Short term tissue response to carbon fibre: A preliminary *in vitro* and *in vivo* study

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Abstract. Carbon in the form of pyrolytic carbon coating is used in a number of implantable medical devices. Carbon-reinforced carbon composite and other forms of diamond-like carbon coatings are being evaluated for their many potential biomedical applications. There is also a possibility of using carbon in fibre form. Though the possibility of using the fibre form of carbon in skeletal and dental implants has been recognized, a detailed study of tissue reaction to carbon fibre has not been reported so far. In this paper, we describe *in vitro* and *in vivo* evaluation of carbon fibre in bone and muscle. Good cell and tissue biocompatibility of the material was observed in bone and muscle. New bone was present in contact with the fibres. Results of this study indicate that carbon fibre has potential in non-load bearing applications, such as skeletal repair and as ligament prosthesis.

Keywords. Carbon fibre; biocompatibility; bone.

1. Introduction

The use of fibrous materials such as braided horse hair, leather strips, and cotton fibres as suture materials was first described in the *Susrutha Samhita* 2,500 years ago (Lyman 1991). The excellent pliability of fibres enable them to be tied, woven or knitted. Nowadays, fibres are being used in extra corporeal devices (fibre reinforcements for dialysis and oxygenator membranes), and as implantables (sewing rings for prosthetic valves, heart patches etc). Carbon as filaments, yarn or fabric is one of the fibrous materials, which is currently used for tissue repair (Benson 1972; Keifer *et al* 1987; Diirsden and Claes 1989).

Carbon was considered as a suitable material for implantable prosthesis only in 1967 (Benson 1972). Technological development led to discovery of new physical forms of essentially elemental carbon (90–99.9% purity) with good physical properties and extremely low chemical reactivity. Pyrolytic carbon, carbon-reinforced carbon composites, and high strength carbon fibres are some of the wide variety of products made from carbon. Since it has a modulus and a density of 1.7–2.0 which are close to that of compact bone, carbon has been recognized as a mechanically dependable material for skeletal and dental implants (Minns *et al* 1982; Yanagisawa 1986; Minns and Muckle 1989). Its anti-thrombogenicity (Yanagisawa 1986) and non-antigenicity (Lymann 1991) confer on it additional advantages for its use in soft

tissue and hard tissue. However, detailed biocompatibility studies of carbon fibres have not been reported.

2. Experimental

Carbon fibres were obtained from the Defence Research and Development Laboratory, Hyderabad. The fibres contained 93% carbon content.

Pieces of fibres 1 cm in length were cleaned thoroughly in an ultrasonic bath with distilled water (two changes of 10 min each) and sterilized by autoclaving. Some of the fibres were gold coated in an ion sputter and surface characterization was carried out by viewing under a scanning electron microscope (SEM) prior to the implantation (figure 1). The morphological changes after the implantation were also observed with SEM.

3. Methods

3.1 *In vitro* study

Cytotoxicity studies of materials were carried out using L929 fibroblasts which were obtained from the National Centre for Cell Science (NCCS). The cells were grown in Eagles medium containing streptomycin and penicillin and supplemented with 10% calf serum. Single strands of sterilized carbon fibres were placed on a monolayer of cells and 24 h later, morphology of cells was examined under phase contrast microscopy (Leitz Model DMIL).

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Osteoblast responses to carbon were investigated using SEM. Single strands of carbon fibres were fixed on to cover slips with sterile, inert silastic glue. An appropriate number of MG 63 osteoblasts obtained from NCCS were laid on to the material. After 8 days, cells and material were fixed in 1% glutaraldehyde in phosphate buffer and dehydrated in acetone. Drying was carried out in a critical point dryer, and the specimen was gold coated in an ion sputter and examined under a SEM.

