P2- 010

A Novel Prosthetic Resin Composite containing Fine Enamel Filler Particles

Kazuhiko Endo[1] and Hiroki Ohno[1]

[1]FDivision of Biomaterials and Bioengineering, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-tobetsu, Hokkaido, 061-0293 JAPAN

Introduction

Mechanical strengths and wear resistance of prosthetic resin composites have recently been improved due to a significant increase in the filler/matrix ratio. However, it may have adverse effects on antagonistic dentition when composite restoratives contain large amounts of hard filler particles[1]. In the present study, we used finely powdered enamel as a filler to develop a prosthetic resin composite that has excellent mechanical properties and no potential to abrade opposing tooth structure.

Materials and Methods

Crowns of bovine teeth were ground into powder by a crusher, and then the enamel particles were separated from the powder by centrifugation in bromoform-ethanol solution at a rotation speed of 3,000 rpm for 10 min. The particle size distribution of the enamel powder was determined by the laser diffraction particle size analyzer. The resin matrix consisted of UDMA (60 mole %) and Tri-EDMA (40 mole %). Camphorquinone (0.5 mass %) was added to the monomer as a photo-initiator. Fillers were incorporated directly into the resin matrix in amounts of 80 or 85 mass %. Eight specimens for each hardness and flexural strength test were cured using a light-curing instrument. Half of the light-cured specimens were subjected to heat treatment at 100 ℃ in air for 15 min to increase the degree of polymerization. The hardness (Hv) of the specimens was measured on a Vickers Hardness Tester under a 1 kg load. The bending strength was measured using a universal testing machine operated at a cross-head speed of 1 mm/min.

Results and Discussion

Scanning electron micrographs showed that the enamel particles are characterized by their prismatic shape, probably due to the structure of the enamel rod. The size of the particles ranged from 0.2 μm to 100 μm. An increase in filler content resulted in an increase in the Hv and the bending strength of the resin composite. The average flexural strength and Hv for specimens having 85 mass % filler that had been subjected to heat treatment after light-curing were 109.8 MPa and 95.2, which were higher than those for most commercially available resin composites. This finding suggests that the experimental resin composite having a large amount of finely powdered enamel would be used clinically for inlays and full-coverage crowns.

[1] S. Suzuki, E. Nagai, Y. Taira, Y. Minesaki: J. Prosthet. Dent., 88, 431-436 (2002).

Modification of Dentin Surface by Coating of Carbon Nanotubes

Tsukasa Akasaka[1], Keiko Nakata[2], Motohiro Uo[1], and Fumio Watari[1]

[1]Graduate School of Dental Medicine, Hokkaido University, Sapporo, Japan

[2]Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

Introduction:

Carbon nanotubes (CNTs) have been attracting considerable attention because of their unique physical properties and potential for a variety of applications. Furthermore, carbon nanotubes may be an important biomedical material for improved cell adhesion, mineralization of hydroxyapatite, and sterilization. However, there have been very few studies on their dental application. In this study, we investigated modification of dentin surface by coating of carbon nanotubes and the effect on tensile bond strength.

Materials and Methods:

Flat dentin slices were prepared by cutting off of human molars with a diamond saw and with running water. The exposed dentin surfaces were etched with a phosphoric acid for 1min and rinsed with distilled water. After rinsing, the dentin slice was mixed with CNTs dispersion in concentration of 10 mg/L. For SEM observation, the slices were fixed with a solution of 2.5% glutaraldehyde and dehydrated following critical-point drying. To estimate tensile bond strength, the slices were bonded with Liner Bond and attached with a resin composite. The bond strength of them was measured by microtensile test.

Results and Discussion:

After mixing with CNTs, the color of dentin area in tooth slice became gray (Fig. 1a). On contrast, the color of enamel area did not changed. SEM image shows that CNTs were adhered to the surface of the etched dentin (Fig. 1b). CNTs seem to be selectively adhered to collagen fibers in the etched dentin. These results indicate that CNTs interact with collagen than hydroxyapatite preferentially. As unexpected, the tensile bond strength of CNT-coated tooth bonded with Liner Bond was similar to that of non-coated tooth. Thus, this modification might play an important role in the marker of the interface between tooth and resin, the mineralization of hydroxyapatite, and the sterilization of dental bacterium.

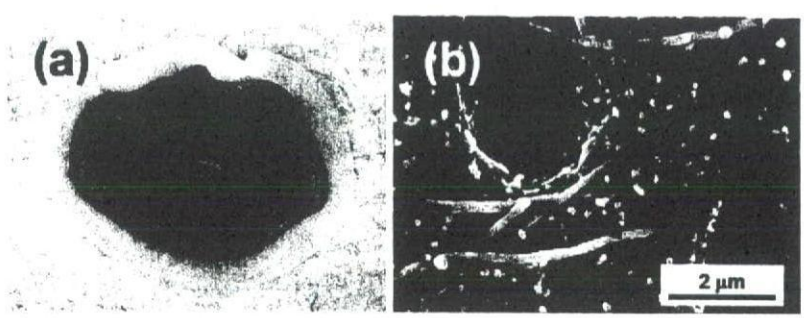

Figure 1 Photograph (a) and SEM image (b) of tooth slice coated with carbon nanotubes.

[1] K. Nakata, *the Journal of the Japan Dental Assocoastion* **57,** 153 (2004).

[2] T. Akasaka, F. Watari, Y. Sato, and K. Tohji, *Mater. Sci. Eng. C*, **26**, 675 (2006).

