit, Wolfson Centre, University of Strathclyde, 106 Rottenrow, Glasgow G4 0NW, UK
(Received 6 June 1989; accepted 10 August 1989)

Native collagen, acetylated collagen, collagen/10% chondroitin sulphate, collagen/2.5% hyaluronic acid and collagen/20% hyaluronic acid were implanted both as film and as sponge into rat lumbar muscle for 7 and 14 d. After 7 d implantation, all materials elicited an acute inflammatory cell response characterized by numerous polymorphs and histocytes. The cell population after 14 d was principally mononuclear, i.e. leucocytes, neutrophils, macrophages, lymphocytes and fibroblasts. Both films and sponges followed a similar pattern. Native collagen elicited a subacute inflammatory response after 7 d. However, 14 d after implantation, a marked infiltration by neutrophils was apparent with subsequent degradation of existing collagen material. Acetylated collagen film evoked a much greater inflammatory cell response than native collagen. Both collagen/hyaluronic acid composites elicited a similar response. The collagen/10% chondroitin sulphate composite elicited the least inflammatory cell response at 7 d, whereas infiltration by host fibroblasts after 14 d implantation was clearly seen.

Keywords: Tissue response, inflammation, collagen, collagen/glycosaminoglycan composites

Collagen has many desirable features, which make it an excellent choice as a naturally occurring biomaterial. Among these are its high tensile strength, controllable biodegradation, haemostatic properties[1], low antigenicity[1-2-3], low inflammatory and cytotoxic properties[4], ability to promote cellular growth and attachment[5-11]. Indeed, in its many reconstituted forms it has found significantly increased utilization in a variety of clinical, cosmetic and biomedical applications[1-18]. Collagen has been produced in a range of physical forms such as sheets, tubes, sponges, powder and fleece to be utilized in medical practice[12].

In a previous paper[19], we evaluated the cellular response to a number of collagen-based substrates, such as native collagen, acetylated, methylated and succinylated collagen, as well as collagen/hyaluronic acid and collagen/chondroitin sulphate composites. Each material was made in the form of both sponge and film and the cellular attachment and growth measured using optical microscopy, cell counting (Coulter counter), and ^{3}H-thymidine incorporation. An established cell line derived from mouse fibroblasts was used in the study which showed that: (a) Polystyrene surfaces provided the most suitable surface for cell attachment, growth and proliferation. (b) Collagen, in comparison to polystyrene, is a relatively poor substrate for cell attachment and growth but can be improved by chemical modification, by incorporating either 5 or 10% chondroitin

Correspondence to Dr S. Srivastava.

 0142-9612/90/030155-07

sulphate or low concentrations (< 5%) hyaluronic acid into the collagen matrix. (c) Concentrations in excess of 5% hyaluronic acid in the collagen matrix appear to inhibit cellular attachment and growth, and these materials appear to be inferior substrates compared with the native collagen itself. (d) The cellular growth and attachment to native collagen appear to be enhanced in the presence of fibronectin, a glycoprotein, which is known to promote the attachment and spreading of many cell types.

While such an *in vitro* survey is necessary in designing biomaterials, there are considerable limitations in extrapolating from the *in vitro* to the *in vivo* situation. Further assessment of the biological properties of these materials was therefore considered desirable.

From the materials tested *in vitro*[20], native collagen, acetylated collagen, collagen/10% chondroitin sulphate, collagen/2.5% hyaluronic acid, and collagen/20% hyaluronic acid were selected for further study in both film and sponge form.

These materials were implanted into the lumbar muscles of rats and the resulting tissue response, such as infiltration by host inflammatory cells, biodegradation, and formation of new collagen, was assessed histologically. The rates of solubilization by bacterial collagenase were also determined and compared with the *in vivo* data.

MATERIALS AND METHODS

The collagen-based materials selected for further assessment by collagenase digestion and implantation *in vivo* were native collagen, acetylated collagen, collagen/10% chondroitin sulphate, collagen/2.5% hyaluronic acid and collagen/20% hyaluronic acid. Collagen sponges were selected for collagenase digestion and both sponges and films were implanted *in vivo*. The preparation of all these materials has been described previously[20].

In vitro collagenase solubilization of collagen sponges

Collagenase digestion was performed on the collagen sponges only, employing the following method. Collagen or modified collagen sponge (15 mg) was suspended in 1 ml of 0.1 M tris/HCl buffer, pH 7.2, containing 0.15 M $CaCl_2$ and 0.02% sodium azide. The whole was allowed to stand for 1 h after which 1 ml of a solution containing 50 units of bacterial collagenase (type V, Sigma Chemicals, UK) in deionized water was added and the whole incubated for 30 or 60 min at 37°C. After incubation, 15% w/v trichloracetic acid (0.4 ml) was added, and the mixture centrifuged for 20 min at 6000 rev/min at ambient temperature. The 1 ml of the supernatant liquor was removed and the amount of solubilized collagen determined from the hydroxyproline content of the sample using the method of Woessner[21].

In vivo implantation of collagen sponges and films

The collagen sponges and films were sterilized by soaking in methanol and further washing in sterile saline before implantation. The lumbar fascia of experimental rats was exposed through a midline incision, and a 10 × 3 mm beaver blade used to make a small pouch about 1 cm long in the lumbar muscle.

