

## Acellular dermal graft for repair of abdominal wall defects in rabbits

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### ABSTRACT

Sixteen clinically healthy New Zealand white rabbits of either sex were divided into 2 equal groups (I and II) of 8 animals each. Under thiopental sodium (2.5 %) anaesthesia a 2 × 3 cm full-thickness abdominal wall defect in the mid-ventral abdominal wall was created and repaired with an acellular dermal graft (ADG) in all the animals of group I (test group). In animals of group II (control group) a full-thickness linear midline abdominal muscular wall incision was made and repaired with a continuous suture pattern using 2-0 nylon.

**Key words:** abdominal wall defects, carbon fibres, rabbits.

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### INTRODUCTION

Defects of the abdominal wall continue to be a significant problem for patients and surgeons. The lack of sufficient tissue may require the insertion of prosthetic material. Common biological materials like small intestine mucosa<sup>9</sup>, *tensor fascia lata* and *lattisimus dorsi* muscle<sup>6</sup>, human dura mater<sup>41</sup>, porcine dermal collagen<sup>12</sup>, diaphragm<sup>21,45</sup>, autologous full-thickness skin and dermis<sup>4</sup> have been used with limitations such as inflammatory oedema, adhesion formation and recurrence. In cellular grafts, the histocompatibility antigens present in cells generally lead to immunological reactions. Xenografts are also known to cause immunological reactions. However, the response of allografts may be satisfactory. Cartmell and Dunn<sup>7</sup> reported the reduced antigenicity of acellular grafts. Acellular dermal grafts have been used successfully in repairing septal perforations in humans<sup>22</sup>. Gulati and Cole<sup>16</sup> observed less immune response and better tolerance of acellular grafts in rats and rabbits. The present study was undertaken to evaluate acellular dermal grafting for the repair of abdominal wall defects in rabbits.

### MATERIALS AND METHODS

#### *Preparation and storage of acellular dermal grafts*

A full-thickness skin of a buffalo calf was procured from an abattoir. After proper

washing with sterile normal saline solution, the epidermis was removed with the help of dermatome and the dermis was split into pieces measuring 3 × 4 cm. These dermal grafts were rendered acellular using the method of Gupta *et al.*<sup>17</sup> (Fig. 1). The dermal pieces were placed in distilled water for 1 hour and agitated using a magnetic bar to lyse the cells and finally to release the intracellular contents. Tissues were then suspended in 4 % sodium deoxycholate for 3 hours followed by treatment with deoxyribonuclease-1 (2000 Kunitz units) suspended in a 1 molar sodium chloride solution and stirred for 1–2 hours. This process was repeated 3 times to extract all cells from the tissue. Samples were then stored in 0.09 % phosphate-buffered saline con-



Fig. 1: Prepared acellular dermal graft.

taining streptopenicillin (Dicrysticin; Sarabhai Zydus Animal Health Limited, Gujrat, India) at 0 °C until grafting. Samples were then stored in 0.09 % phosphate-buffered saline containing streptopenicillin. The grafts were stored at 4 °C and utilised within 2 weeks.

#### *Experimental procedure*

The experimental procedure was approved by the Indian Veterinary Research Institute's ethical committee. The study was conducted on 16 clinically healthy New Zealand white rabbits. The animals were randomly divided into 2 equal groups (I and II) of 8 animals each. Food for 18 hours and water for 6 hours were withheld before operating. The ventral abdominal area was prepared for aseptic surgery and the operation was performed under general anaesthesia. Thiopental sodium (2.5 %) was injected intravenously in the ear vein 'till effect' for anaesthesia. The animals were secured in dorsal recumbency. After skin incision, a full-thickness defect of the abdominal wall (2 × 3 cm) in the mid-ventral abdominal region was created and repaired with acellular dermal grafting in all the animals of group I. The graft was implanted at the site using a continuous suture pattern with nylon no. 2-0 suture material (Fig. 2). In the control group, a full-thickness linear midline abdominal muscular wall incision was made and repaired with a continuous suture pattern using 2-0 nylon. Postoperatively, streptopenicillin at the rate of 100 mg/kg body weight and diclofenac sodium at the rate of 2 mg/kg body weight were administered intramuscularly for 5 days in all animals. Daily dressing of the suture line with 5 % povidone iodine was carried out. The skin sutures were removed on the 9th postoperative day or when the skin had completed healing. Clinical examination of the implantation site was carried out daily for up to 14 days and included the following observations:

a) General behavioural changes: feeding pattern and general behavioural changes in all the animals were noted daily.