Destruction of HIV and Growth Restriction of Cancer Cell by Nano-Hydroxyapatite

Hideki Aoki and Tatsuhiko Yajima
Advanced Science Research Center,
Saitama Institute of Technology

Abstract

Since our success in sintering hydroxyapatite(HA) in 1972, the HA with various shapes including powder, granule, dense or porous sintered body and coated on metals have been used as bone and dental cements, bone filling materials, bone spacers, tooth roots, joints and composite materials of medical devices. In 1992, we prepared a nano-HA as a drug or a drug carrier by a wet method using a solution reaction. Since then, many medical applications using the nano-HA have developed. Among them we presented a paper of influence of the nano-HA on P388 leukemia cell behavior in this meeting.

In this paper a destruction of HIV and growth restriction of cancer cells by the nano-HA will be presented. Mixtures of the nano-HA with 0, 0.05, 0.5, and 50mg/ml and media containing HIV-1 of $4x10^3TCUD_{50}$/ml were shaken at 37℃ for 10 and 60 minutes, then after centrifugal separation the mixture were added into media wells containing MT-4 cells and incubated at 37℃ for days on a 5 % CO_2 incubator. As a control the mixture without centrifugal separation were added into MT-4 cell media and incubated at 37℃ for days. After the incubation, the HIV-1 of $TCID_{50}$/ml in the mixtures was calculated by the counting of CPE-positive wells, that is syncytium formation positive wells. The HIV-1 drastically decreased with increasing amounts of nano-HA by chemical or physical destruction. Influence of the nano-HA on cancer cell Ca-9 growth was investigated by a cell culture technique and the cancer cell growth was drastically restricted by the nano-HA.

P2- 013

Preparation of mono-dispersed carbon nanotubes for exposure and risk assessment experimental studies

Bunshi Fugetsu[1,a] and Fumio Watari[2,b]

Graduate School of Environmental Science, Hokkaido University, Sapporo 060-0810;
Graduate School of Dental medicine, Hokkaido University, Sapporo 060-8586, Japan
[a]email: hu@ees.hokudai.ac.jp; [b]email: watari@den.hokudai.ac.jp

Developments in nanotechnology have led to predictions of great benefits as well as to warnings of great dangers to humanity and the environment. Carbon nanotubes (CNTs) have been the most extensively used nano-materials due to their excellent physical and chemical properties, including the high electrical conductivity, excellent mechanical strength, superior flexibility, and large surface areas. The increase in CNT production and application will undoubtedly increase the exposure of humans and the environment to CNTs. If CNTs are toxic, their influence in the environment will be long lasting and could spread throughout the food chain. In fact, several studies on biocompatibilities of CNTs have suggested that CNTs can adversely affect both humans and animals. However, the toxicological definition for CNTs is still highly controversial. CNTs, due to their high tendency to aggregate, have been examined commonly as micro-meter sized particles; the experimental data, therefore reflecting the biocompatibilities of the micro-meter sized particles. CNTs dispersed with the synthesized surfactants were found to be highly sensitive to the external conditions; changes in the ionic strength and pH often result in re-aggregation of the dispersed CNTs. In other worlds, the intrinsic aggregation properties of CNTs have been the most critical barriers for evaluating the possible risk of the truly nano-sized materials. In this study, we have established a novel dispersing system by using phosphatidylcholine mixed with phosphatidylinositol, the naturedly occurred amphipathic species as the essential dispersants for preparing the mono-dispersed CNTs. The dispersants are highly biocompatible and moreover, the mono-dispersed CNTs have been found to be highly stable even after being introduced into the biological systems. The truly nano-sized CNTs have shown stronger adverse-effects than that of the aggregated CNTs to *Arabdopsis* T87 cells.

P2- 014

Distribution imaging of magnetic particles in mice compared with magnetic resonance imaging and X-ray scanning analytical microscope

Mitsue Esaki[1], Shigeaki Abe[1], Tsukasa Akasaka[1], Motohiro Uo[1], Manabu Morita[2], Toshiaki Hosono[3], Yoshinori Sato[3], Balachandran Jeyadevan[3], Yoshinori Kuboki[1,4], Kazuyuki Tohji[3], Fumio Watari[1]

[1]Graduate School of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan

[2]Graduate School of Medicine, Dentistry and Pharmaceutical Science, Oayama University, Okayama 700-8525, Japan

[3]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan

[4]Koken Bioscience Institute, Tokyo 115-0051, Japan

Introduction: Nanosized particles has been received much attention in view of electronic, cosmetic and medical applications. For example, magnetite nanoparticles have been investigated for medical applications such as hyperthermic oncology. In this study, we determined the distribution of administered magnetic particles in mice using MR imaging (MRI) and X-ray scanning analytical microscope (XSAM).

Materials and Methods: Male mice (Jcl:ICR) were used at ages from 8 to 12 weeks. The magnetic particles (ca. 20 nm in diameter) were dispersed into saline. The particle dispersion was injected into the tail veins under pentbarbital anesthesia. MRI data were obtained using a 7-T horizontal bore spectrometer (Varian Inc., CA). Radiofrequency pulses were transtimmed and MRI signals were received using a 4-cm volume coil. Spin-echo images were acquired with a data matrix of 256 x 128, field of view of 80 x 40 mm, slice thickness of 1 mm, echo time of 5 ms, and repetition time of 5000 ms. The magnetic particles injected to mice were also applied for XSAM analysis. Their organs were excised at several post injection time. The specimens of excised organs were subjected to elemental distribution analysis using XSAM (XGT-2000V, Horiba, Japan). The XSAM observation was carried with the incident X-rays generated from an Rh anode under conditions of 30kV and 1 mA.

