

Reconstruction of large abdominal wall defects with carbon mesh in rabbits: gross, histopathological and histochemical studies

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Successful repair of an abdominal wall defect requires closure of defect without undue tension on the sutured line. Primary closure under tension promotes wound ischemia that predisposes to dehiscence and evisceration. The animal tissues are composed of carbon compounds; since, carbon implants may be used in repair of defects in tissues. Carbon fibres are well tolerated by animal tissues (Nurse and Jenkins 1980) and have no sign of any inflammatory change in animal tissues (Tayton *et al.* 1982). Experimental studies in rats revealed that it could be used for reconstruction of abdominal wall defects (Morris *et al.* 1998). When carbon fibre is implanted into tissue, it is capable of producing dense fibrous tissue resembling tendon (Kumar *et al.* 2002). So we have studied the carbon mesh in the repair of large abdominal wall defects in rabbits.

Clinically healthy adult New Zealand white rabbits (12) of either sex were divided randomly into groups 1 and 2, having 8 animals in group 1 and 4 animals in group 2 (control). Under thiopental sodium (2.5%) anaesthesia a full thickness 2×3 cm defect in mid-ventral abdominal wall was created and immediately repaired with carbon mesh in animals of group 1. In animals of group 2, the linear abdominal muscular wall incision was made and repaired with nylon. Two rabbits from group 1 and one from group 2 were sacrificed at 7, 14, 30 and 60 days. Macroscopic observations were recorded at each time interval. The adhesions, if recorded, at the site were graded as 0–No adhesion; 1–Minimal adhesions, that could be freed by gentle blunt dissection; 2–Moderate adhesions, that could be freed by aggressive blunt dissection; 3–Dense adhesions; that required sharp dissection to free the graft from the underlying

abdominal viscera. The healing tissue was collected at time each interval for histopathological and histochemical studies.

The tissue sections were stained with haematoxylin and eosin for healing studies and with special staining, viz. Masson's trichome stain (MTS), Verhoeff's stain and Periodic Acid Schiff-Alcian blue stain (PAS) for evaluating various tissue changes at different time intervals. For alkaline phosphatase enzyme activity the cryostat sections were cut at 10–15µ thick using cryoform for matrix formation, fixed in acetone at 4°C for 5 min and stained by Gomori's method for alkaline phosphatase enzyme activity (Pearse 1968) using beta-sodium glycerophosphate as substrate. The intensity of reaction of alkaline phosphatase and its distribution in the tissue sections was rated and compared with those of healthy control.

Gross observations in group 1, on day 7 revealed that the carbon mesh was clearly visible and could be easily located. The area of repair was highly vascularized and the thin layer of granulation and fibrous tissue surrounded the carbon mesh. Adhesions were present between the carbon mesh and caecum whereas in group 2 the defect site was hyperemic and filled with granulation tissue. On day 14, in group 1 moderately strong adhesions with caecum were observed. The gap between the carbon mesh and host tissue was filled with fibrous tissue. On day 30, in group 1 adhesion seen were more or less similar to those observed on day 14. On day 60, the adhesions with caecum were minimum. The mesh was surrounded by relatively thick and dense fibrous connective tissue. Few adhesions with the skin were present at the reconstruction site. In group 2 however the adhesions were not formed with visceral organs. Presence of relatively more vascularity in group 1 suggested that the proliferation of tissue was faster in this group of animals as compared to group 2. As the healing progressed, the vascularity decreased and the granulation tissue became more organized. Peritonization began with the appearance of glistening membrane, which covered the inner surface of the material

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both at its periphery and around the adhesions to the foreign materials. Complete peritonization occurred with graft/implant by postoperative day 14 as also reported by Arnaud *et al.* (1977). The implants were completely covered by white fibrous tissue at day 60. In most of the animals in present study the adhesions between the graft and caecum were observed. Adhesions between reconstructed site and intraperitoneal organs after hernioplasty with different prosthetic materials reported by Bellon *et al.* (1996).

Histological observations at day 7, in group 1 showed that the carbon mesh was surrounded by neutrophils and macrophages. The carbon fibres had generated profuse young

fibrous connective tissue reaction within the exudate of necrotic cell debris, erythrocytes and few inflammatory cells. The supporting stroma of the muscle and its fascia revealed extensive granulation tissue along with mild infiltration of neutrophils and mononuclear cells. On day 14, the above changes became more chronic with laying of thick mature fibrous connective tissue between the junction of muscle and the mesh. The ingrowths of fibroblasts and collagen fibers were seen in stroma of degenerated and contracted muscle fibres. The reaction around the carbon mesh fibres consisted of fibroblasts and mononuclear cells followed by thick mature fibrous connective tissue. MTS sections revealed interwoven



Figs 1–8. **1.** Extensive fibroblastic reaction around the carbon sheet and within the necrosed muscle fibres. Mason's Trichome $\times 100$ (group 1, day 7). **2.** Fibrous connective tissue along with mononuclear cells around the carbon mesh. H&E. $\times 100$ (group 1, day 14). **3.** Fairly noticeable PAS positive material in the around matric of the healing tissue. PAS $\times 160$ (group 1, day 14). **4.** macrophages, foreign body giant cells and free neutrophils around the fibrils of the carbon sheet in healing tissue. H&E. $\times 250$ (group 1, day 30). **5.** Fibro cellular reaction with few mononuclear, foreign body giant cells and neocapillaries at the interface of muscle and carbon sheet. H&E. $\times 400$ (group 1, day 60). **6.** Muscle giant cells with piled up nuclei. H&E $\times 400$ (group 2, day 7). **7.** Suture fibres encircled with dense mature fibrous connective tissue and hyalinized muscle fibres. H&E $\times 100$ (group 2, day 30). **8.** Wavy and irregular collagen fibres of healing tissue. Mason's Trichome $\times 100$ (group 2, day 30).

