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Characterization and biological behavior of a carbon fiber/carbon composite scaffold with a porous surface for bone tissue reconstruction

CAO Ning[1], WANG Qiang-xiu[2], DONG Jian-wen[3], HAO Guang-zheng[4], LI Mu-sen[1]

(1. *Key Laboratory for Liquid-Solid Structural Evolution and Processing of Materials (Ministry of Education), Shandong University, Jinan* 250061, *China*;
2. *Department of Pathology, Provincial Hospital Affiliated to Shandong University, Jinan* 250021, *China*;
3. *The Second Affiliated Hospital, Shandong University of Chinese Traditional Medicine, Jinan* 250001, *China*;
4. *Yantai Metallury Institute of New Materials, Yantai* 264006, *China*)

Abstract: A carbon/carbon composite scaffold for bone tissue reconstruction was prepared. The surface morphology and trace elements of the scaffold were analyzed and its biological behavior was studied both *in vitro* and *in vitro*. It was found that the scaffold had a good biocompatibility, not only resulting from its high purity and mild cell toxicity, but also from the excellent integration of the bone tissue with the composite scaffold during the reconstruction.

Keywords: Microstructure; C/C composites; Biocompatibility; Bone

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1 Introduction

Carbon fiber reinforced carbon composites (CFRC) consist of reinforcing carbon-fiber skeletons and a homogeneous carbon matrix[1]. They have great potential as substitute for human bone material because of their similar elastic modulus (Table 1) to cortical bone. A CFRC implant has better biomechanical performance than an internal fixation cortical plate for bone fracture because it allows elastic deformations at the fracture site, which may benefit the bone tissue reconstruction. Owing to its small atom number and low density, CFRC is radiolucent, thus allowing a visualization of new bone formation when used as scaffold material for tissue reconstruction. CFRC can also be used in artificial hip joints, intervertebral cages, and tooth roots for its excellent biocompatibility[2-3]. Earlier researches indicate that the surface morphology and chemical state of the biomaterials play important roles on the interaction at the interface between their surfaces and living tissue cells[4-6].

Table 1 Comparison of elastic modulus of CFRC and some commonly used biomaterials with human bone[7-9]

Materials	CFRC	Pure titanium	Ti6Al4V alloy	316L stainless steel	CoCrMo alloy	Human bone
Elastic modulus *E*/GPa	45-47	102.7-103.4	101-114	200	200-230	10-40

2 Experimental

2.1 Sample preparation

In this study, polyacrylonitrile-based CFRC was prepared using chemical vapor deposition and then processed into open-box scaffolds through machining, the size of which is 6×9×13 mm (Fig. 1). The surface of the scaffold was made porous through carborundum sand (average grit size 11 μm) blasting. All the scaffolds were ultrasonically cleaned in deionized water, acetone, and ethanol for 30 s.

2.2 Material characterization and *in vitro* cell coculture test

The surface morphology of the scaffolds was observed using scanning electron microscopy (SEM)

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Corresponding author: LI Mu-sen, Professor. E-mail: msli@sdu.edu.cn

Author introduction: CAO Ning(1982-), male, Doctor Student, engaged in research of biomedical material. E-mail: caoning1982@mail.sdu.edu.cn

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with an acceleration voltage of 20 kV. And the deleterious trace elements in the CFRC were measured by X-ray atomic fluorescence analyzer (S/MAX3080B, Rigaku Company, Japan).

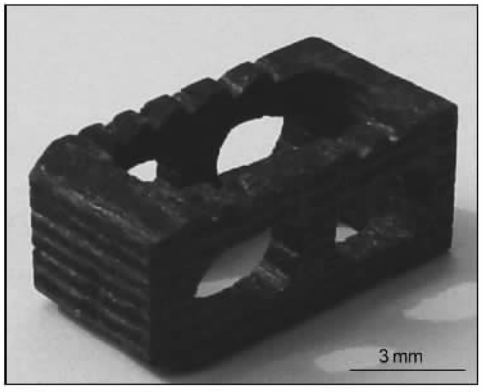

Fig. 1　The scaffold made of CFRC

The cell compatibility of CFRC was evaluated by means of coculture with L929 fibroblasts. The experiment selected the alumina ceramic and poly(vinylidene chloride) (PVC) as negative and positive contrast materials, respectively. Three parallel test specimens and contrast parameters were adopted.