Results & Discussion: The signal intensity of magnetic resonance images decreased with magnetic particles injection. Especially the phenomena were obtained in the liver and kidney tissue. It suggested that the magnetite particles without any modification of the surface was circulated in the body and arrived at particular organs. In the XSAM analysis, the localization of Fe was observed at lung and liver tissue. The fluorescence of Fe was also determined in the kidney. Then, the MRI and XSAM results were in good agreement. This phenomenon was depended upon the size of particles because negligibly weak fluorescence was determined in the kidney when micro-sized particles were administered.

Conclusions: In this study, we succeeded to visualize of distribution of magnetic particles in mice. Determination of the biodistribution property of magnetic nano-particles is helpful for achievement to apply its medical field.

P2- 015

Microstructure and biological influence of environmental exposure of asbestos

Keiji Yada* and Norihiko Kohyama*

*

*Tohken Co., LTD, 2-27-7 Tamagawa Choufu, Tokyo 182-0025, Japan

**Faculty of Economics, Toyo University, 5-28-20 Hakusan Bunkyo-ku, Tokyo 112-8606, Japan

Asbestos minerals are very thin fiber type of minerals. They become like cotton and can be woven on thread or cloth when pulverized. They excel in tensile strength, wear resistance, heat resistance, insulation properties, soundproofing, chemical resistance, etc., other than the spinning-and-weaving nature. They were honorably said as "the mineral of the miracle" because of their valuable nature and they were also strategic goods. On the other hand, the relation between asbestos exposure and lung cancer and malignant mesothelioma are proved by epidemiology and an animal experiment by around 1970, and thereafter has been feared as "a quiet time bomb."

Here, microstructures of chrysotile asbestos which makes the mainstream in asbestos substances are described. It is also described that in what kind of environment people are exposed to asbestos and what kind of biological or epidemical things happens after asbestos exposure. Recent epidemic prediction of asbestos related diseases in Japan is reviewed. Many kinds of fibrous materials as the substitutes of asbestos are described in relation to their carcinogenicity.

P2- 016

New geometrical matrix for bone regeneration: a honeycomb-shaped □-TCP ceramics induced straight longitudinal bone inside the tunnels

Michiko Terada[1], Shouhei Iku[2,3], Yoshimasa Kitagawa[1], Mariko Takayama[4], Tohru Kaku[5], Motohiro Uo[1], Fumio Watari[1] and Yoshinori Kuboki[1]

[1] Graduate School of Dental Medicine, Hokkaido University, Japan

[2]Koken Bioscience Institute, Japan

[3]Department of Basic Medicine, Xinxiang Medical University, China

[4]Pilot Precision, Japan

[5]Oral Pathology, Health Sciences University of Hokkaido, Japan

Introduction. Hydroxyapatite and □-TCP ceramics are often used as the bone grafting materials, but their geometrical structure of micro-meter order was proposed to be one of the key factors controlling their functions of new bone growth [1]. We have already shown that the optimal pore size of the porous hydroxyapatite ceramics for osteogenesis was 300-400 □m. But the pores of the hydroxyapatite used those experiment was irregular shape, though they were interconnected. Furthermore, we found that honeycomb-shaped hydroxyapatite ceramics with the straight tunnels efficiently induced bone or cartilage, depending upon the size of pores of the tunnels [2]. In this study, we demonstrate that not only in the hydroxyapatite ceramics, but also in the □-TCP ceramics, honeycomb geometric structure gives effective spaces for osteoblasts growth and bone tissue development.

Materials and methods. The honeycomb-shaped □-TCP ceramics in a disc-form equipped with 37 tunnels, each diameter of which was 300 □m, and the non-honeycomb-shaped □-TCP (discs without tunnels) were combined with BMP-7. Both □-TCP were implanted subcutaneously into the back of the rat for 1 to 4 weeks. Extracted □-TCP were observed histologically and analyzed for alkaline phosphatase (ALP) activity.

Results. The honeycomb-shaped □-TCP showed remarkable osteogenesis inside the tunnels. The bone was formed on the inner surface of tunnels with one or several capillaries at the central area of the tunnel. ALP activity of honeycomb-shaped □-TCP was higher than non-honeycomb-shaped □-TCP.

Conclusion. The honeycomb-shaped □-TCP with tunnel size of 300 □m showed more efficient osteogenesis than without the tunnels. It was conclude that geometry of the honeycomb-shaped □-TCP is feasible for bone formation, which is useful in future clinical application with its biodegradable ability.

[1] Y.Kuboki, Q.Jin, and H.Takita: J. bone. Joint. Surg. Am. Vol. 83 (2005), p. 105-115

[2] E.Tsuruga, H.Takita, H.Itoh, Y.Wakisaka and Y.Kuboki: J. Biochem. Vol. 121 (1997), p. 317-1324.

Enhanced osteoinduction by controlled release of bone morphogenetic protein-2 from Hydroxyapatite and β-tricalcium phosphate

Junichi Tazaki[1], Masaru Murata[1], Toshiyuki Akazawa[2], Masaya Yamamoto[3]
Yasuhiko Tabata[3], Katsutoshi Ito[1], Makoto Arisue[1] and Takanori Shibata[1]

[1] Health Sciences University of Hokkaido, 1757 Kanazawa Tobetsu-cho 061-0293, Japan

[2] Hokkaido Industrial Research Institute, Nishi-11 Kita-19 Kita-ku, Sapporo 060-0819, Japan

