Technical Note

Enhanced functions of osteoblasts on nanostructured surfaces of carbon and alumina

R. L. Price K. M. Haberstroh T. J. Webster

Department of Biomedical Engineering, Purdue University, West Lafayette, IN, USA

Abstract—*It is of the utmost importance to increase the activity of bone cells on the surface of materials used in the design of orthopaedic implants. Increased activity of such cells can promote either integration of these materials into surrounding bone or complete replacement with naturally produced bone if biodegradable materials are used. Osteoblasts are bone-producing cells and, for that reason, are the cells of interest in initial studies of new orthopaedic implants. If these cells are functioning normally, they lay down bone matrix onto both existing bone and prosthetic materials implanted into the body. It is generally accepted that a successful material should enhance osteoblast function, leading to more bone deposition and, consequently, increased strength of the interface between the material and juxtaposed bone. The present study provided the first evidence of greater osteoblast function on carbon and alumina formulations that mimic the nano-dimensional crystal geometry of hydroxyapatite found in bone.*

Keywords—*Adhesion, Nanomaterials, Carbon fibres, Alumina*

Med. Biol. Eng. Comput., May 2003, **41**, 372–375

1 History of the orthopaedic implant

PRIOR TO a few years ago, the top priority for an orthopaedic implant was to possess mechanical properties that could support normal physiological loading events. For example, the first total hip replacements were made of steel and did not take many of the individual needs of the patient (such as immune response, cytocompatibility, foreign body response, amount of physiological activity, age etc.) into account. Needless to say, these implants were successful for only a limited number of years (PARK and LAKES, 1992).

Frequently, problems with orthopaedic implants occur owing to either loosening of the implant, osseo-degradation of bone surrounding the implant or hypersensitivity of the patient to the particular metal (initially steel, but, in recent years, titanium or cobalt chromium alloys) that formed the implant (PARK and LAKES, 1992). All of these conditions usually lead to acute pain for the patient that can only be alleviated by removal of the failed implant and surgical re-insertion of a new bone prosthesis. As revision surgeries require a large amount of healthy bone removal, most people can only undergo one such replacement. This finite number of allowable surgeries makes it imperative for younger, more active patients with bone and joint problems to have an implant that will last for 20–60 years or more. This problem has driven engineers and scientists to investigate possible improvements to traditional materials and to design new materials for orthopaedic implants.

To begin with, the materials used today (namely, titanium and cobalt chromium alloys) are lighter and stronger than those used in the first hip implants (PARK and LAKES, 1992). The change in materials, along with several other modifications to the initial design, has increased the average lifetime of an implant, but still not satisfactorily.

Eventually, modifications became more innovative, and several coatings have been attempted. For example, compelling *in vitro* data have been provided by several investigators describing the benefits of protein, hydroxyapatite, peptide sequences and other bio-active surface chemistries as implant coatings (DEE *et al.*, 1996; WEBSTER, 2001). These studies provided evidence that osteoblast responses to many surface modifications are favourable (DEE *et al.*, 1996). For example, *in vivo*, hydroxyapatite-coated implants have been successful for the most part. It has been found that bone apposition on the surface of porous hydroxyapatite-coated implants is better than that on metallic porous coatings (COATHUP *et al.*, 2001).

However, numerous problems are still encountered with these approaches. For example, the average lifetime of hydroxyapatite-coated implants needs improvement, owing to high solubility rates that can lead to degradation of the coating and loosening at the bone–hydroxyapatite interface (OGISO *et al.*, 1998). Similarly, the efficacy of protein and/or peptide coatings remains to be realised, owing to the macromolecular interactions that can sterically hinder these coatings from being in direct contact with osteoblasts once implanted (ANDERSON *et al.*, 1996). It is therefore necessary to continue the search for a material surface that will effectively allow for increased osteoblast function.

Correspondence should be addressed to Dr Thomas J. Webster; email: twebster@ecn.purdue.edu