Owing to the collapsible nature of the sponges, a somewhat different procedure was adopted to that used for the films (described below). Presterilized collagen sponges were sectioned as carefully as possible to fit into 1 cm lengths of medical-grade silicone tubing, internal diameter 2 mm (Portex). A pouch was made in the lumbar muscle of rats, the tubes containing the sponges inserted and the pouch and skin incision closed. The animals were killed at 7 and 14 d, after which the whole muscle was dissected out and transferred into 10% buffered formalin for 24 h. Following fixation, the extraneous muscle was pared to within 1 mm of the tube, an incision was made through to the surface of the tube, and sufficient tube exposed to allow the sponge to be removed. The recovered sponge was then fixed in neutral buffered formalin for 48 h and processed for histology using HPS (haematoxylin phloxine saffron) trichrome staining. The implanted collagen fibres stain yellow/orange, nuclei stain blue/black and other tissue elements stain shades of pink. Newly synthesized collagen stains light orange. As the collagen matures, it stains darker orange/brown.

Presterilized pieces of collagen films, measuring 10 × 2.5 mm, were soaked in sterile saline and then the leading edge was inserted into the pouch. The pouches were closed with a single fine gauze Prolene suture, and the skin incision closed with interrupted fine gauze Prolene sutures 5–7 mm apart.

The rats were killed after 7 and 14 d. Tissue sections were fixed in 10% buffered neutral formalin and then processed for histological examination using HPS trichrome staining.

RESULTS

In vitro collagenase digestion

The results of the bacterial collagenase digestion *in vitro* are presented in *Table 1*. After 60 min digestion, the amounts of collagen solubilized from the native collagen sponge and collagen containing 20% hyaluronic acid were greater than for the other materials. At 30 min, more solubilization appeared to occur with the collagen/20% hyaluronic acid composite under the conditions employed.

The collagen/2.5% hyaluronic acid and collagen/10% chondroitin sulphate materials were degraded at similar levels after 60 min but the former was solubilized to a greater extent after 30 min. A much lower rate of solubilization was observed for the acetylated collagen at both time periods.

Implantation of sponges into rat lumbar muscle

The *in vivo* cellular response to each of the selected sponges and films is summarized in *Tables 2* and *3*.

The *in vivo* response to collagen/2.5% hyaluronic acid sponge, is shown in *Figure 1* after 14 d implantation. This represents a general view of the implant area. At higher magnification (*Figure 2*) the implanted material is seen to be extensively invaded by histocytes, polymorphs and a number

Table 1 Results of bacterial collagenase solubilization; materials in sponge form

Material	Hydroxyproline (μg) liberated per mg collagen	
	30 min	60 min
Collagen/20% hyaluronic acid	48.4	59.7
Native collagen	39.5	58.7
Collagen/2.5% hyaluronic acid	34.8	43.5
Collagen/10% chondroitin sulphate	27.4	43.9
Acetylated collagen	13.9	27.2

Table 2 In vivo *response to collagen sponges*

Material	Implantation period	
	7 d	14 d
Native collagen	Limited invasion by granulation tissue; little cellular invasion	Acute, chronic inflammatory cell infiltration; giant cell, histocytes and macrophages
Acetylated collagen	Greater response evoked; PMN leucocytic infiltrate, some implant degradation	Marked acute infiltration by PMN leucocytes and histocytes. Material degradation and maturation of granulation tissue
Collagen/10% chondroitin sulphate	Little cellular infiltrate into implant. Granulation tissue present	Maturation of granulation tissue. Marked new collagen deposition and survival of existing implant
Collagen/2.5% hyaluronic acid	Marked acute infiltration by PMN and histocytes. Oedema causes dispersion of implant fibres	Implant degradation, chronic infiltration by histocytes, polymorphs and mainly lymphocytes
Collagen/20% hyaluronic acid	Acute infiltration by PMNs. Fibrin extending meshes of implant material	Similar response to above. Greater proportion of polymorphs

Table 3 In vivo *response to collagen films*

Material	Implantation period	
	7 d	14 d
Native collagen	Markedly convoluted film; subacute infiltrate. Early formation of granulation tissue	Degradation of film by fibroblasts causing material fragmentation
Acetylated collagen	Greater degree of subacute infiltrate causing expansion and destruction of convoluted film	Near-complete degradation of film by chronic inflammatory cells
Collagen/10% chondroitin sulphate	Convoluted film sparsely infiltrated by acute cells. Early fibroblasts present	Advanced degradation of film by chronic type cells, mainly fibroblasts
Collagen/2.5% hyaluronic acid	Moderately convoluted film infiltrated around its periphery by subacute inflammatory cells	Marked infiltration by mononuclear cells. Early formation of granulation tissue and material degradation
Collagen/20% hyaluronic acid	Convolutions fairly extended. Infiltrate mainly mononuclear and histocytes present	Marked chronic infiltrate. Greater degree of survival of implant and some new collagen formation

of lymphocytes. A high degree of inflammatory cell infiltration within the collagen sponge matrix is thus observed at this period. *Figure 3* shows at 14 d both acute and chronic cellular infiltration to native collagen sponge. This is indicated by the presence of polymorphs and numerous mononuclear cells (histocytes or macrophages). Existing collagen appears to be integrated with early formation of new collagen. A more pronounced chronic infiltration can be observed for the collagen/10% chondroitin sulphate sponge where the cell population is mainly leucocytic including a number of polymorphs and fibroblasts (*Figure 4*).