3.2 *In vivo studies*

Two adult mongrel dogs were used. Dogs were quarantined prior to the experiment. Soft and hard tissue response were evaluated in the dorsolateral skeletal muscle (this is one of the sites used conventionally for evaluation of biomaterials (Turner 1973) and in the forearm and hind leg long bones, respectively. Animals were pre-medicated with 0.05 mg/kg of atropine sulphate (Bengal Immunity Ltd., Calcutta) and 0.5 mg/kg of Diazepam (Ranbaxy, Thane) intramuscularly. They were anaesthetized using a combination of 2 mg/kg of xylazine (India Immunological, Hyderabad) and 15 mg/kg of ketamine hydrochloride (Ketmin 50: Themes Chemicals Ltd., Hyderabad) intramuscularly. The dorsolateral surface along the left lateral edge of spine was prepared for intramuscular implantation. Skin and fascia were incised longitudinally and muscles were separated along fascial lines. The sterilized implant was dipped in alcohol and placed deep within the muscle. Muscle, fascia and skin were sutured. A sham incision was also made at a separate site. For implantation in bone, the antero-lateral aspect of the skin over the bone was shaved and cleaned with ethanol. A 5–10 mm skin incision was made in close proximity to the diaphysis. The periosteum was elevated gently. Transcortical holes were drilled into the

bone using a slow speed dental handpiece fitted with a round burr, under constant cold saline irrigation. Holes were 5 mm in diameter, 1 cm in depth and 1 cm apart. Fibres were tightly packed into the defect. The surgical site was flushed with saline. Muscle and skin were sutured with silk.

The two animals were killed after one and six months, respectively following implantation. The implant with surrounding skeletal muscle was removed. Part of the specimen was fixed in a 3% glutaraldehyde in phosphate buffer for scanning microscopic studies. The rest of the specimen was fixed in 10% buffered formalin for light microscopy (LM). The whole bone was removed at both joints. One inch blocks of bone with implant were cut on a diamond disc (ISOMET, Beuhler). Parallel sections of 1 cm thickness were cut, fixed and processed separately for viewing under SEM and LM.

Soft and hard tissues for SEM were washed in phosphate buffered saline, post-fixed in 1% osmium tetroxide

Figure 1. Surface morphology of carbon fibres prior to implantation ($\times 1.0$ K, 12 KV).

Figure 2. Phase contrast photomicrograph of **a.** L 929 cells and **b.** osteoblasts with carbon fibre. Cells have normal morphology ($\times 100$).

Figure 3. Photomicrograph of tissue reaction to carbon fibres implanted for six months in dog skeletal muscle. Macrophages (*); fibroblasts (◆) and foreign body type of giant cells (←) are seen. Haematoxylin-Eosin ($\times 300$).

in phosphate buffer and dehydrated in graded concentrations of acetone. Critical point drying with carbon dioxide was carried out in a HCP-2 CPD at a pressure of 77 mm Hg, 72.8 atmosphere and 31°C. Samples were mounted on aluminium stubs, sputtered with gold in a HITACHI EIOI ion sputter and examined in a Hitachi SEM. Decalcified and undecalcified sections of bone were prepared for light microscopy. Decalcification was carried out in 10% formic acid with constant agitation. Point of decalcification was checked chemically and decalcified tissues were processed similar to soft tissue specimens. Briefly, samples were dehydrated in graded concentrations of alcohol, cleared in chloroform, and embedded in paraffin. Five microns thick sections were sectioned, stained with haematoxylin and eosin, and viewed under a NIKON Optiphot light microscope. Undecalcified sections were dehydrated in ascending grades of alcohol, infiltrated with poly methyl methacrylate (PMMA) and embedded in PMMA in vacuum. Sections 300 μm thick, were sectioned on a diamond disc, ground down to a thickness of 275 μm on a 400 grit aluminium carbide paper and polished with aluminium oxide powder to a final thickness of 150 μm . Sections were stained by Steventhal's Blue and examined under LM.

4. Results

4.1 Cytotoxicity studies

Normal cellular morphology was observed in most of the fibroblasts placed on the material, when examined

after 24 h (figure 2a). Osteoblasts in direct contact with the carbon fibres for eight days had normal morphological features (figure 2b). The osteoblasts showed excellent contact with the fibre when viewed under SEM.

4.2 *In vivo* studies

4.2a Soft tissue response: At one month following the implantation, the fibres were seen well circumscribed in the skeletal muscle. A mild chronic inflammatory response and evidence of repair were evident around cross sections of the fibres. The cellular infiltrate was composed of macrophages, lymphocytes, fibroblasts and fibrocytes. Foreign body type of giant cells were also observed. There was no evidence of necrosis or acute inflammation in the surrounding muscles. At 6 months a mild degree of chronic inflammation and evidence of repair persisted (figure 3). Under SEM, carbon fibres were seen to maintain their morphology. Cells and a layer of extracellular matrix were observed in close approximation at one and six months (figures 4a and b).