2.3 *In vivo* test and histological observation

Two healthy male hybrid goats (50-60 kg) were selected as animal experimental models. An autogenous bone filled scaffold was implanted into the lumbar of each goat under sterilized conditions. Euthanasia was conducted for the goats at the 60th day and the scaffolds were taken out from the surrounding tissues. After dehydration, embedded with methyl methacrylate, the cross-sections (80 μm thick each) of the scaffolds were made by a LEICA sp1600 saw microtome (Leica Biosystems Nussloch GmbH, Germany) and stained with both methylene blue and hematoxylin and eosin (HE). The histological observation was carried out using the optical microscope (Olympus B51, Japan).

3 Results and discussion

3.1 Impurity contents and cell compatibility evaluation

Commonly, CFRC constitutes more than 99% of carbon and less than 1% of impurities by weight. Therefore, these materials have an excellent chemical stability and are biologically inert[10-11]. According to ASTM F1185-03 standard, as scaffold materials for tissue engineering, the species and concentrations of the deleterious elements in CFRC should be limited as follows: As ≤ 3 mg/kg, Cd ≤ 5 mg/kg, Hg ≤ 5 mg/kg, and Pb ≤ 30 mg/kg. However, the results indicated that the concentration of As, Cd, Hg, and Pd was 0.004 9, ≤0.10, 0.009 1, and 1.8 mg/kg, respectively, which can completely meet the requirement of the standard mentioned.

The cell compatibility test results indicated that more than 90% of the cells could survive for 24 h. The CFRC was evaluated to have a mild cell toxicity in accordance with the standard for biological evaluation of medical devices (ISO 10993-5-1999).

3.2 Radiograph evaluation

Compared with the observation before surgery, radiological fusion was definitely inside and outside the scaffold on standard radiographs on the 60th day post-implantation. For the low atomic weight of carbon element, the implant is radiolucent. Moreover, the adjacent vertebral endplates were fixed by the calcified anterior longitudinal ligament (Fig. 2).

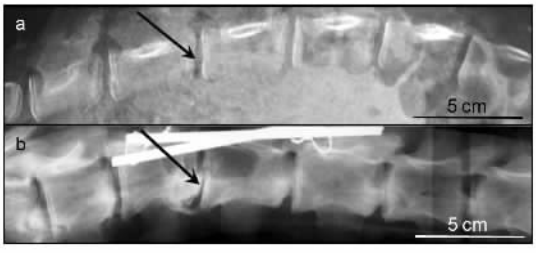

Fig. 2　Radiological images of the experimental animal intervertebral bone (a) before surgery and (b) on the 60th day post-implantations. (Arrowheads pointed to the implantation position.)

3.3 Histological observation and microstructure of the scaffolds of in vivo test

Fig. 3 shows the optical observation of histological cross-sections on the 60th day. It was demonstrated that the tissue, which was in direct contact with the scaffold, was a fibrous tissue about 50 μm thick. The tissue contained many unevenly distributed small-sized carbon particles. No inflammatory cell infiltration or remarkable lymphocyte accumulation was observed[12-14]. It was revealed that although the thickness of the tissue around CFRC was similar to that of the metal implant[15-16], CFRC formed a tighter bonding with the tissues than the metal implants did. The bone tissue reconstruction occurred between the filled autogenous bone and the original lumbar cancellous bone. The newly formed trabecula in the scaffold was observed to be connected to the original lumbar trabecula at some places. The original lumbar trabeculas were arrayed along the stress direction and the growth direction of new bone trabeculas in the autogenous bone powders were at random, which indicated that the scaffold had an obvious shear support function during the 60 days. In the methylene blue stained samples, new lamellar bone was found to be formed

between the surface of scaffolds and the fiber tissue, and osteoblasts proliferated on the surface of the new bone trabecula.