In all cases, the biological response as evidenced by the marked cellular infiltration into the collagen matrices (*Figure 1,* top field, right) contrasted strongly with the apparent absence of cellular infiltration of the silicone rubber tubing (*Figure 1,* lower field, right).

Implantation of films into rat lumbar muscle

Compared with the implanted sponges, the area occupied by collagen film materials could be detected more readily microscopically. For example, *Figure 5,* illustrating marked convolutions of the native collagen film (7 d implantation), shows extensive inflammatory cell infiltration between the tubular film structure and around its periphery. This has resulted effectively in a more advanced degradation of the implanted film after 14 d as observed in *Figure 6,* where the convoluted tubular structure is less pronounced. This response when viewed at higher magnification (*Figure 7*) shows a section of the implanted film in the process of degradation as indicated by the abundant chronic infiltration associated with fibroblasts. Apparent expansion and degradation of the acetylated collagen film can be observed in *Figure 8* (after 7 d). This material shows a much greater degree of infiltration by acute and subacute inflammatory cells around the implant area.

Once again, expansion of the convoluted film can be observed for the collagen/2.5% hyaluronic film (*Figure 9*). However, less destruction of the convolutions after 7 d is seen. This material elicited a fairly marked inflammatory infiltrate, although less pronounced than the acetylated film. Higher magnification (taken of a section of the convolutions) reveals the nature of the infiltrate (*Figure 10*); subacute with numerous fibroblasts aligning in between the convolutions and along the periphery.

The near disappearance associated with marked degradation of the same implant can be observed following 14 d implantation (*Figure 11*). Higher magnification (*Figure 12*) reveals the type of chronic infiltrate, mainly mononuclear, histocytes, which has resulted in marked degradation of the implant. An interesting difference in the *in vivo* response was observed for the collagen/10% chondroitin sulphate film (*Figure 13*). After 7 d implantation, the material showed a relatively sparse infiltrate between the convolutions and around its periphery. This material appeared to elicit the least inflammatory response and, at higher magnification, the infiltrate was of subacute inflammatory cell type associated with some fibroblasts indicating new collagen formation.

DISCUSSION

Collagen-based materials in the form of sponges, sheets, sutures and coatings have been used in animal and human studies as dermal wound dressings[9, 22, 23] and as a supporting matrix for the growth and maintenance of cell-cultured skin components such as epidermal and fibroblast cells[24]. Porous collagen sponges provide a scaffold for tissue ingrowth in animals[25, 26] and man[27-29] and also in combination with extracellular components such as chondroitin sulphate forming a biodegradable skin substitute for the coverage of third-degree burns[22, 30]. The presence of hyaluronic acid combined with collagen sponges appears to enhance the repair of animal dermal wounds[9] and increases fibroblast cell replication and biosynthesis *in vitro*[10, 11].