[3] Kyoto University, 53 Kawahara-cho, Seigoin, Sakyo-ku, Kyoto 606-8507, Japan

Biomaterial is indispensable for tissue engineering. Hydroxyapatite and β-tricalcium phosphate have been clinically applied to biomaterials of living hard tissues because of their excellent biocompatibility and osteoconduction. Critical characteristics of biomaterials will include surface geometry, hydrophobicity and hydrophilicity, crystallinity, biodegradation rates, and release pharmacokinetics (PK) of incorporated molecules such as BMP-2. We forcused on *in vivo* local BMP-2 PK and bone induction in two ceramics systems, based on different composition. Spongy bones of bovine femur (Holstein, bull, Hokkaido, Japan) were used as starting materials, boiled and calcined at 800℃ for 24h in air to obtain crystalline HAp, so called true bone ceramics. By the calcination process, all prion proteins and organic residues of bovine bone were completely burned out. The HAp ceramics are characterized by high porosities (60~80%) and low specific surface area ($1m^2/g$) [1]. □-TCP (Osferion®, Olympus, Japan) (porosity: 75%, pore size: 100 to 400□m, sintering temperature: 1050℃, surface area: $4m^2/g$) were used as a control. The HAp (3×3×3mm) and □-TCP (3×3×3mm) containing 0.0, 0.05, 0.1, 0.3, 0.5, 1.0, 5.0□g of BMP-2 were implanted into the back subcutis of 4 week old Wistar rats. At 3 weeks after implantation, the ceramics were explanted, fixed with 10 vol% neutral formalin solution, dehydrated with ethanol, and embedded in paraffin. The fixed tissues were cross-sectioned to 4□m thickness with a microtome and stained with Mayer's hematoxylin-eosin (H-E) solution. The histological sections were observed with an optical microscope. Ectopic bone induction occurred in the □-TCP/BMP-2 (0.3, 0.5, 1.0, 5.0□g) system at 3 weeks, while only in the HAp/BMP-2 (5.0□g) , bone induction was found. Furthermore, we analyzed the *in vivo* release profile of ^{125}I–labeled BMP-2 from β-TCP and HAp. BMP-2 was gradually released with time from ceramics. The amount of BMP-2 remaining in the β-TCP at 1 day after implantation was 49.6%, while the amount was 43.0% in the HAp. By using β-TCP, it is likely that an extremely low dose of BMP-2 is enough to enhance bone induction if BMP-2 is appropriately delivered to the site of action.

Literature References

[1] T. Akazawa, M. Murata, T. Sasaki, J. Tazaki, M. Kobayashi, T. Kanno, K. Nakamura and M. Arisue: J. Biomed. Mater. Res., Vol. 76A, No. 1 (2006), p. 44

P2- 018

Geometric property of rod-like molecules: Interaction mechanism of collagen triple-helix with carbon nanotubes

Yoshinori Kuboki[1)], Michiko Terada[2)], Yoshimasa Kitawawa[2)], Shigeaki Abe[2)], Motohiro Uo[2)] and Fumio Watari[2)]

[1]Emeritus Professor, Hokkaido University, N-13, W-7 Kita-ku. Sapporo, Japan 090-8586
[2]Hokkaido University, Graduate School of Dental Medicine, N-13, W-7 Kita-ku. Sapporo, Japan 090-8586

Introduction.
Exceptionally high mechanical strength, electric conductivity and unique geometrical structures of carbon nanotubes (CNT) have been attracting attentions from various fields, including biological application, but the interactions with important biomolecules, above all, collagen molecules have not been studied in details. This situation partly dues to the fact that CNT is a solid entity, while the most of the biomolecules can be prepared into soluble entity. Thus, we introduced to evaluate turbidity in order to analyze the interaction between CNT and collagen molecules. It was found that native collagen induced distinct aggregation with CNT, while denaturation of this protein deprived the molecules of the ability to aggregate with CNT.

Materials and Methods
To a stable suspension of CNT (10 ppm in 0.1% Triton), collagen solution was added to obtain final concentration of 25 ppm. Degree of aggregation was evaluated by measuring the turbidity of the suspension at 660 nm on a Sienco aggregation meter (Morrison Co., USA).

Results.
It was found that addition of collagen induced remarkable aggregation immediately and reached plateau after 15-20 minutes. Furthermore, when the collagen solution was denaturated at 60 degree beforehand, no aggregation was observed; just like the other globular molecules albumin and lysozyme which were added under the same conditions. Thus, it was clearly shown that collagen molecules in their native triple helical structure could interact with CNT to form aggregation. However, after the triple helical structure of collagen is destroyed by heat denaturation into the random polypeptide chains that is so-called "parent gelatin", this protein no longer could interact with CNT to form aggregation.

Discussions.
The result indicated that a rigid rod-like structure of the native collagen triple helix is essential for interaction with CNT to form aggregation. Once the characteristic triple helix was destroyed by a mild heat denaturation, in that no peptide bond cleavage would occur, the random-coiled gelatin could not function to support aggregation with CNT. The phenomenon suggested that rod-like geometry is essential for this interaction. A similar phenomenon has been reported in the interaction of platelets with collagen, in that only the certain polymeric states (oligomers) of collagen molecules are able to induce the platelets aggregation, but the monomers of collagen are not, suggesting the effect of three-dimensional geometry of the collagen oligomers. Though the scale of platelets-collagen interactions is larger than those of CNT-collagen, the essential mechanisms of both cases are considered to be dependent upon geometric properties of rod-like collagen molecules. The findings in this paper will open a new avenue to clarify the detailed mechanism of interaction between collagen molecules and CNT.

Multi-walled carbon nanotube blocks cross-linked by de-fluorination

Sashida Naoki[1], Yoshinori Sato[1], Masaru Namura[1], Shinji Hashiguchi[2], Kenichi Motomiya[1], Balachandran Jeyadevan[3], Kazuyuki Tohji[3]

[1] Graduate School of Environmental Studies, Tohoku University, Sendai, 980-8579, Japan.

[2] Stella Chemifa Co., 1-41, Rinkai-cho, Izumiotsu, Osaka, 595-0075, Japan.