Paper received 27 March 2002 and in final form 17 January 2003

MBEC online number: 20033773

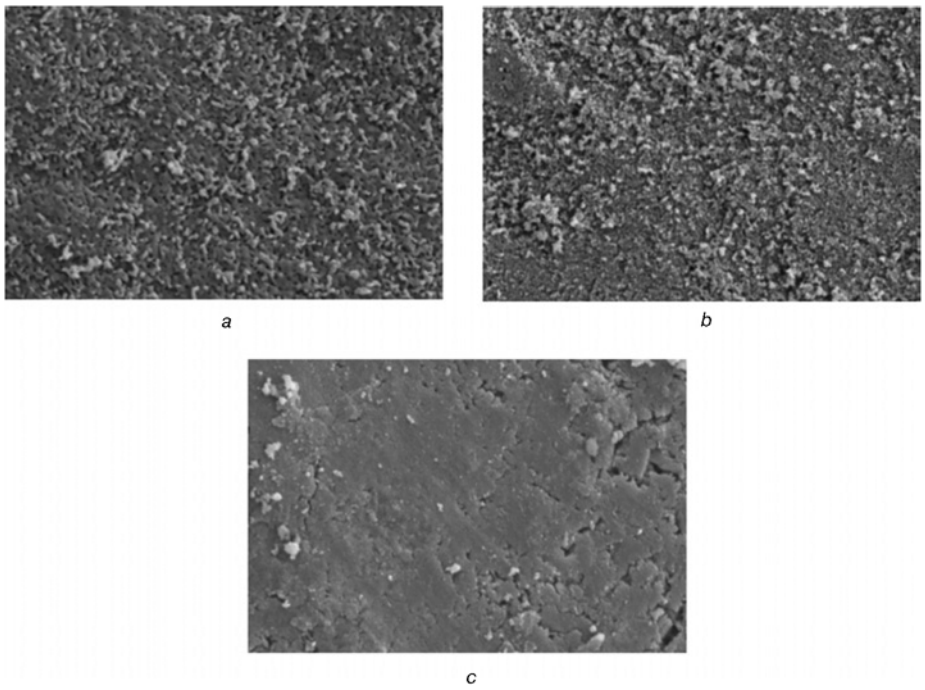


Fig. 1 *Alumina particle compacts at 10 000× magnification (scale bar — = 1 μm). Fibres* and spherical grain sizes† were pressed with uni-axial pressing cycle (0.2 GPa–1 GPa over 10 min period) at room temperature. (a) Spherical alumina particles with nanometre (23 nm) grain sizes. (b) Spherical alumina particles with conventional (167 nm) grain sizes. (c) Alumina fibres 2 nm in diameter and around 50 nm in length*

2 Benefits of nanostructured materials

Current pro-active materials used for bone prostheses have surface roughness values on the micrometre scale. In contrast, the sizes of the inorganic particles in natural bone are in the nanometre range. This provides a nanostructured surface roughness for bone cell function *in vivo*. For this reason, progress has been made in the past few years using a whole new line of materials for overall implant design and orthopaedic/dental research, i.e. nanostructured materials (that is, materials possessing constitutive components with dimensions less than 100 nm) (MA and ZHANG, 1999; WEBSTER, 2001).

2.1 *Nanometre spherical-grain ceramic substrates*

In these studies, ceramics such as alumina, titania and hydroxyapatite were synthesised to produce grains (which are separated by grain boundaries or the edge planes of groups of atoms) that were nanometres in dimension as opposed to conventional ceramics that possess micrometre-sized grains (WEBSTER *et al.*, 2001*b*). Nanophase and larger, conventional, spherical grain size alumina compacts are shown in Figs 1*a* and *b*, respectively. Osteoblast studies indicated that a decrease in the grain size of these ceramics correlated with an increase in adhesion, proliferation, alkaline phosphatase activity and calcium deposition (WEBSTER *et al.*, 2001*a*; *b*; WEBSTER, 2001). These exciting results suggested that osteoblasts prefer a nanostructured surface roughness. Moreover, these cells continue to function normally and produce bone matrix components at a higher level on nanophase ceramics compared with on conventional ceramics with micrometre-sized grains (WEBSTER *et al.*, 2001*a*; *b*; WEBSTER, 2001).

Competitive cell lines (fibroblasts, which are cells that produce soft tissue that can encapsulate an implant, and endothelial cells, which compete with osteoblasts for implant space and available nutrients) were also tested for adhesion to the nano-sized grain ceramics. However, it was found that increased nanometre-surface roughness had a negative effect on competitive cell function compared with respective conventional grain size ceramic formulations. In this manner, ceramics with grain sizes less than 100 nm represent truly unique materials that selectively enhance osteoblast functions without delicate, protein-based surface modifications.