Studies on the cellular response to collagen-based

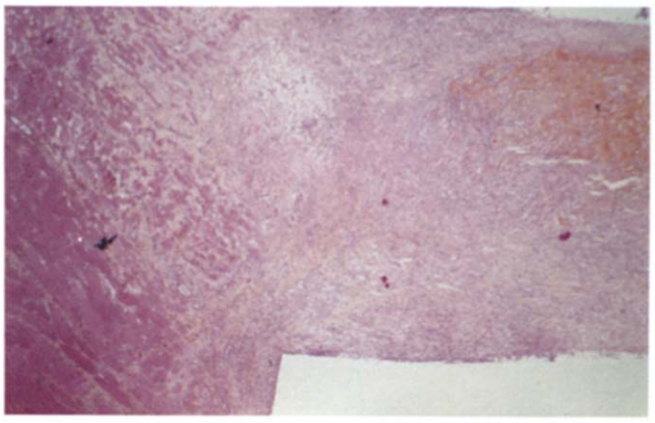

Figure 1

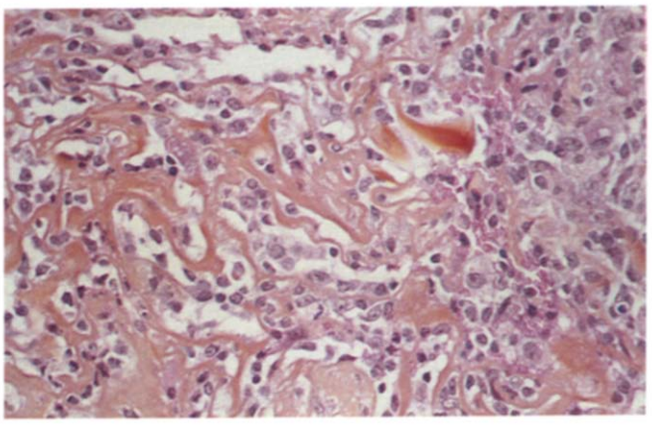

Figure 2

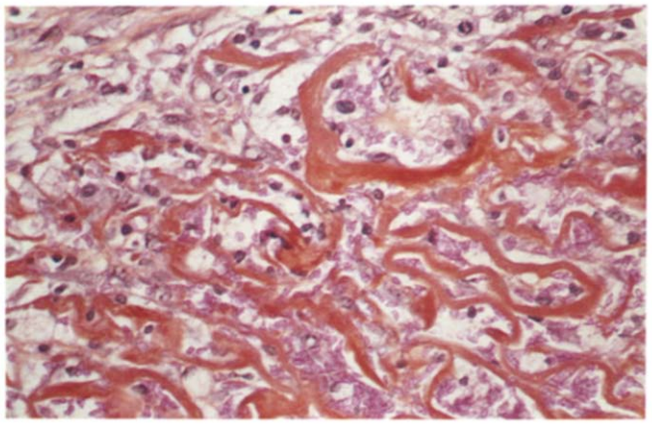

Figure 3

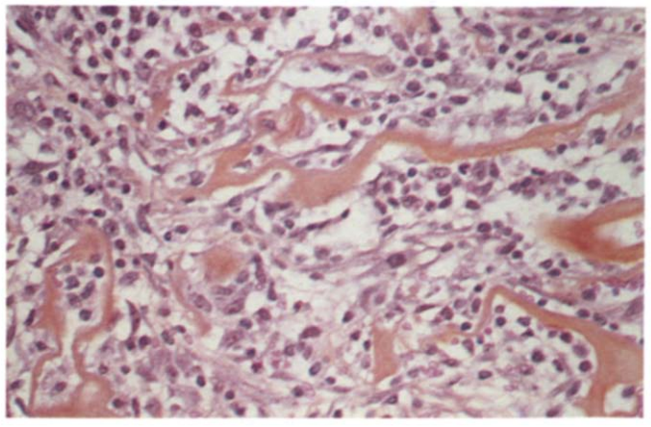

Figure 4

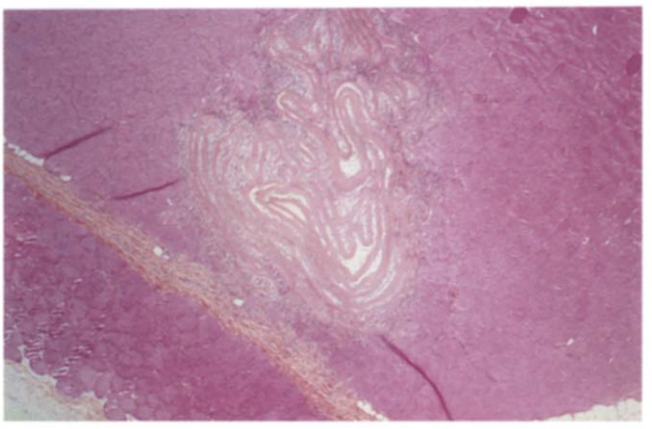

Figure 5

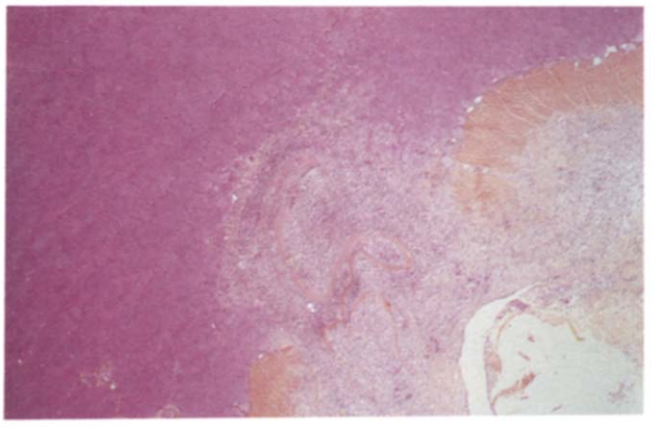

Figure 6

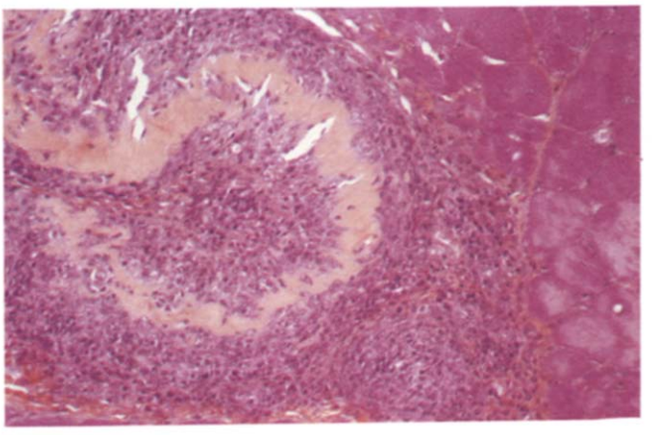

Figure 7

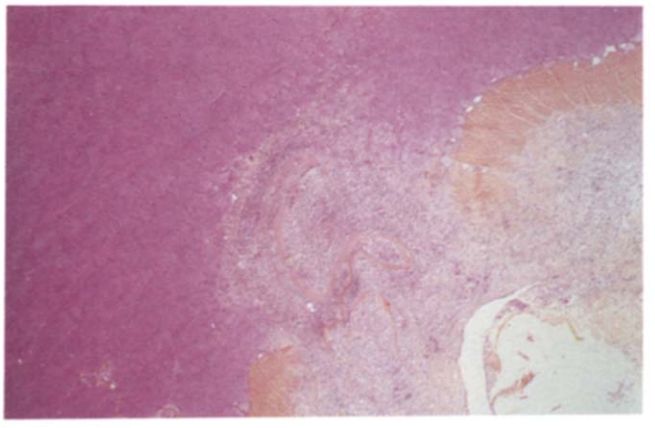

Figure 8