b) Rectal temperature: rectal temperature was recorded daily in the morning

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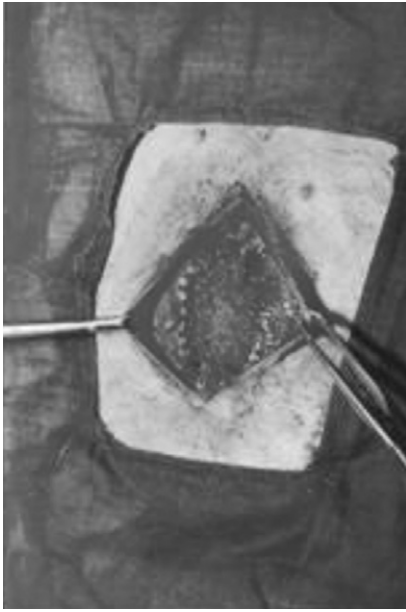


Fig. 2: Repair of a 2 x 3 cm abdominal wall defect with an acellular dermal graft in a rabbit.

up to day 14 in all animals.

c) Wound condition: on the basis of the following parameters, the surgical wounds were examined for their gross appearance and status of healing for up to 14 days after the operations.

i) *Swelling*: the degree of swelling at the site of repair was graded on a scale of 0–3: 0 = no swelling; 1 = mild swelling; 2 = moderate swelling; 3 = severe swelling.

ii) *Exudation*: the degree of exudation at the site of repair was graded on a scale of 0–3: 0 = no exudate; 1 = mild exudate; 2 = moderate exudate; 3 = severe exudate.

iii) *Warmth*: the warmth at the site of repair was graded on a scale of 0–2: 0 = normal; 1 = mildly warm; 2 = moderately warm.

iv) *Pain*: the degree of pain was assessed by gently pressing the operation site and was graded on a scale of 0–3: 0 = no pain; 1 = mild pain; 2 = moderate pain; 3 = severe pain.

For estimation of plasma glucose, blood samples were collected on days 0, 7, 14, 30 and 60. Blood smears were made on days 0, 1, 3, 7, 14 and 30 for differential leucocyte counts.

#### Immunological reactions

The immunoreactivity of the grafted material was assessed by ELISA. The serum samples were collected on days 7, 14, 30 and 60 post-transplantation to detect the extent of antibody generated towards the graft component. A fixed dilution of antibody of 1:400 was used throughout the study. A microtitre ELISA plate (Nunc, Denmark) was coated with 0.1  $\mu\text{g}$  of protein (derived from grafted

material) in 100  $\mu\text{l}$  of 0.05 molar sodium carbonate buffer (pH 9.6) per well. The plate was incubated at 4 °C overnight. After incubation the plate was washed with PBST [0.15 molar sodium chloride – 0.02 molar phosphate buffer (pH 7.2) containing 0.005 % Tween 20]. Subsequently, blocking was performed with 5 % skimmed milk powder in PBST and further incubated at 37 °C for 2 hours. The plate was washed with PBST followed by the addition of a 1:400 dilution of sera obtained from different rabbits grafted with various graft materials. The plate was incubated again for 2 hours at 37 °C followed by washing. Peroxidase-labelled anti-rabbit conjugate in a 1:500 dilution was made in PBST and each well received 100  $\mu\text{l}$  and then further incubated at 37 °C for 2 hours. Finally the plate was washed as before and peroxidase substrate was added [100  $\mu\text{l}$  of 17 millimolar sodium citrate buffer, pH 6.3, containing 0.2 % (wt/vol.) *O*-phenylene diamine and 0.015 % (wt/vol.) hydrogen peroxide] per well. The substrate was allowed to react for 10 minutes at 37 °C, keeping the plate in the dark. Absorbance was recorded at 492 nm using an ELISA reader (ECIL, Hyderabad, India).

Two rabbits from each group were euthanased at 7, 14, 30, and 60 days for macroscopic, biochemical, histopathological and histochemical observations. Macroscopic observations included the presence or absence of adhesions at the site and were graded as 0 = no adhesion, 1 = minimal adhesion (could be freed by gentle blunt dissection), 2 = moderate adhesion (could be freed by aggressive blunt dissection), 3 = dense adhesion (requiring sharp dissection to free the graft from the underlying abdominal viscera). Biochemical parameters included the estimation of collagen<sup>25</sup>, hydroxyproline<sup>30</sup>, hexosamine<sup>33</sup> and elastin<sup>25</sup> in the healing tissue. Zinc and copper in the healing tissue were analysed by an atomic absorption spectrophotometer.