2.2 *Fibre substrates*

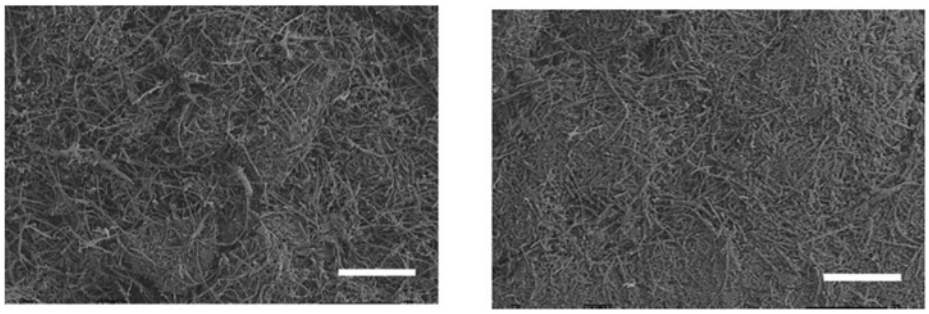
The previously mentioned nanostructured materials have nanospherical grain sizes and shapes. However, the main component of naturally occurring bone is hydroxyapatite fibres that possess dimensions between 2 and 5 nm in width and lengths of around 50 nm (KAPLAN, 1994). Nanospherical particles, although mimicking nanometre dimensions of bone, do not accurately depict the fibre-like forms that come into contact with the cellular component of bone. Therefore studies similar to those mentioned previously were performed on alumina and carbon fibres, with diameters of 2 nm and 60–125 nm, respectively, to attempt to determine if this geometric similarity would have an effect on osteoblast function. A nanofibre alumina compact is shown in Fig. 1*c*. Conventional and nanofibre carbon compacts are shown in Figs 2*a* and *b*, respectively. The data presented in this manuscript have been discussed separately in previous works (PRICE *et al.*, 2001; GUTWEIN *et al.*, 2002); however, this is the first attempt to combine, compare and contrast the results on similar dimension, yet chemically different, bone prosthetic materials.

3 Materials and methods

3.1 *Materials*

Alumina nanofibres, approximately 2–4 nm in diameter and nm in length, were synthesised* using proprietary sol-gel synthesis, followed by ageing, filtration and subsequent drying and heat treatment. The fibres used were heat treated at 400°C. The resultant crystals were mostly boehmite alumina (AlOOH) (TEPPER *et al.*, 2001). Alumina nanofibre powders were compacted serially in a steel-tool die via a uni-axial pressing

*By the Argonide Corporation
†Nanophase Technologies, Corp.



a b

Fig. 2 *Carbon nanofibre compacts at 2500× magnification (scale bar — = 10 μm). Nanofibres* were pressed with uni-axial pressing cycle at 27.5 MPa of pressure for 2 min at room temperature. (a) Conventional-sized 125 nm-diameter fibres. (b) Nano-sized 60 nm-diameter fibres*

**Applied Sciences, Inc.*

Table 1 Increased osteoblast adhesion on alumina and carbon nanofibre substrates

Substrate	Average cell density, cells cm^{-2}	Standard error of mean (SEM)	*p*-value (compared with respective nanofibre material)
conventional alumina	917	39	0.003
nanosphere alumina	1557	52	0.043
nanofibre alumina	1805	37	
conventional carbon fibre (125 nm)	1340	325	0.151
nanophase carbon fibre (60 nm)	1796	105	

cycle (0.2–1 GPa over a 10 min period) at room temperature. Resulting compacts were approximately 1–1.5 mm thick and 1.25 cm in diameter. Compacts were autoclaved for sterilisation purposes at 250°C for 30 min.

Carbon fibres†, synthesised using chemical vapour deposition, were obtained, with diameters ranging from 60 to 125 nm. These fibres were grown using an iron catalyst (introduced as a gas) with small amounts of sulphur to increase production and a hydrocarbon gas (namely methane), at temperatures of between 600 and 1100°C depending on the gases used (RODRIGUEZ, 1993). The fibres were pressed uni-axially for 2 min at 6 t of pressure, at room temperature, in a stainless-steel die, and were sterilised by exposure to UV light for 1 h prior to cell experiments. The resulting compacts were approximately 1 mm thick and 1.25 cm in diameter.

3.2 *Cells*

Human osteoblasts (bone-forming cells; CRL-11372 American Type Culture collection, population numbers 3–16) in Dulbecco's modified eagle medium‡ (DMEM) supplemented with 10% fetal bovine serum** (FBS) and 1% penicillin/streptomycin** were separately seeded at a density of 3500 cells cm^{-2} on carbon fibres and 2500 cells cm^{-2} on alumina fibres and placed in standard cell culture conditions (that is, a humidified 5% CO_2/95% air environment) for a period of 1 h for carbon fibres and 2 h for alumina fibres.

After the prescribed time period, substrates were rinsed in phosphate buffered saline (PBS) to remove any non-adherent cells. The remaining cells were fixed with formaldehyde, stained with Hoescht 33258 dye†† and counted under a fluorescent microscope.