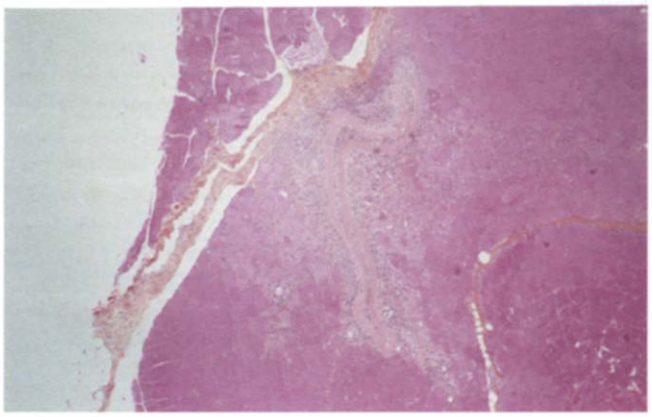

Figure 9

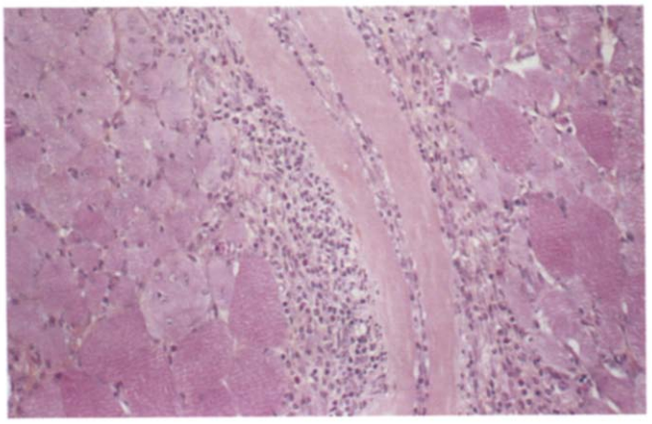

Figure 10

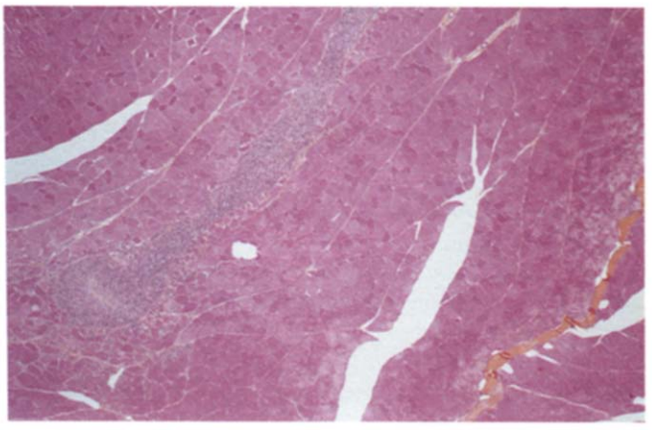

Figure 11

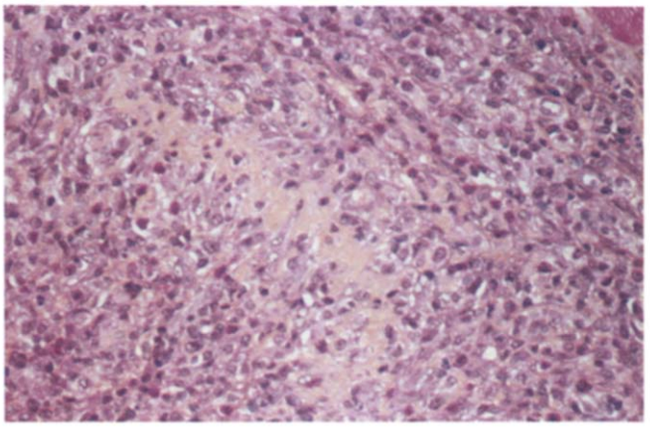

Figure 12

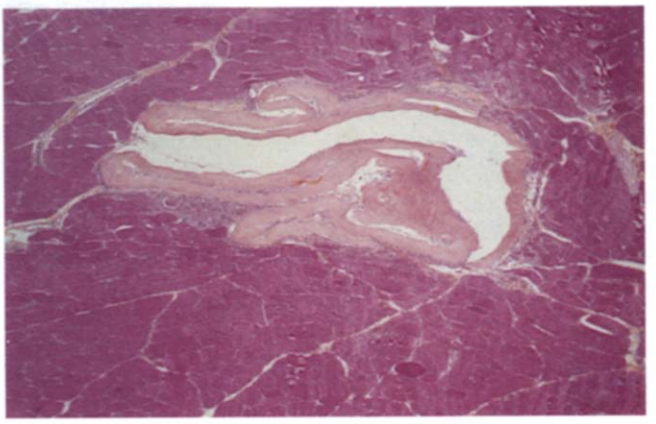

Figure 13

Figure 1 Collagen/2.5% hyaluronic acid sponge, 14 d after implantation. A general view shows a mainly inflammatory response invading the implant (top right). The spaces (upper and lower field) are artefactual. (Original magnification × 10.)