#### Histopathological and histochemical observations

The specimens for histopathological examination were preserved in 10 % formalin. The tissues were processed by routine paraffin embedding and sections were cut at a thickness of 5  $\mu\text{m}$ . The sections were stained with haematoxylin and eosin for healing studies<sup>44</sup>, Masson's trichrome for collagen fibres<sup>28</sup>, Verhoeff's for elastic fibres<sup>27</sup> and Periodic Acid Schiff for polysaccharides and mucosubstances<sup>23</sup>. For histochemical study and for studying alkaline phosphatase activity, the tissue samples and normal healthy abdominal wall tissue were preserved at

–20 °C. Cryostat sections of 10–15  $\mu\text{m}$  thicknesses were cut and stained by Gomori's method<sup>32</sup> using  $\beta$ -sodium glycerophosphate as substrate. For enzymatic controls, the parallel sections were incubated in distilled water without a substrate.

#### Statistical analysis

Student's paired *t*-test was used to compare the means at different time intervals<sup>39</sup> and nonparametric observations were analysed with Friedman's 2-way analysis of variance by ranks<sup>37</sup>.

## RESULTS

### Clinical observations

All the animals from both groups remained slightly anorexic and dull for 2 days after the operation. Food and water intake normalised by the 4th post-operative day. A significant reduction in rectal temperature was observed in both groups of animals. Significant hypothermia ( $P < 0.05$ ) up to days 4 and 7 was recorded in the test and control groups, respectively. The peak values of rectal temperature in group I ( $39.79 \pm 0.07$  °C) and group II ( $39.74 \pm 0.07$  °C) were observed on days 12 and 11, respectively. The surgical wounds appeared healthy throughout the period of observation in both groups. None of the wounds showed any complications such as gaping or infection. Maximum swelling scores were recorded on day 1 in both groups, and thereafter swelling at the operation site continued to decrease gradually, and completely subsided on days 6 and 4 in groups I and II, respectively. Mild exudation at the site was noticed up to day 3 in group I, whereas no exudation was seen in the control group. Warmth scores in group I showed significant increases ( $P < 0.05$ ) up to day 2 and thereafter reduced, and normal warmth was recorded from day 5 onwards. Normal warmth in the control group was observed on day 3. The animals in the test group exhibited pain at the site up to day 4, whereas in the control group no pain at the site was observed after day 2. From day 6 onwards, no pain was recorded at the wound site in any of the animals from either group.

### General observations

In group I, on day 7, the host-graft junction was filled with pink, firm granulation tissue. Thin, filamentous and easily separable adhesions to the caecum were observed. On day 14 the junction was filled with white fibrous tissue and was relatively firmer than in animals from the control group. On day 30 the graft was

Table 1: Differential leucocyte count (%) at different intervals after reconstruction of an abdominal wall defect with an acellular dermal graft in the test group (I) versus that of the control group (II).

Name of leucocyte	Group	Day					
		Preoperative	Postoperative				
			0	1	3	7	14
Neutrophil	I	37.00 ± 1.85	51.63 ± 1.18*	54.00 ± 5.10*	49.75 ± 3.51*	47.67 ± 5.93	50.50 ± 10.84
	II	34.50 ± 1.19	54.25 ± 2.06*	56.75 ± 1.50*	50.50 ± 3.62*	42.00 ± 5.51	38.00 ± 1.00
Lymphocyte	I	60.13 ± 1.88	45.75 ± 1.19*	33.63 ± 5.33*	47.00 ± 3.62*	49.67 ± 5.41	46.17 ± 3.00
	II	62.00 ± 1.78	41.25 ± 3.50*	40.75 ± 1.00*	47.25 ± 3.99	55.33 ± 5.84	55.00 ± 4.00
Eosinophil	I	0.13 ± 0.13	0.00 ± 0.00	0.25 ± 0.16	0.25 ± 0.16	0.17 ± 0.17	0.00 ± 0.00
	II	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.50
Monocyte	I	2.25 ± 0.45	2.38 ± 0.42	2.00 ± 0.46	2.25 ± 0.49	2.00 ± 0.58	2.50 ± 0.65
	II	2.50 ± 0.50	1.75 ± 0.25	0.20 ± 0.41	1.50 ± 0.50	2.33 ± 0.33	4.50 ± 2.50
Basophil	I	0.50 ± 0.27	0.25 ± 0.25	0.13 ± 0.13	0.75 ± 0.31	0.50 ± 0.22	0.25 ± 0.25
	II	0.75 ± 0.48	0.25 ± 0.25	0.50 ± 0.29	1.00 ± 0.40	0.33 ± 0.33	2.00 ± 0.00