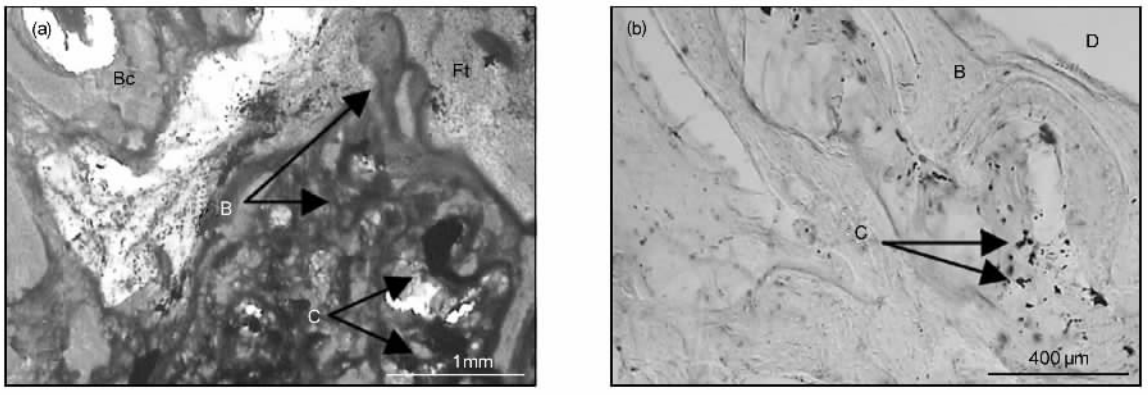

Fig. 3　The optical observation of nondecalcified histological cross-sections on the 60th day. (a) HE-stained, 40, (b) methylene blue stained, 100. B: newly formed bone; Bc: cancellous bone; Ft: fibrous tissue; C: carbon particle; D: the position of the CFRC scaffold.

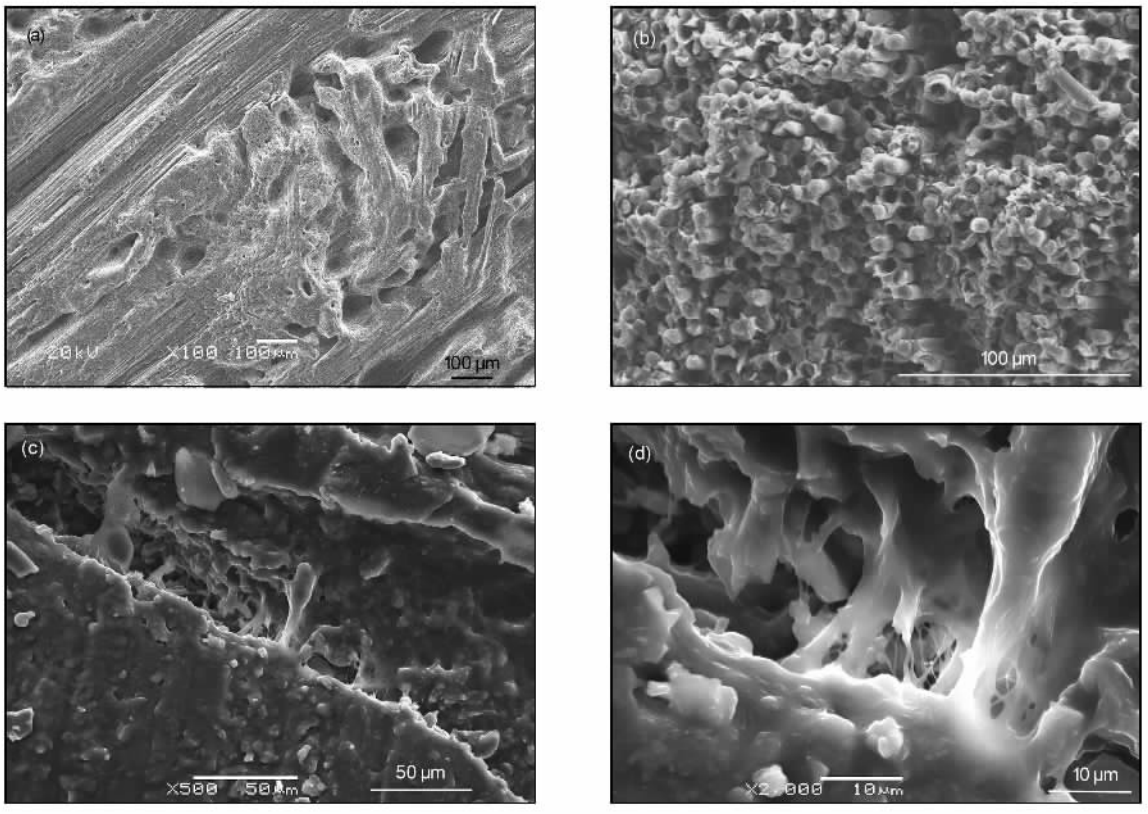

Fig. 4　The SEM observation of the CFRC surface and the interface between CFRC and the grafted autogenous bone on the 60th day after the implantation. (a) porous structure of CFRC, arrowheads indicate carbon fiber bubbles and porous surface structure. 100, (b) carbon fiber exposure on the surface, 500. (c, d) the interface between CFRC and the grafted autogenous bone