All experiments were run in triplicate and repeated on at least three separate occasions. Numerical data were analysed using one-tailed, paired standard Student *t*-test techniques (MONTGOMERY, 1991). A standard normal distribution was assumed for all data.

†Applied Sciences, Inc., Cedarville, OH, USA

‡Gibco

**Hyclone

††Sigma

3.3 *Alumina*

Adhesion studies of osteoblasts on alumina nanofibres sintered at 400°C showed increased numbers of osteoblasts compared with spherical alumina with either nanometre-sized or micrometre-sized grains, as seen in Table 1 (GUTWEIN *et al.*, 2002). Specifically, after 2 h, about 1805 osteoblasts cm^{-2} were counted on the surfaces of alumina fibres, compared with 1557 cells cm^{-2} for alumina compacts with spherical nanometre grain sizes and 917 cells cm^{-2} for alumina compacts with spherical conventional sized grains. Although these results could not be linked to the shape of the particles exclusively, owing to changes in the surface chemistry during the sintering process (nanospherical alumina was gamma-phase whereas nano-fibre alumina was boehmite phase), the data are still useful when considered for the first time in conjunction with the following carbon nanofibre data.

3.4 *Carbon*

Osteoblast function was also analysed on carbon with nanometre and conventional fibre dimensions (PRICE *et al.*, 2001). These results showed an increase in the number of adherent osteoblasts on such fibres compared with titanium and cobalt chromium alloy substrates. More importantly, this study also provided evidence that the smaller-dimension carbon fibres had the largest number of adherent osteoblasts after 1 h (as shown in Table 1). Specifically, those with 60 nm diameters (nanophase) had an average of 1796 adherent osteoblasts cm^{-2}, whereas 125 nm diameter (conventional) carbon fibres had an average of 1340 cells cm^{-2} adherent osteoblasts after 1 h. At the same time, the adhesion of competitive cells (chondrocytes, which are cartilage-producing cells, fibroblasts and smooth muscle cells, which compete with osteoblasts for implant space and available nutrients) was not significantly affected by the dimension of the carbon fibres. The surface areas of the two carbon fibre materials were 52.6 $m^2 g^{-1}$ for the 60 nm carbon fibre compacts and

$62.2\ m^2\ g^{-1}$ for the conventional (125 nm diameter) carbon fibre (ELIAS *et al.*, 2001). These results provide evidence that the surface area differences are not responsible for the observed change in osteoblast adhesion. Further study of osteoblast function provided evidence that proliferation, alkaline phosphatase activity and calcium deposition were increased on the smaller-diameter carbon nanofibre compacts as well (PRICE *et al.*, 2001; ELIAS *et al.*, 2001).

4 Discussion

Previous findings have shown that osteoblasts, fibroblasts, smooth muscle cells, chondrocytes and endothelial cells all respond to differences in nanometre compared with conventional surface roughness (KAY *et al.*, 2002; MILLER *et al.*, 2002; THAPA *et al.*, 2002; WEBSTER, 2001). These results appear to be virtually independent of the material chemistry used, whether ceramic, polymer, carbon or composites of these materials. In particular, despite variations in shape, surface chemistry and composition, osteoblast functions were consistently enhanced on alumina and carbon fibre materials, which extends to other materials as well, with smaller nanometre surface dimensions approximating those of hydroxyapatite found in bone. In short, nanoscale materials allow for the rapid and select adhesion, as well as increased functions, of osteoblasts on the surface of these substrates, compared with materials with microscale surface dimensions. It is because of these variations that it can be suggested that nanostructured surfaces may provide a better overall design parameter for orthopaedic implants than those currently being used.

In summary, the present study provides the first evidence that faster bonding with juxtaposed bone may be achieved through the use of different nanofibred materials. If this is possible, orthopaedic implant life may be prolonged and may lead to increased physical activity for the patient.

Acknowledgments—The authors would like to thank Luke Gutwein, Applied Sciences, for providing the carbon nanofibres, the Argonide Corporation for supplying the alumina fibres, the NSF Integrative Graduate Education and Research Training (IGERT) Program for the Therapeutic and Diagnostic Devices Grant DGE-99-72770 and the Whitaker Foundation for financial support.