Figure 2 Collagen/2.5% hyaluronic acid sponge, 14 d after implantation. Implant material in course of degradation, extensively infiltrated by histocytes and inflammatory cells including a few polymorphs but mainly lymphocytes. (Original magnification ×100).

Figure 3 Native collagen sponge, 14 d after implantation. Detail from residual implant material can be observed. Moderate numbers of polymorphs are associated with numerous mononuclears (histocytes or macrophages), one of which is seen in mitosis (upper field, right). Early formation of new collagen (upper field, left) and occasional fusiform nuclei indicate a fibroblastic component (arrow). (Original magnification × 100.)

Figure 4 Collagen/10% chondroitin sulphate sponge, 14 d after implantation, showing a mainly leucocytic infiltrate including a few polymorphs. Occasional fusiform fibroblasts are seen lower field (left), indicating early collagen formation and progressive degradation of the implant. (Original magnification × 100.)

Figure 5 Native collagen film, 7 d after implantation. Low-power view showing marked convolution of the film with oedema and inflammatory cell infiltration. (Original magnification × 10.)

Figure 6 Native collagen film, 14 d after implantation. A general view which illustrates the more advanced degradation of implanted material. (Original magnification × 10.)

Figure 7 Native collagen film, 14 d after implantation. The convoluted material is fragmented and shows greater degradation. An abundant chronic infiltration is associated with fibroblasts showing early fasciculation. (Original magnification × 50.)

Figure 8 Acetylated collagen film, 7 d after implantation. General view showing expansion and destruction of convolutions as a result of the acute inflammatory cell invasion. (Original magnification × 10.)

Figure 9 Collagen/2.5% hyaluronic acid film, 7 d after implantation. A general view shows a moderately convoluted implant with a fairly marked inflammatory infiltrate. (Original magnification × 10.)

Figure 10 Collagen/2.5% hyaluronic acid film, 7 d after implantation. At higher magnification, the infiltrate is of subacute inflammatory cell type. Fibroblasts are also fairly conspicuous (right and centre field). (Original magnification × 50.)

Figure 11 Collagen/2.5% hyaluronic acid film, 14 d after implantation. A general view, at low magnification, emphasizes the near-complete disappearance of the implant amid the marked cellular infiltration. (Original magnification × 10.)

Figure 12 Collagen/2.5% hyaluronic acid film, 14 d after implantation. High magnification shows the implant virtually destroyed and the area densely infiltrated by a mainly mononuclear infiltrate with very early formation of granulation tissue. (Original magnification × 100.)

Figure 13 Collagen/10% chondroitin sulphate film, 7 d after implantation. The convoluted implant shows a relatively sparse infiltration by inflammatory cells. (Original magnification × 10.)

substrata are extremely useful for the design of new implant material.

In a previous paper[19], we evaluated a number of chemically modified collagen and collagen/glycosaminoglycan composite films as potential substrates for cellular adhesion and growth. While invaluable information can be obtained from such experiments, it is always difficult to extrapolate the results obtained to the *in vivo* situation, and as a natural progression from these studies the work presented here was carried out.

In considering the results of the implant studies, the possibility of animals producing a variable response has to be accepted. Accordingly, repetition might not yield an identical pattern. However, the data obtained indicate the following general features:

1. In the acute phase the material was invaded by polymorphs and histocytes (after 7 d implantation). Following 14 d implantation, the infiltrate was mainly leucocytes, macrophages, occasional lymphocytes and numerous fibroblasts.
2. Biodegradation of existing collagen.
3. Fibroblastic response indicating the formation of new collagen.
4. The presence of hyaluronic acid causing the fibres of collagen films and sponges to spread apart, presumably on account of its high water-binding capacity.

The *in vivo* performance of these selected collagen-based materials was assessed by implanting collagen films and sponges into rats for 7 and 14 d. Interesting differences in the type of cell response were observed. Following an implantation period of 7 d, all the materials elicited an acute inflammatory response, characterized by the presence of PMNs and histocytes. After 7 d, the cell population was principally mononuclear, i.e. leucocytes, macrophages, lymphocytes and fibroblasts. Implanted films and sponges followed a similar pattern. Native collagen elicited a subacute inflammatory response with considerable infiltration of fibroblast cells after 7 d. However, after 14 d, a marked infiltration by neutrophils was apparent with subsequent degradation of the existing collagen material.

Surface charge on collagen biomaterials is often influential on collagen–cell interactions. Chemical modification of collagen side-chains such as succinylation, methylation and acetylation has been shown to affect the adhesion of platelets to chemically modified substrates[31] and a few studies have reported the interaction of other cell types such as fibroblasts and macrophages with such substrates[32–5]. Most of the information has been obtained from *in vitro* studies and little has been documented regarding the *in vivo* performance of chemically modified collagens.

When implanted in the lumbar muscle of rats, the acetylated collagen film appeared to evoke a much greater inflammatory cell response after 7 d compared with native collagen film. The infiltrate was mainly mononuclear and some muscle giant cells were also present. In effect, this resulted in expansion and destruction of the film.

Although in general the collagen/hyaluronic acid composite films containing 2.5% and 20% glycosaminoglycan elicited a similar type of cell response to each other, *in vivo* the collagen/2.5% hyaluronic acid composite showed a more marked inflammatory cell response, more so than the other collagen-based substrates. Infiltration was mainly mononuclear, although some fibroblast cells were visible after 7 d. Following 14 d implantation, the material was virtually degraded.