Blocks containing pure carbon nanotubes (CNTs) in the absence of a binder or a matrix are expected to be applied in the fabrication of electrodes and biomaterials for supercapacitors, bioelectrodes and biosensors, cell growth scaffolding, dental implants and artificial bone structures. These structures could then take advantage of the outstanding characteristics of individual CNTs, which could possess a large specific surface area and fascinating electronic and mechanical properties [1]. Additionally, covalent 2D and 3D network-carbon nanomaterials from 1D building blocks are gaining more importance due to their fascinating mechanical and electronic properties that have been predicted recently [2]. However, it is difficult to only solidify pure CNTs in the absence of polymer binder. Multi-walled carbon nanotubes (MWNTs) are normally inert and reactions on their surface are difficult. In this context, numerous scientists have faced various difficulties in incorporating the MWNTs into polymeric, metallic or ceramic matrices due to their weak adhesion (interaction) which do not permit the establishment of sp^3 hybridized carbon bonding between the tube walls and the matrix. Therefore, pure MWNT composite assemblies have the disadvantage that the load is not efficiently transferred to the tubes due to the weak adhesion. This results in tube slipping within the matrix, and the presence of sp^3-hybridized covalent bonds among nanotubes is significantly inhibited [3].

In this study we demonstrate that an advantageous method for producing active cross-linking sites on the surface of nanotubes is de-fluorination of MWNTs [4]. We report on the production and excellent mechanical and electronic properties of free-standing solidified MWNT blocks by de-fluorinating fluorinated MWNTs. The method is able to introduce a large number of sp^3-hybridized cross-linking among the nanotubes, via a spark-plasma sintering (SPS) apparatus operating under 80 MPa at 1273 K for 10 minutes. The result will be reported in detail and discussed.

[1] R. H. Baughman, A. A. Zakhidov, W. A. de Heer, *Science* **2002**, *297*, 787.

[2] J. M. Romo-Herrera, M. Terrones, H. Terrones, S. Dag, V. Meunier, *Nano Lett.* **2007**, *7*, 570.

[3] P. Calvert, *Nature* **1999**, *399*, 210.

[4] Y. Sato *et al*, *ACS Nano* **2008**, *2*, 348.

P2- 020

Maturation of osteoblast-like SaoS2 induced by carbon nanotubes

Xiaoming Li[1], Hong Gao[2], Motohiro Uo[1], Yoshinori Sato[3], Tsukasa Akasaka[1], Shigeaki Abe[1], Qingling Feng[4], Fuzhai Cui[4], Fumio Watari[1]

[1] Department of Biomedical Materials and Engineering, Graduate School of Dental Medicine, Hokkaido University,Sapporo, Japan

[2] Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University,Sapporo, Japan

[3] Graduate School of Environmental Studies, Tohoku University, Sendai, Japan

[4] Department of Materials Science and Engineering, Tsinghua University, Beijing, China

Introduction

Nanostructures of biomaterials have been shown to affect cell behaviour [1, 2]. In this study, multiwall carbon nanotubes (MWNTs) and graphite (GP) were pressed as compacts. Human osteoblast-like SaoS2 cells were cultured on the two kinds of compacts with and without adsorbing rhBMP-2. The cell responses, especially cell differentiation on the two kinds of compacts, were described and compared in *vitro*.

Materials and Methods

Fabrication of compacts: MWCTs (about 90 nm in diameter) and GP (about 4.5□m in diameter) with the same weight were separately compacted serially in a steel-tool die via a uniaxial pressing cycle.

Cell culture: SaOS2 cells were respectively seeded on the samples, with and without adsorbing rhBMP-2 in advance, with a cell density of 5.0×10^4 per sample in culture plates. Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 1% penicillin/streptomycin was used as culture medium. After 1, 7 and 14 days, the DNA, alkaline phosphatase (ALP), proteins and the osteogenic gene expression of osteonectin, osteopontin, osteocalcin of the cells were analyzed.

Results

During the conventional culture (without the adsorption of rhBMP-2 in advance), significantly higher osteonectin, osteopontin, osteocalcin gene expression level, ALP/DNA, protein/DNA on MWNTs were found. Furthermore. comparing with on GP, osteonectin, osteopontin, osteocalcin gene expression level, ALP/DNA, protein/DNA of the cells tested increased more on MWNTs after the compacts adsorbed rhBMP-2.

Discussion and Conclusion

Carbon nanotubes might improve the differentiation process of the cultured osteoblasts by adsorbing more specific proteins due to their larger surface area, unique electronic, catalytic and chemical properties, which provided new information for the understanding of the biocompatibility and bioactivity of carbon nanotubes and indicated that carbon nanotubes may be a candidate for scaffold material for bone tissue engineering.

References

[1] X. Li, C.A. Van Blitterswijk, Q. Feng, F. Cui, F. Watari: Biomaterials Vol. 29 (2008), p: 3306.

[2] X. Li, Q. Feng, X. Liu, W. Dong, F. Cui: Biomaterials Vol. 27 (2006), p: 1917.

P2- 021

The difference of the effect of multi-walled carbon nanotubes on human hepatic normal and cancer cells

Sachiko Itoh[1], Yasutaka Yawaka[1], Tsukasa Akasaka[1], Fumio Watari[1]

[1]Graduated School of Dental Medicine, Hokkaido University, Sapporo ,Hokkaido, Japan

Introduction

Carbon nanotubes(CNT) have been known as new material in various industries. In this study, we cultured the human hepatic normal and cancer cells in the medium with CNT to assess the effect of CNT on the cells. In addition, we cultured hepatic cells on the CNT to compare with the cells cultured on the other dishes.