thick collagen fibres around the carbon mesh fibres. PAS positive material was fairly noticeable at the reaction site. At day 30, scanty fibrocellular reaction with few mononuclear and foreign body giant cells was seen at the interface of muscle and the carbon sheet. The carbon mesh further encased in thick mature fibrous connective tissue. MTS sections revealed parallel collagen fibres in abundance around the mesh and in between the muscle fibre. PAS activity in the healing tissue was not very much appreciable. At day 60, the above tissue sections still persisted and not much difference was noticed. In control group, on day 7 postoperative the incision site and suture material was surrounded by healing tissue consisted of compactly arranged active fibroblasts having plump/spindle shaped elongated nuclei and pale cytoplasm and less wavy collagen fibres. The stroma showed little exudation infiltrated with moderate number of neutrophils and lymphocytes. Within the necrosed muscle fasciculi, the regeneration of muscle fibres was evident by the presence of muscle giant cells having numerous central nuclei. On day 14, tissues close to the suture material became necro-calcified. There was moderate fibroblastic tissue reaction around the suture consisted of large number of fibroblasts linearly arranged in the collagen fibres. The exudate in the matrix predominated by moderate number of neutrophils and few monocytes. On day 30, the bluish pink necro-calcified debris still persisted close to the suture fibres encircled by heavy amount of irregularly arranged active fibroblasts/fibrocytes and capillaries within the peritoneum. The stroma appeared, eosinophilic and infiltrated with mild number of neutrophils distributed focally or diffusely. The muscle fibres were apparently normal at places. On day 60, the mature wavy, dense and parallel collagen fibres with nestled inactive fibrocytes were observed in the form of thin stripes around the suture material. No inflammatory cells were visible in the stroma.

Postoperative inflammatory and healing tissue reaction mounted by the host under the influence of prosthetic implants was assessed histopathologically and histochemically. In group 2, the incised abdominal wall reconstructed by braided nylon showed moderate inflammatory reaction and laid down of mild to moderate 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 noticed with formation of mature connective tissue and muscle repair. On the contrary, in group 1, the carbon fibre implant in the rabbit abdominal wall defect had induced extensive fibrous tissue reaction around the carbon fibres and also severe muscle necrosis. The inflammatory cells were almost found negligible in the stroma, which indicates the host tissue tolerance to carbon fibers as described by Rallis and Forster (1981). The deposition of more collagen fibres in the healing tissue and chronic cell reaction adjacent to the carbon fibres/fibrils along with macrophages, few foreign

body giant cells and microgranulomas have similarly been observed by earlier workers in relation to carbon fibres and mersilene mesh (Nurse and Jenkins 1980, Cameron and Taylor 1985). Similar findings were also reported by Bellon *et al.* (1996) subsequent to repair of abdominal wall defect with PTFE and Marlex mesh. The chronic cellular reaction persisted throughout the observation period of 60 days in the form of microgranulomas and foreign body giant cells around spilled carbon fibrils, but in long term study by Rallis and Forster (1981), it has been reported that the fibrils gradually undergo disintegration. The carbon fibre itself did not induce inflammatory reaction (Tayton *et al.* 1982).

Histochemical studies on day 7, in group 1 showed that the activity of enzyme was very faint at the suture site and mild in muscles. On day 14, the activity of enzyme varied from mild to moderate which became intense on day 30. The muscle enzymatic activity did not change much. On day 60, the alkaline phosphatase activity was similar to day 30. In group 2 at day 7, the alkaline phosphatase activity in various cellular (neutrophils, macrophages, giant cells, fibroblasts) tissue elements at healing/suturing area varied from mild to moderate (+/++). Thereafter, on day 14, it increased moderately (++) in the fibrous connective tissue (fibroblast), muscle fascicles, inflammatory exudate and in between the stroma. Later it remained more or less same on day 30 and 60 at the healing site. Various tissue enzymes play a crucial role in wound-healing process. The alkaline phosphatase (a zinc-containing enzyme) is located in cell membranes of various cells (neutrophils, macrophages, giant cells, fibroblasts etc.) of the body. The increased levels of alkaline phosphatase in traumatic area as a result of tissue injuries help in the proliferation of fibroblasts (Soni *et al.* 1976). The alkaline phosphatase activity gradually increased up to 14 days and continued to increase up to 30 days in both groups. However, the intensity was more in group 1 animals. These results indicated the proliferation of fibroblast in early stages up to 30 days (Patel and Hardenbrook 1970). Comparatively lesser rise in alkaline phosphatase activity in control group might be due to lesser damage of tissues.

SUMMARY

The present study was conducted on 12 clinically healthy New Zealand white rabbits divided into group 1 and 2 having 8 and 4 animals respectively. Under thiopental sodium (2.5%) anesthesia a 2 cm × 3 cm full thickness abdominal wall defect in the mid-ventral abdominal wall was created and repaired with carbon mesh in animals of group 1. In animal of group 2 (control group), a full thickness linear midline abdominal muscular wall incision was made and repaired with a continuous suture pattern using 2-0 nylon. Gross examination revealed increased vascularity and adhesions with caecum in group 1 animals. Whereas no adhesions were seen in group 2 animals. Histopathologically, the carbon mesh implant induced extensive fibrous tissue reaction. Carbon mesh was

well tolerated by host tissue. Histochemically, increased alkaline phosphatase activity in group 1 suggested the proliferation of fibroblast in early stages.

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