Incorporation of low levels of hyaluronic acid (1% and 5%) into a collagen sponge has been shown to result in increased chemoattraction, replication of fibroblasts and collagen deposition in an *in vivo* wound-healing model[9], a result which was also observed during *in vitro* studies[10]. Our own *in vitro* cell culture studies[20] also showed that collagen film composites incorporating 2.5% hyaluronic acid promote cellular growth and attachment significantly more than native collagen after a 6 d incubation period.

The most important observation following implantation of the collagen/hyaluronic acid sponges was the actual spreading apart of the collagen fibres by the cellular population. This could be explained by the presence of hyaluronic acid where this glycosaminoglycan on account of its high water-binding capacity causes the tissue to swell and expand, pushing the collagen fibres apart. The separation creates spaces which may act as avenues for the cell migration that follows tissue swelling[36].

The modified collagen material shown to elicit the least acute inflammatory cell response was the collagen/10% chondroitin sulphate composite film. The infiltration was mainly mononuclear and relatively sparsely distributed compared with the other implanted materials. After 7 d, fibroblasts were present. Following 14 d implantation, this material showed the most advanced biological effect with considerable infiltration of fibroblasts synthesizing new collagen. However, the implanted collagen was still present at this stage suggesting that this material elicited the longest persistence time *in vivo*. In this respect, it may well be significant that it also showed the least inflammatory response.

Parallel to the implant study, rates of solubilization were measured using bacterial collagenase. Both the native collagen and collagen/20% hyaluronic acid composite were solubilized by bacterial collagenase at a rate faster than the other three collagen materials. The collagen composites containing 10% chondroitin sulphate and 2.5% hyaluronic acid were degraded at similar levels after 60 min solubilization, but the collagen/2.5% hyaluronic acid composite was degraded faster after 30 min. The material which showed the least rate of solubilization by bacterial collagenase was acetylated collagen.

Chondroitin sulphate has been combined with a porous collagen sponge for the coverage of third-degree burns[22, 30] in animals and later in humans for 40–50 d. It was found that the enzymatic degradation of the collagen was strongly suppressed for cross-linked collagen/glycosaminoglycan composites in which the mucopolysaccharide component contains a sulphate group[30]. Once the material was sutured in place, fibroblasts from the underlying recipient connective tissue appeared to migrate into the composite sponge matrix and synthesize a neodermis while simultaneously degrading the original composite. This appears to imply that this form of composite is well tolerated *in vivo* and thus acts as a biodegradable template for the synthesis of a neodermal tissue. In our studies, the maximum period of implantation was 14 d and interestingly enough, this material was shown to elicit the least inflammatory reponse, as described above. *In vitro* this collagen/chondroitin sulphate composite material evoked the highest cellular response[20].

When considering the results presented in these implantation studies of collagen-based materials, it must be borne in mind that there are difficulties in attempting to correlate those findings with the earlier *in vitro* studies. Such difficulties are compounded by the variations in foreign body

response to implanted materials encountered among individual animals.

Nevertheless, the results presented are relevant for predicting how individual and composite materials will react when implanted *in vivo*.

ACKNOWLEDGEMENTS

We would like to thank C. Mirtle and D. Bewsey (Ethicon Ltd, Edinburgh) for assistance in the preparation of histological photographs and F. Aitken for typing the manuscript.

REFERENCES

1 Chvapil, M., Kronenthal, R.L. and Van Winkle, W. Jr., Medical and surgical applications of collagen, in *International Reviews of Connective Tissue Research,* Vol. 6 (Eds D.A. Hall and D.S. Jackson), Academic Press, London, 1973, pp 1-61

2 Michaeli, D., Benjamin, E. and Leung, D.Y.K., Immunochemical studies on collagen. II. Antigenic differences between guinea pig skin collagen and gelatin, *Immunochemistry* 1971, **8**, 1-6

3 Etherington, D.J., Silver, I.A. and Restall, D.J., Resorption of insoluble heterologous fluoroscein — collagen sponges in sensitised and non-sensitised rats. *Br. J. Exp. Pathol.* 1979, **60**, 549-559

4 Stenzel, K., Miyata, T. and Rubin, A., Collagen as a biomaterial, *Ann. Rev. Biophys. Bioeng.* 1974, **3**, 231-253

5 Elsdale, T. and Bard, J., Collagen substrata for studies on cell behaviour, J. Cell. Biol. 1972, **54**, 626-637

6 Schor, S.L., Cell proliferation and migration on collagen substrate in vitro, *J. Cell. Biol.* 1980, **41**, 159-175

7 Kleinman, H.K., Klebe, R.J. and Martin, G.R., Role of collagenous matrices on the adhesion and growth of cells, *J. Cell. Biol.* 1981, **88**, 473-485

8 Yang, J. and Nandi, S., Growth of cultured cells using collagen as a substrate, *Int. Rev. Cytol.* 1983, **81**, 249-286

9 Doillon, C.J. and Silver, F.H., Collagen-based wound dressing. Effects of hyaluronic acid and fibronectin on wound healing, *Biomaterials* 1986, **7**, 3-8

10 Doillon, C.J., Silver, F.H. and Berg, R.A., Fibroblast growth on a porous collagen containing hyaluronic acid and fibronectin, *Biomaterials* 1987, **8**, 195-200