References

ANDERSON, J. M., GRISTINA, A. G., HANSON, S. R., HARKER, L. A., JOHNSON, R. J., MERRITT, K., NAYLOR, P. T., and SCHOEN, F. J. (1996): 'Host reactions to biomaterials and their evaluation', in RATNER, B. D., HOFFMAN, A. S., SHOEN, F. J., and LEMONS, J. E. (Eds): 'Biomaterials science: an introduction to materials in medicine' (Academic Press, London, 1996), pp. 165–215

COATHUP, M. J., BLUNN, G. W., FLYNN, N., WILLIAMS, C., and THOMAS, N. P. (2001): 'A comparison of bone remodeling around hydroxyapatite-coated, porous-coated and grit-blasted hip replacements retrieved at post-mortem', *J. Bone & Joint Surg. Brit.*, **83**, pp. 118–123

DEE, K. C., RUEGER, D. C., ANDERSEN, T. T., and BIZIOS, R. (1996): 'Conditions which promote mineralization at the bone-implant interface: a model *in vitro* study', *Biomaterials*, **17**, pp. 209–215

ELIAS, K. E., PRICE, R. L., and WEBSTER, T. J. (2001): 'Enhanced functions of osteoblasts on carbon nanofiber compacts', *Biomaterials*

GUTWEIN, L. G., TEPPER, F., and WEBSTER, T. J. (2002): 'Increased osteoblast function on nanofibered alumina', *American Ceramic Society 26th Annual Meeting Conf. Proc.*

KAPLAN, F. S., HAYES, W. C., KEAVENY, T. M., BOSKEY, A., EINHORN, T. A., IANNOTTI, J. P., and SIMON, S. P. (1994): 'Orthopaedic basic science' (American Academy of Orthopaedic Surgeons, Columbus, Ohio, 1994), pp.127–185

KAY, S., THAPA, A., HABERSTROH, K. M., and WEBSTER, T. J. (2002): 'Nanostructured polymer: nanophase ceramic composites enhance osteoblast and chondrocyte adhesion', *Tissue Eng.*, **8**, pp. 753–761

MA, P. X., and ZHANG, R. (1999): 'Synthetic nano-scale fibrous extracellular matrix', *J. Biomed. Mater. Res.*, **46**, pp. 60–72

MILLER, D. M., THAPA, A., HABERSTROH, K. M., and WEBSTER, T. J. (2002): 'An *in vitro* study of nano-fiber polymers for guided vascular regeneration'. Materials Research Society Symposium Proc., **711**, pp. GG3.2.1–GG3.2.4

MONTGOMERY, D. C. (Ed) (1991): 'Design and analysis of experiments' (John Wiley & Sons, Inc., New York, 1991), pp. 28–30

OGISO, M., YAMASHITA, Y., and MATSUMOTO, T. (1998): 'Differences in microstructural characteristics of dense HA and HA coating', *J. Biomed. Mater. Res.*, **41**, pp. 296–303

PARK, J. B., and LAKES, R. S. (1992): 'Biomaterials: an introduction' (Plenum Press, New York, London, 1992)

PRICE, R. L., ELIAS, K. L., HABERSTROH, K. M., and WEBSTER, T. J. (2001): 'Small diameter, high surface energy carbon nanofiber formulations that selectively increase osteoblast function', Mater. Res. Soc. Spring 2001 Meeting Conf. Proc.

RODRIGUEZ, N. M. (1993): 'A review of catalytically grown carbon nanofibers', *J. Mater. Res.*, **8**, pp. 3233–3250

TEPPER, F., LERNER, M., and GINLEY, D. (2001): 'Nanosized alumina fibers', *Am. Ceramic Soc. Bull.*, **80**, pp. 57–60

THAPA, A., WEBSTER, T. J., and HABERSTROH, K. M. (2002): 'An investigation of nano-structured polymers for use as bladder tissue replacement constructs', *Materials Research Society Symposium Proceedings*, **711**, pp. GG3.4.1–GG3.4.6

WEBSTER, T. J. (2001): 'Nanophase ceramics: the future orthopedic and dental implant material', *Adv. Chem. Eng.*, **27**, pp. 125–166

WEBSTER, T. J., SIEGEL, R. W., and BIZIOS, R. (2001*a*): 'Nanoceramic surface roughness enhances osteoblast and osteoclast functions for improved orthopaedic/dental implant efficacy', *Scripta Materialia*, **44**, pp. 1639–1642

WEBSTER, T. J., ERGUN, C., DOREMUS, R. H., SIEGEL, R. W., and BIZIOS, R. (2001*b*): 'Enhanced function of osteoblasts on nanophase ceramics', *Biomaterials*, **21**, pp. 1803–1810

Author's biography

RACHEL L. PRICE received a B.S. degree in Physics and Chemistry from the University of North Alabama in 2000 and is currently in the PhD program at Purdue University in the Biomedical Engineering program. She is working under the guidance of Dr. Thomas J. Webster and as such is interested in cell reaction to orthopedic and dental nanomaterials. Specifically, she is studying the effects of the size and shape of such materials on osteoblast and other orthopedic-related cell lines.