4.2b Hard tissue response: At one and six months post implantation, formation of new woven bone around bundles of carbon fibres was evident (figure 5a). Intensely stained long trabeculae of osteoid (figure 5b) and collagen fibres (figure 5c) were seen in between carbon fibres. Osteoid was in close contact with carbon in some areas (figure 6a) and in other areas it was separated by collagen (figure 6b). SEM photographs show the fibres surrounded

by extracellular matrix (figure 7a) as well as accretions (possibly osteoid) (figure 7b).

5. Discussion

In this experimental study, the biocompatibility of carbon fibres has been studied in terms of its cytotoxicity, and soft and hard tissue response to the material.

Two cell types viz. fibroblasts, and osteoblasts, were used in the *in vitro* studies. Both are known to have a similar ontogeny. Fibroblasts are one of the early cells to appear in response to wound healing in both soft and hard tissue, and osteoblasts are the cells responsible for osteoid formation. The lack of cytotoxicity in both the types of cells, following close contact with the material, and preservation of their normal and cellular morphology suggest that carbon fibres are non-cytotoxic. Osteoblasts grown on the carbon fibre had normal morphological

features after 8 days. Future work is necessary to assess the functional status of these cells in long-term cultures. Histological evaluation of soft tissue response to the fibres showed the expected mononuclear cellular response at one and six months. Degree of response is known to depend on the form of the material, the response being

Figure 4. Scanning electron micrograph of carbon fibres implanted in skeletal muscle for **a.** one month and **b.** six months. Cells and extracellular matrix are observed.

Figure 5. Photomicrographs of undecalcified sections of bone showing deposition of woven bone (*) in contact with **a.** carbon, **b.** trabeculae of osteoid (→) and **c.** fibres of collagen in between the carbon fibres (*). Steventhal's blue stain (x350).

more to braided forms than around monofilaments (Lord 1986). Presence of foreign body type of giant cells and fragments of carbon fibres, fibre disintegration and production of wear debris from carbon fibre-reinforced composites have been demonstrated earlier in case of carbon fibre ligament prosthesis (Jenkins 1977; Wolter *et al* 1977; Rushton *et al* 1983). Phagocytosis of these fragments by macrophages and their transport by lymphatic

and vascular system to remote tissues, resulting in chronic inflammation is known (Foster *et al* 1978; Neugebauer *et al* 1981; Tayton *et al* 1982; Brandwood *et al* 1992). Persistent chronic inflammation around these fragments may lead to a vicious cycle of recruitment of more cells, more cytokine production and more fibre degradation. Long-term studies on the relevant draining lymph nodes, pulmonary, splenic and hepatic tissues need to be

Figure 6. Photomicrographs of undecalcified sections of bone. **a.** Osteoid deposition at the carbon interface (+) and **b.** collagen between new bone and carbon fibres (←). Steventhal's Blue ($\times 350$).

Figure 7. Scanning electron microphotographs of carbon fibres implanted in bone for six months. **a.** Layers of extracellular matrix enveloping individual as well as groups of carbon fibres and **b.** localized accretions are also observed.

performed. Fibres with high purity and high strength may show less degradation in tissues.

Response of hard tissue to injury or implantation usually involves a series of processes with clot formation, inflammation, fibrous tissue invasion, osteoid formation and mineralization. However, the successful repair of the tissues depends to a large extent on type of injury and post-injury treatment, as well as type of material used in case of implants with evidence of remodelling. In the latter, the interface between the host bone and implant may be fibrous tissue or bone. In the present experiment, the picture varied in different areas of bone surrounding the implant. Collagen was prominent in between individual fibres. Newly formed osteoid was also found in close contact with the fibres. Trabeculae

of mineralized bone were also noted extending from the surrounding compact bone in between carbon fibres. These features point to the probable good bone carbon fibre contact at 6 months.

6. Conclusion

The results indicate that there is no adverse tissue response to the carbon fibre upto six months. The biocompatibility needs to be validated by long term implantation studies for specific applications.

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