\*Differs significantly ( $P < 0.05$ ) from day 0 values.

covered by white fibrous tissue. Adhesions between the graft and the caecum were moderate and could be freed by blunt dissection. On day 60 the graft was completely buried under newly formed tissue. A few, easily separable, band-like adhesions were seen in the caecum. In the control group, on day 7, the defect site was hyperaemic and filled with granulation tissue. On day 14 the incision site was covered with thin fibrous tissue. On day 30 the site was completely covered with white fibrous tissue. This became more organised by day 60.

#### Immunological observations

The immunoreactivity of the grafted material was assessed by ELISA. The serum samples collected at various time intervals post-transplantation were checked to detect the extent of antibody generated towards the graft component. After several trials, a fixed dilution of antibody of 1:400 was found to be optimal. Beyond this dilution the absorbance was too low and above this dilution (1:50) no appreciable change in absorbance was found. The absorbance ranged from  $0.154 \pm 0.011$  to  $0.0733 \pm 0.016$  in the test group, whereas absorbance in the control group ranged from  $0.056 \pm 0.0002$  to  $0.065 \pm 0.0007$ .

#### Haematological observations

A significant ( $P < 0.05$ ) increase in neutrophil count up to day 7 after the operation was observed in both the groups (Table 1). The peak values were observed on day 3 in the acellular graft (group I). At day 14 the neutrophil count decreased non-significantly ( $P > 0.05$ ). At day 30, a slightly elevated neutrophil count ( $P > 0.05$ ) was observed in the test group, whereas values close to baseline were recorded in the control group. In contrast to increased neutrophil count, a significant decrease ( $P < 0.05$ ) in lympho-

cytes was observed in animals from group I, whereas in the control group significant ( $P < 0.05$ ) lymphocytopenia was observed for up to 3 days. The changes observed in eosinophil, basophil and monocyte counts were not significant ( $P > 0.05$ ).

#### Biochemical observations

**Plasma:** after the operation, hyperglycaemia was recorded in both groups of animals (Fig. 3). Significantly higher ( $P < 0.05$ ) values of glucose were recorded on day 7 in both groups (Table 2). Thereafter, the values decreased and returned to near baseline by day 60 in both groups.

**Healing tissue:** there was a gradual increase in collagen content in both groups of animals for up to day 30 (Table 2), thereafter it reduced and returned to near baseline values. The hydroxyproline content in healing wounds also showed a gradual increase up to 30 days, followed by a decrease at 60 days in both groups (Table 2). Comparison between the groups showed a significant difference ( $P < 0.05$ ) in hydroxyproline values at day 14, whereas a non-significant ( $P > 0.05$ ) difference was observed at subsequent time intervals. The highest concen-

tration of hexosamine was observed on day 7 in both the groups. Thereafter it gradually decreased up to day 30. A slight rise at day 60 was observed in both groups but the values still remained lower than the day 7 values. Elastin percentage in healing tissue showed an increase up to day 14, and thereafter no distinct trend in elastin percentage was observed. The level of zinc in healing tissue showed a slight increase at day 14 in group I (and subsequently) but the values had decreased by days 30 and 60 (Table 2). The zinc content in group II showed significant increases at days 14 and 30, and slight decreases at day 60. The copper content in the healing tissue at day 7 was higher in animals treated with an acellular graft than in the control group. The copper content showed a significant decrease ( $P < 0.05$ ) at days 14, 30 and 60 compared to day 7 in the test group. In the control group no significant rise in copper content was observed.