Material and methods

The human hepatic normal cells, Hc cells, were cultured in CSC-medium(Cell systems), while the cancer cells,HepG2 in DMEM, incubated for 10 days. Then, CNT were added to each of them and they were incubated for 7 more days. The incubation were carried out at 37°C in 5% CO_2 atmosphere. After that, the cells were observed by a phase contrast microscopy and we evaluated the differences between the results of Hc cells and HepG2.

Hc cells were labeled with QTracker, then cultured on the CNT and collagen-coated dish, observed by the fluorescent microscope.

Results

Adhesion of CNT to Hc cells were observed immediately after the addition of CNT, however, it did not happen in HepG2, no significant adhesion. The morphology of Hc cells exposed to CNT was almost the same as that of the control, but the number of the cells of HepG2 exposed to CNT remarkably decreased compared to that of the control. (Fig.1)

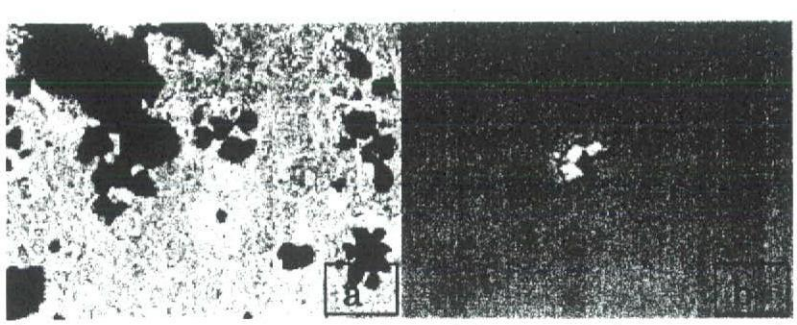

Fig.1 Microscope images after CNT addition. a:Hc cell ; b HepG2

Multi wall carbon nanotube coating of 3D collagen cell culture scaffold

Eri Hirata, Motohiro Uo, Hiroko Takita, Tsukasa akasaka,

Fumio Watari, Atsuro Yokoyama

Graduate School of Dental Medicine, Hokkaido University, Sapporo, 060-8586, Japan

Objectives:

Carbon nanotubes (CNTs) have high biochemical properties such as strong cell adhesion and the protein absorption, which is very useful as the cell cultivation scaffold. In addition, the authors found that CNTs have high affinity to collagen.In this study we prepared the CNTs-coated collagen sponge and applied to the 3D scaffold of cell culture.

Methods:

Collagen sponge honeycomb (AteloCell®;Koken Co.,Ltd.) was treated with carboxylated multi wall carbon nanotubes (MWCNTs) dispersed aqueous solution. MC3T3-E1 cells were cultured on the MWCNTs-coated sponge. After 3 weeks cultivation, the cell proliferation and biochemical properties (alkaline phosphatase (ALP) activity and DNA quantity) were estimated. The cell morphology and the surface structure of the sponges were observed by optical microscope and SEM.

Result:

The collagen sponge surface was homogeneously coated with MWCNTs (Fig 1).

MC3T3-E1 cells were spread around and inside the MWCNTs-coated sponge (Fig 2). Amount of DNA contained in the cells on the MWCNTs-coated sponges was significantly higher than those on the collagen sponges ($p<0.05$).There was no significant difference between the estimated ALP activity normalized by DNA quantity on MWCNTs-coated sponge and that on the non-coated collagen sponge.

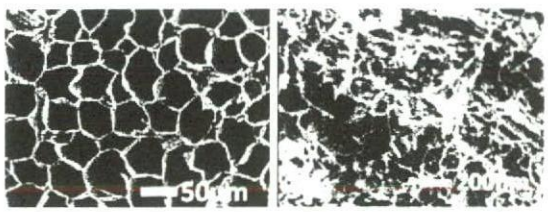

Fig.1 SEM images of the CNTs coated

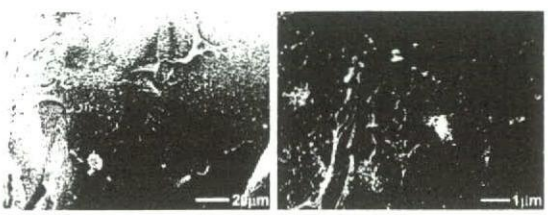

Fig.2 SEM images of the cells after 1 week cultivation on the CNTs coated sponge

Conclusion:

The collagen sponge is one of the most affinitive scaffolds for cell cultivation. MWCNTs treatment would improve the various properties of the scaffold such as mechanical and electric properties. In addition, MWCNTs-coated surface shows strong cell adhesion. Therefore, the MWCNTs-coated collagen sponge is expected to be a useful 3D scaffold for cell cultivation.

P2-023

Establishment of a novel antisense-oligonucleotide therapy using a natural DNA carrier molecule and its effectiveness on murine model of arthritis

Shin Onodera[1], Harukazu Tohyama[1], Kazunori Yasuda[1], Yoshikazu Koyama[2], Atsushi Uno[3], Hironori Ando[4], and Kazuo Sakurai[5]

1) Department of Sports Medicine and Joint Reconstruction Surgery, Hokkaido University Graduate School of Medicine
2) Department of Medical Bioinformatics, Hokkaido Information University
3) Napa Jenomics
4) Department of Chemical Process and Environments, the University of Kitakyusyu