11 Doillon, C.J., Wasserman, A.J., Berg, R.A. and Silver, F.H., Behaviour of fibroblasts and epidermal cells cultivated on analogues of extracellular matrix, *Biomaterials* 1988, **9**, 91-96

12 Chvapil, M., Reconstituted collagen, in *Biology of Collagen,* Vol. 22 (Eds A. Viidik and J. Vuust), Academic Press, London, 1980, pp 313-324

13 Knapp, T.R., Development of an injectable collagen for soft tissue restoration, in *Biomaterials in Reconstructive Surgery,* Vol. 58 (Ed. L.R. Rubin), Mosby Company, St Louis, 1983, pp 882-910

14 Arem, A., Collagen modifications, *Clin. Plast. Surg., 1985,* **12**(2), 209-220

15 Burke, K.G., Naughton, G. and Cassai, N.A., Histological, immunological and electron microscope study of bovine collagen implants in the human, *Ann. Plast. Surg.* 1985, **14**, 515-522

16 Ford, C. and Bless, D.M., Clinical experience with injectable collagen for vocal cord augmentation, *Laryngoscope* 1986, **8**, 863-869

17 Huc, A., Collagen biomaterials characteristics and applications, *J. Am. Leather Chem. Assoc.* 1985, **80**, 195-212

18 Klopper, P.J., Collagen in surgical research, *Eur. Surg. Res.* 1986, **18**, 218-223

19 Srivastava, S., Gorham, S.D. and Courtney, J.M., The attachment and growth of an established cell line on collagen, chemically modified collagen, and collagen composite surfaces, *Biomaterials* 1990, **11**, 162-168

20 Srivastava, S., Implantable collagen-based biomaterials: influence of polymer modification on cellular interactions, PhD thesis, Bioengineering Unit, University of Strathclyde, 1988

21 Woessner, J.F. Jr, Determination of hydroxyproline in connective tissues, in *The Methodology of Connective Tissue Research,* Vol. 23 (Ed. D.A. Hall), Joynston-Bruvvers Ltd, Oxford, 1976, pp 227-234

22 Yannas, I.V., Burke, J.F., Gordon, P.L., Huang, C. and Rubenstein, R.N., Design of an artificial skin. II. Control of chemical composition, *J. Biomed. Mater. Res.* 1980, **14**, 107-131

23 Chvapil, M., Collagen sponge: theory and practice of medical applications, *J. Biomed. Mater. Res.* 1977, **11**, 721-741

24 Bell, E., Sher, S., Hull, B., Merrill, C., Rosen, S., Chamson, A., Asselineau, D., Dubertret, D., Coulomb, B., Lapiere, C., Nusgens, B. and Neveux, Y., The reconstitution of living skin, *J. Invest. Dermatol.* 1983, **81**, 2-10

25 Oliver, R.F., Grant, R. A. and Kent, C.M., The fate of subcutaneously implanted trypsin purified dermal collagen in the pig, *Br. J. Exp. Pathol.* 1972, **53**, 540-549

26 Oliver, R.F., Hulme, M.J., Mudie, A. and Grant, R.A., Skin collagen allografts in the rat, *Nature* 1975, **258**, 537

27 Shakespeare, P.G. and Griffiths, R.W., Dermal collagen implants in man, *Lancet* 1980, **1**, 795-796

28 Holl-Allen, R.T.J., Porcine dermal collagen implants in man. *J. R. Coll. Surg. Edinb. 1984,* **29**(3), 151-153

29 Holl-Allen, R.T.J., Porcine dermal collagen repair of inguinal hernias, *J. R. Coll. Surg. Edinb.* 1984, **29**(3), 154-157

30 Yannas, I.V., Crosslinked collagen-glycosaminoglycan composite materials. Biological and mechanical properties, Annual Report, PB24372/AS, National Technical Information Service, Springfield, 1975

31 Miyata, T., Schwartz, A., Wang, C.L., Rubin, A.L. and Stenzel, K.H., Deposition of platelets and fibrin on chemically modified collagen hollow fibres, *Trans. Am. Soc. Artif. Int. Organs.* 1976, **22**, 261-267

32 Wang, C.L., Miyata, T., Weksler, B., Rubin, A.L. and Stenzel, K.H., Collagen-induced platelet aggregation and release. II. Critical size and structural requirements of collagen, *Biochim. Biophys. Acta* 1978, **544**, 568-577

33 Kasai, S., Miyata, T., Takehiko, K. and Nitta, K., Enhanced effect of chemically modified collagen on attachment and growth of fibroblasts in serum-free medium, *Biomed. Res.* 1983, **4**(2), 147-154

34 Kasai, S., Akaike, T., Miyata, T., Kunimoto, T. and Nitta, K., Separation of macrophages from mouse peritoneal exudate cells with substrata coated with chemically modified collagens, *J. Biomed. Mater. Res.* 1984, **18**, 243-253

35 Miyata, T. and Noishiki, Y., Biological properties of native and modified collagen as biomaterials, in *The Future of Collagen,* 1st Atlantic Conference, Lyon, 1985, pp 135-140

36 Toole, B.P., Glycosaminoglycans in morphogenesis, in *Cell Biology of the Extracellular Matrix,* Vol. 9 (Ed. E.D. Hay), Plenum Press, New York, USA, 1982, pp 259-288