#### Histopathological and histochemical observations

On day 7 the host muscle-acellular dermal graft junction revealed moderate numbers of lymphocytes and few neutro-

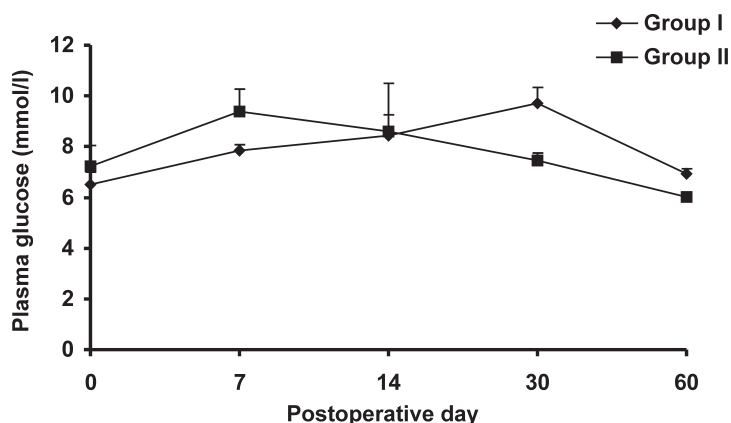


Fig. 3: Plasma glucose (mmol/l) levels after creation an abdominal wall defect with an acellular dermal graft in test group I versus those of the control group.

Table 2: Mean  $\pm$  SE of collagen, hydroxyproline, hexosamine, elastin, zinc and copper at different intervals after reconstruction of an abdominal wall defect with an acellular dermal graft in the test group (I) versus that of the control group (II).

Parameter	Group	Postoperative day			
		7	14	30	60
Collagen (%)	I	28.925 $\pm$ 2.11 <sup>a</sup>	31.010 $\pm$ 0.59 <sup>a</sup>	75.526 $\pm$ 1.66 <sup>a</sup>	29.205 $\pm$ 1.40 <sup>a</sup>
	II	33.988 $\pm$ 0.69 <sup>b</sup>	38.243 $\pm$ 0.59 <sup>*b</sup>	60.524 $\pm$ 1.25 <sup>*b</sup>	14.822 $\pm$ 1.52 <sup>b</sup>
Hydroxyproline (mg/g of wet tissue)	I	3.877 $\pm$ 0.28 <sup>a</sup>	4.157 $\pm$ 0.08 <sup>a</sup>	10.124 $\pm$ 0.22	3.915 $\pm$ 1.13
	II	4.556 $\pm$ 0.00 <sup>b</sup>	5.126 $\pm$ 0.00 <sup>*b</sup>	8.113 $\pm$ 0.00 <sup>*</sup>	1.987 $\pm$ 0.16
Hexosamine (mg/g of wet tissue)	I	0.137 $\pm$ 0.02 <sup>a</sup>	0.090 $\pm$ 0.01 <sup>a</sup>	0.055 $\pm$ 0.01 <sup>a</sup>	0.095 $\pm$ 0.01 <sup>a</sup>
	II	0.634 $\pm$ 0.05	0.339 $\pm$ 0.01	0.237 $\pm$ 0.01	0.452 $\pm$ 0.00
Elastin (%)	I	7.24 $\pm$ 0.52	11.59 $\pm$ 0.35	10.66 $\pm$ 0.56	12.12 $\pm$ 1.01
	II	8.33 $\pm$ 0.68	8.36 $\pm$ 0.25	8.57 $\pm$ 0.42	8.69 $\pm$ 0.31
Zinc (fg/g of wet tissue)	I	42.3641 $\pm$ 9.38	50.9574 $\pm$ 8.09	30.3126 $\pm$ 9.96	19.2957 $\pm$ 11.25
	II	16.2361 $\pm$ 0.30	18.0250 $\pm$ 0.41	27.2499 $\pm$ 0.86	25.3815 $\pm$ 10.84
Copper (fg/g of wet tissue)	I	4.5827 $\pm$ 0.21	2.4878 $\pm$ 0.07	2.2516 $\pm$ 0.08	3.4465 $\pm$ 0.17
	II	1.2081 $\pm$ 0.09	1.3877 $\pm$ 0.23 <sup>b</sup>	1.8676 $\pm$ 0.85	1.6086 $\pm$ 0.19

\*Differs significantly ( $P < 0.05$ ) from day 7 values.

<sup>ab</sup>Values with different letters differ significantly ( $P < 0.05$ ) between the groups at particular time intervals.

phils in the loose fibrinous connective tissue. The muscle fascia towards the peritoneum was prominently thickened with regularly arranged fibroblasts; collagen fibres and fine blood capillaries were arranged parallel to the fibroblasts. The incised abdominal wall in the control group showed healing tissue consisting of compactly arranged, active fibroblasts with plump/spindle-shaped, elongated nuclei and pale cytoplasm, and less wavy collagen fibres. The stroma showed little exudation, which was infiltrated with moderate numbers of neutrophils and lymphocytes. The tissue in the healing site had a light rose appearance on PAS reaction.