Schizophyllan (SPG) is a natural β-(1->3)-D-glucan that can form complexes with single stranded homo-polynucleotides. To determine the efficacy of SPG as a delivery system for nucleic acid-based therapeutics, we examined the stability of oligodeoxynucleotide (ODN)/SPG complexes in serum. Both *in vitro* and *in vivo*, uncomplexed (naked) ODN instantly disappeared from serum, while ODN/SPG complexes showed a significantly prolonged half-life, indicating that SPG protected complexed ODN from processing by endogenous nucleases in blood. We next examined the activity of a tumor necrosis factor-α (TNF-α)-specific antisense ODN with native phosphodiester bond structure in a murine model of type II collagen antibody-induced arthritis. Mice that received naked antisense ODN developed severe arthritis, and were indistinguishable from mice that received saline alone as a control. In contrast, administration of SPG/ODN complexes resulted in weaker footpad swelling compared with control animals, indicating that SPG/ODN complexes were effective in protecting mice from arthritis. These results suggest that, upon forming complexes with SPG, native DNA acquires a prolonged half-life in blood, and can potentiate the efficacy of antisense drug therapy. Thus, the use of SPG for the delivery of nucleic acid-based therapeutics represents an ideal application for natural β-(1->3)-D-glucans.

Application of Platinum Nanomaterials for SALDI-MS

Tetsu Yonezawa[1], Hideya Kawasaki[2] and Ryuichi Arakawa[2]

[1]Department of Chemistry, School of Science, The University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

[2]Department of Chemistry, Faculty of Engineering, Kansai University,
Yamate-cho, Suita, Osaka, Japan

The MALDI-MS system has become an indispensable tool for mass analyses of bio-related molecules. However, the MALDI-MS system with organic matrices has some disadvantages. (1) Laser pulse irradiation introduces decomposition of organic matrix molecules and obstacle peaks are detected in the *m/z* region < 1000. (2) 2D mass mapping is difficult according to the sweet-spot problems. (3) "*Soft*" ionization of some unstable biomolecules without decomposition is difficult. To overcome these problems, we have developed structure-controlled platinum nano-materials for "*soft*" ionization of bio related molecules.

The platinum nanomaterials were prepared by chemical reduction of H_2PtCl_6 under ambient condition. The structure was observed by HR-SEM (JEOL JSM-7401). As shown in Figure 1, the platinum nanomaterials showed an aggregated form with thin platinum plate on the surface. AFM observation revealed the thickness of the plate is as small as 1-2 nm. We then call these platinum nanomaterials as "platinum nanoflower".

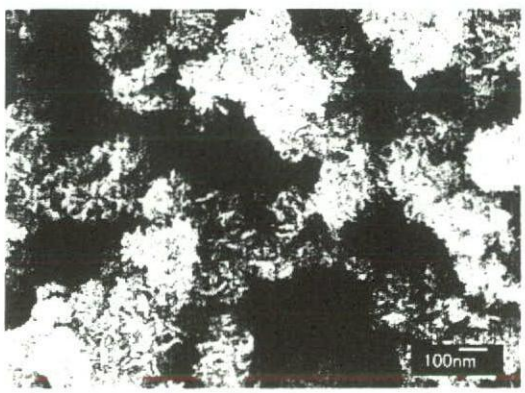

Figure 1. HR-SEM image of "platinum nanoflower"

These nanoflowers are very effective as SALDI-MS matrix (substrate). Angiotensin I, substance P and cytochrome C can be ionized at a very low sample amount. Samples with a low molecular weight could be also measured without obstacle peaks which are observed MALDI-MS system.

This work is financially supported in part from Grant-in-Aid for Scientific Research in Priority Area (477) from MEXT, Japan (20050007).

Reference [1] H. Kawasaki, T. Yonezawa, T. Watanabe, R. Arakawa, *J. Phys. Chem. C*, **111**, 16278-16283 (2007).

Size dependence of interaction of materials with cells and tissue

Fumio Watari, Shigeaki Abe, Eri Hirata, Atsuro Yokoyama, Tsukasa Akasaka, Motohiro Uo, Makoto Matsuoka, Noriyuki Takashi, Yasunori Totsuka, Kosuke Ishikawa, Sachiko Itoh, Yasutaka Yawaka

Graduate School of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan

Biological effect of nanosizing of materials was investigated by both in vitro biochemical cell functional test and in vivo animal implantation test. Dependence of reaction of cells and tissue on particle size was studied. Fig.1, 2 and 3 are the reaction of tissue to macroscopic size, cell to sub-micron and long-term tissue to sub-micron for Ni, soluble metal, and Ti, insoluble metal, respectively. The increase of specific surface area causes the enhancement of chemical reactivity and toxicity for stimulative materials such as Ni, which is most usually and easily recognized. However even for biocompatible materials such as Ti, stimulus was increased with the decrease of particle size and pronounced below 10 μm where phagocytosis was induced to cells (Fig.2b) and inflammation to tissue (Fig.3b). For the size below 200nm, particles invade into the internal body through the respiratory (Fig.4) or digestive system and diffuse inside body (Fig.5). Thus nanosizing induces the intrinsic functions of biological organism and results in the conversion of functions such as from biocompatibility to stimulus.

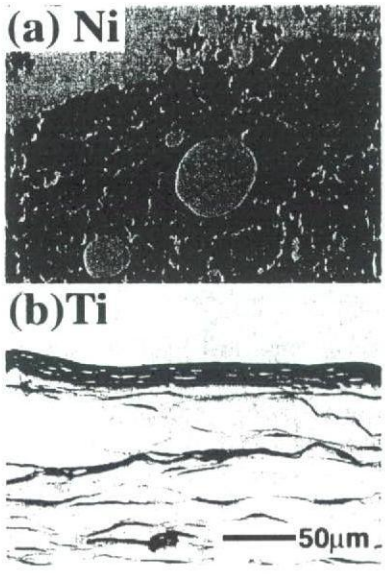

Fig.1 Histological image of rat soft tissue inserted with Ni (a) and Ti (b) of macroscopic size for 1 week.