The SEM morphology indicated that the porous surface structure was characterized by a fiber exposure and tissue penetration on the interface between CFRC and the grafted autogenous bone during the 60 days. The micropore sizes were from 10 to 100 μm, with a high porosity at CFRC surface. It has been reported that the high-porosity biomaterial is more conducive to bone growth than the low-porosity one[17]. Upon implantation, the porous biomaterial exhibits strong bonding performance and superior osteoconduction because the pores contribute to mechanical interlock, which result in a firmer fixation with tissue in vivo[18]. Also, the porous surface structure of scaffold material is beneficial to a direct adhesion, proliferation, and differentiation of cells[19-20]. This could be why a high interface bonding strength could be

achieved between the CFRC surface and the bone tissue. Furthermore, the quantity and density of newly formed bone around the scaffolds can be improved. In Fig. 4c, we found that, in some places, osteoplasts were tightly adhered to the carbon fiber surface by the agency of cell pseudopodiums, which grew into the pores. The grafted autogenous bone was connected to the scaffold through the osteoplast adhesion at a high cell-adhesion ratio. Radiate bone matrix could be seen among the cells under a higher magnification, which indicated that the osteoplasts were at a functionally vigorous stage and both the proliferation and differentiation were active.

4 Conclusions

Both in vitro and in vivo tests revealed that the CFRC scaffold had a good biocompatibility, which resulted from a mild cell toxicity, high purity, and excellent integration of the bone with the CFRC scaffold during the bone reconstruction. The porous surface structure was greatly beneficial for a direct adhesion, proliferation, and differentiation of cells. A strong mechanical interlock might be formed between CFRC and the cells formed within its surface pores.

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表面多孔炭/炭复合材料骨组织改建支架的生物学性能及其表征

曹　宁[1]，王强修[2]，董建文[3]，郝广政[4]，李木森[1]

（1. 山东大学 材料液固结构演变与加工教育部重点实验室，山东 济南 250061；
2. 山东大学 附属省立医院病理科，山东 济南 250021；
3. 山东中医药大学 第二附属医院，山东 济南 250001；
4. 烟台冶金新材料研究所，山东 烟台 264006）

摘　要： 采用炭/炭复合材料制备了骨组织改建支架，并对所制支架进行了表面形貌观察、痕量元素分析和体内生物学性能评价。结果表明：所制支架能够有效实现对自体移植骨的支撑和骨性融合，生物相容性良好。亦即，表面多孔炭/炭复合材料能够满足作为骨组织改建支架材料的成分要求。

关键词： 微观结构；炭/炭复合材料；生物相容性；骨

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通讯作者：李木森，教授. E-mail：msli@ sdu. edu. cn

作者介绍：曹　宁（1982 – ），男，山东滨州人，博士研究生，主要从事生物医用材料的研究. E-mail：caoning1982@ mail. sdu. edu. cn

2008 年材料科学类期刊影响因子排序表

序号	刊名	影响因子	序号	刊名	影响因子
1	**新型炭材料**	**1.000**	19	材料科学与工艺	0.373
2	中国稀土学报	1.000	20	航空材料学报	0.368
3	复合材料学报	0.854	21	材料热处理学报	0.366
4	无机材料学报	0.788	22	耐火材料	0.363
5	JOURNAL OF RARE EARTHS	0.758	23	材料保护	0.351
6	材料导报	0.681	24	合成材料老化与应用	0.330
7	玻璃钢/复合材料	0.680	25	宇航材料工艺	0.323
8	稀有金属材料与工程	0.574	26	材料科学与工程学报	0.313
9	稀有金属	0.571	27	兵器材料科学与工程	0.309
10	中国腐蚀与防护学报	0.569	28	腐蚀与防护	0.308
11	材料工程	0.555	29	稀土	0.306
12	高分子材料科学与工程	0.537	30	腐蚀科学与防护技术	0.280
13	钛工业进展	0.535	31	机械工程材料	0.259
14	材料研究学报	0.519	32	材料开发与应用	0.255
15	功能材料	0.489	33	磁性材料及器件	0.233
16	贵金属	0.474	34	金属功能材料	0.209
17	JOURNAL OF MATERIALS SCIENCE & TECHNOLOGY	0.451	35	功能材料与器件学报	0.204
18	绝缘材料	0.416	36	JOURNAL OF WUHAN UNIVERSITY OF TECHNOLOGY MATERIALS SCIENCE EDITION	0.193

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