On day 14, there was evidence of distinct union between host and graft tissue in the test group. The granulation tissue consisted of closely packed fibroblasts, collagen fibres and parallel-arranged capillaries with moderate numbers of neutrophils and lymphocytes. The fibroblastic tissue invaded the acellular dermal graft to variable distances. The graft collagen at the interface exhibited resorption (Fig. 4). The peritoneal side of the muscle was markedly thickened with young granulation tissue along with infiltrated neutrophil and mononuclear cells. A small amount of PAS-positive material was visible in the healing tissue. In the control group there was moderate fibroblastic tissue reaction around the suture, consisting of a large number of fibroblasts linearly arranged in the collagen fibres. The elastic fibres in the healing tissue were not visible on Verhoeff's elastin staining. PAS reaction was mild at the healing site.

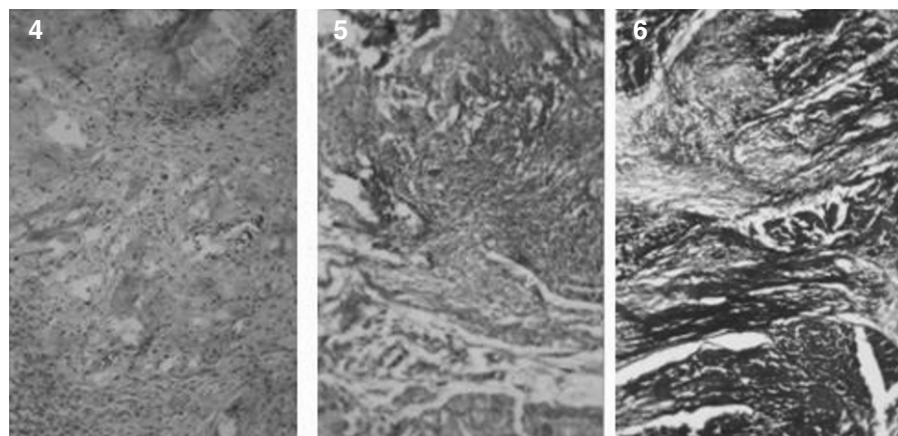
By day 30 the interface between muscle and the acellular dermal graft in group I animals had extensive fibroblastic tissue reaction with a large number of neutro-

phils, lymphocytes and few macrophages. The host-graft junction showed deposits of fibrocytes/fibroblasts and collagen fibres having discrete lymphoid aggregates and neutrophils (Fig. 5). Ingrowths of fibrous connective tissue were seen within the graft at variable distances (Fig. 6). PAS activity was comparatively less than at day 14. In the control group, the site showed a large amount of irregularly arranged, active fibroblasts/fibrocytes and capillaries (angioblasts). The stroma appeared eosinophilic and was infiltrated with a small number of neutrophils. The fascia of the muscle fascicles was denser, having overlaid parallel-arranged fibroblasts and fine collagen. A few muscle fibres also showed a positive PAS reaction.

By day 60, thick ingrowths of mature fibrocytes and wavy collagen fibres in the graft all along its periphery were observed. The mature connective tissue matrix contained a moderate number of neutrophils and lymphocytes. On day 60,

mature wavy, dense and parallel collagen fibres with nestled inactive fibrocytes were observed. No inflammatory cells were visible in the stroma. The dense fibrous tissue with fewer fibrocytes was noticed in the perimycium and endomycium regions of the muscle. The muscle fibres did not show any appreciable changes. The PAS-stained section did not show any activity.

Alkaline phosphatase activity was evaluated in histological sections. At day 7, moderate enzymatic activity at the host graft junction was encountered. The nearby muscle fascicles showed moderate enzyme activity comparable to that of the control (group II). At day 14, slightly increased reaction was seen at the healing site. Thereafter, at 30 and 60 days, it remained mild to moderate. The localisation of alkaline phosphatase activity at day 7 in tissue elements at the healing area in the positive control varied from mild to moderate compared with the negative control. Thereafter, at day 14, it



Figures 4–6: 4: Collagen of a graft undergoing resorption, H & E,  $\times 100$  (group I, day 14); 5: fibrous connective tissue ingrowth within an acellular dermal graft and lymphoid aggregates, H & E,  $\times 100$  (group I, day 30); 6: fibrous connective tissue (fine collagen fibres and fibroblasts) invading an acellular dermal graft, Masson's trichrome,  $\times 400$  (group I, day 30).

increased moderately in the fibrous connective tissue (fibroblast), muscle fascicles and inflammatory exudates. Later it remained more or less the same at days 30 and 60 at the healing site.

## DISCUSSION

Dullness, depression and partial anorexia in the immediate postoperative period was attributed to surgical trauma and inflammation at the site of reconstruction. A significant decrease ( $P < 0.05$ ) in rectal temperature was observed in both groups of animals up to the 4th day. However, a decrease in rectal temperature was seen up to day 7 in the control group. Decreased metabolic rate, muscular activity and muscle relaxation contributed to the fall in rectal temperature<sup>26</sup>. Mild swelling, pain, warmth and exudation were observed in both groups. The presence of swelling at the site may be attributed to inflammation in response to surgical trauma<sup>3,10</sup>. As the healing progressed, the inflammation subsided gradually and no exudation or warmth was observed in any animal from day 6 onwards. Injury to tissues causes a number of changes in the nociceptive system. The injured nociceptors become highly sensitised to stimuli. Inflammatory mediators released during and after surgery also sensitises the peripheral nociceptors to further stimuli<sup>43</sup>.

In the test group, thin filamentous adhesions with the caecum were observed throughout the study period. Moderate adhesions (grade = 2, that could be freed by blunt dissection) were observed on day 30. No adhesions were observed in the control group. Adhesions between the reconstructed site and intraperitoneal organs after hernioplasty with different prosthetic materials have been reported<sup>15,44</sup>. Uniform covering of prosthetic material with a layer of white connective tissue of variable thickness, as seen in the present study, was also observed by Shoukry<sup>36</sup>.

The grafted tissue (acellular dermal graft) showed a very nominal immune response in the recipient. An ideal biomaterial should be immunologically acceptable to the host or should incite minimum reaction in order to avoid being rejected. The acellular dermal graft, rich in collagen, showed poor immunological reaction due to its low antigenicity. This may be a possible reason for the low immunoreaction observed in ELISA. Nevertheless, the chemical components released from collagen fibre, although not antigenic on their own, may behave as a hapten (incomplete antigen). This hapten has ample potential to react with other cellular components of host tissue, result-

ing in a high molecular weight conjugated protein, which can act as a complete antigen and evoke an immune response against it<sup>29</sup>. Another possibility may be the low degradation of collagen, which is a very slow process. Thus, there may not have been sufficient time to elicit the hapten conjugate reaction, as the maximum period of study was only 60 days. Technical modifications that can reduce the antigenicity of graft components are well tolerated by the recipient<sup>2</sup>. On histopathology there was evidence of residual collagen that had yet to be degraded by the host defence mechanism to support the immunological finding. Collagen is known to be a poor immunogen due to its structural stability and poor degradability that acts as a scaffold for tissue regeneration and angiogenesis. Long-term survival of the graft under immunosuppressive therapy should be avoided in non-life-threatening situations. An alternative approach for reducing the antigenicity of grafted material should rather be one like that demonstrated in the present study; within our study period, the grafted materials were found to have low immunological reactivity.

A significant ( $P < 0.05$ ) increase in neutrophil count and decrease in lymphocyte count up to day 7 after the operation was observed. The neutrophilia that occurred briefly suggested that surgical trauma rather than the implant provoked the response. Similar observations in humans have been reported by Vita *et al.*<sup>46</sup> using polypropylene prosthetic material.

Significantly higher ( $P < 0.05$ ) values of glucose were noticed at day 7 in both of the groups. Hyperglycaemia persisted for up to 30 days in the test group. Gaynor<sup>14</sup> also reported an increased level of glucose associated with the stress response due to surgery/trauma.

Increased levels of hydroxyproline is indicative of an increased amount of collagen deposition. The collagen content in healing tissue is directly correlated with the laying down of fibroblasts. There was a gradual increase in collagen and hydroxyproline contents in both groups of animals up to day 30. Thus, the results were in accordance with the findings of Jadon *et al.*<sup>19</sup> and Kanade *et al.*<sup>20</sup> Maximum amounts of collagen and hydroxyproline were observed on day 30 and the increase was significant in the control group. An increased level of hydroxyproline is indicative of an increased amount of collagen deposition and is suggestive of an early healing response. In our study, a higher concentration of hexosamine was seen on day 7 and it gradually decreased up to day 30. Young fibroblasts are

responsible for the secretion of mucopolysaccharides, which accumulate in the granulation tissue in large quantities at the beginning of the healing process. As the healing progresses, the concentration of hexosamine gradually decreases<sup>20,38,42</sup>. The higher concentration of hexosamine content in the initial stage of repair could be attributed to its non-utilisation since granulation tissue was being laid down<sup>11</sup>. There was no definite pattern in the level of elastin in healing tissue of the test group, whereas in the control group the elastin percentage increased slightly up to day 60. Similar observations in healing tissue have been reported in buffalo calves<sup>19,21</sup>. Levenson *et al.*<sup>24</sup> also demonstrated that elastin plays no detectable role in wound healing.

Zinc is the metal moiety of alkaline phosphatase. A slight increase ( $P > 0.05$ ) in zinc level was recorded in group I, but in group II the rise in zinc level was statistically significant ( $P < 0.05$ ) up to day 30. Thereafter, values decreased by day 60. An initial rise in the value of zinc in granulation tissue has been reported by Schilling<sup>35</sup> and Hanzel *et al.*<sup>18</sup>. The increase in zinc level up to day 30 may be due to increased activity of carbonic anhydrase and alkaline phosphatase. A decrease in zinc level at day 60 has been attributed to the increased amount of mature collagen on account of decreased alkaline phosphatase<sup>31</sup>. A significant reduction ( $P < 0.05$ ) in the values of copper were noticed from day 14 onwards, up to day 60 in the test group, and this decreased copper level may be due to its utilisation in promoting the structural integrity of tissue collagen as has also been reported in the case of bone collagen<sup>5</sup>. Increased levels of copper may be attributed to its role in the formation of blood vessels<sup>1</sup> and increased level of inflammation during the day following wounding<sup>19,47</sup>.

Postoperative inflammatory and healing tissue reaction by the host under the influence of prosthetic implants was assessed histopathologically and histochemically. The control group showed moderate inflammatory reaction and mild to moderate laying down of healing tissue. The amount of muscle necrosis was moderate and showed regeneration on day 7. The above changes declined gradually and by day 60 complete healing was apparent with the formation of mature connective tissue and muscle repair. The acellular dermal graft (group I) animals initially showed little incorporation of healing tissue, and contained a moderate number of neutrophils and lymphocytes, originating from the host graft junction. Subsequently, these ingrowths into the voided spaces of

collagen graft became thicker and longer, and there was evidence of graft collagen resorption. Fibroblastic incorporation and small capillary ingrowths into grafts have also been reported in humans<sup>9</sup>. The acellular dermal graft also induced a higher lymphocytic response, in the form of lymphoid aggregates, within the fibrous tissue ingrowth and along the circumference of the graft until day 60. Similar observations were made by Chaplin *et al.*<sup>8</sup>. The graft collagen bundles remained apparently normal. The appearance of static incorporation of a porcine collagen implant by host collagen in the later stages of healing was also reported by Frankland<sup>12</sup>.

In the healing process of wounds, various tissue enzymes play a crucial role. Alkaline phosphatase (a zinc-containing enzyme) is located in the cell membranes of various cells (such as neutrophils, macrophages, giant cells and fibroblasts) of the body. Increased levels of alkaline phosphatase in traumatised areas as a result of tissue injuries have been reported to promote the proliferation of fibroblasts<sup>40</sup>. This enzyme also appears to be associated with the metabolic process of collagen formation<sup>13</sup>. Alkaline phosphatase activity gradually increased up to day 14 in group I, which is suggestive of the proliferation of fibroblasts in the early stages. Fibroblastic proliferation has been reported up to day 30<sup>13,31</sup>. In the control group, alkaline phosphatase activity in healing tissue continued to increase for up to 30 days. The alkaline phosphatase reaction was greater in some of the neutrophils in the healing area and suture site, and appeared to be associated with fibroblasts. A similar pattern of alkaline phosphatase activity was recorded by Salthouse and Williams<sup>34</sup>.

## CONCLUSIONS

The results of this study have shown that a dermal graft, after having been rendered acellular, can be used clinically for the repair of abdominal wall defects. Acellularity reduces the inflammogenicity and immunogenicity of the graft.

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