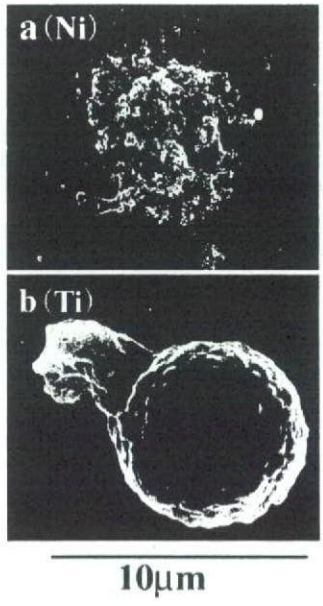

Fig.2 SEM images of human neutrophils exposed to the 500 nm particles of Ni (a) and Ti (b).

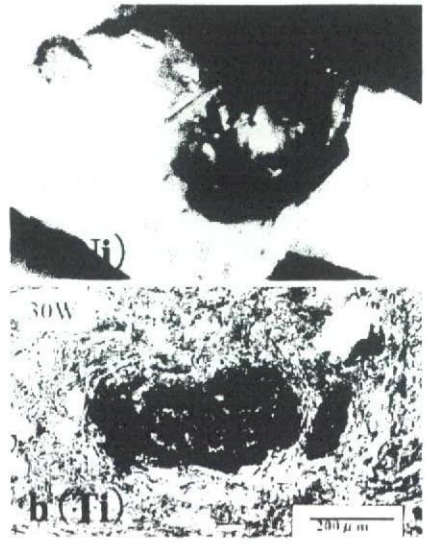

Fig.3 Tissue reaction for long –term implantation to 0.5 μm Ni and 3 μm Ti particles. Tumor induced after 1 year implantation of 0.5 μm Ni particles.

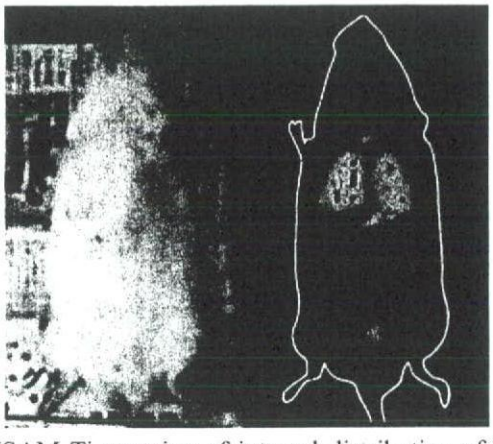

Fig.4 XSAM Ti mapping of internal distribution of 30 nm TiO_2 particles after compulsory exposure test

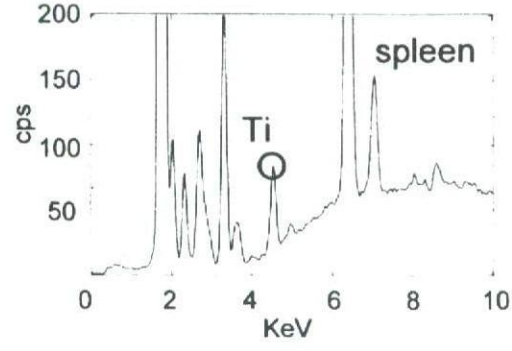

Fig.5 Elemental analysis of spleen after 10 days of oral administration of 30nm TiO_2

Geometry of artificial ECM: Parallel inductions of bone and vasculature within the tunnels of honeycomb-shaped beta-tricalcium phosphate ceramics *in vivo*

Yoshinori Kuboki[1)], Shouhei Iku[2, 3)], Michiko Terada[1)], Yoshimasa Kitawawa[1)], Mariko Takayama[3)], Makiko Kono[2)], Yu Aso[2)], Tohru Kaku[5)], Motohiro Uo[1)] and Fumio Watari[1)]

[1]Hokkaido University, Graduate School of Dental Medicine, N-13, W-7 Kita-ku. Sapporo, Japan 090-8586
[2]Koken Bioscience Institute, 2-13-10, Ukima, Kita-ku, Tokyo, Japan 115-0051
[3]Dep of Basic Medicine, Xinxiang Medical University, Xinxiang City, Jinsui Road, Henan, China 453003
[4]Pilot-Corporation Co., 3-5-57 Tamura, Hiratsuka-Shi Japan 254-0013
[5]Health Science University of Hokkaido 1757 Kanazawa, Toubetsu-Cho, Ishikari-Gun, Hokkaido

Introduction.
We have been proposing a new science: "Geometry of artificial extracellular matrix (ECM)", as one of the important backgrounds in of tissue engineering [1]. In this science, essential properties of ECM were classified into four categories: (1) physical, (2) chemical, (3) biochemical and (4) geometrical properties, the last one of which, so far, has been poorly elucidated. For this reason we have shown by various instances that geometry of artificial ECM can definitely direct cell differentiation and tissue formation. Ten categories of geometries of artificial ECM were proposed as a strategic classification, and individual category was verified by each example, leading to the concept of optimal space provided by the artificial ECM. It was hypothesized that every tissues has its "optimal space" of artificial ECM for regeneration and reconstruction *in vivo* and *in vitro*. The optimal spaces were exampled by many geometry-directed phenotype expressions, including: vasculature-inducing geometry for bone formation [1], vasculature-inhibitory geometry for cartilage formation [2], optimal size and shape of pores for bone formation [3], optimal tunnel size for collagen fibrils direction [4], and honeycomb geometry feasible for directing blood vessels, nerve, cartilage and bone [5]. In this presentation, we will demonstrate that honeycomb-shaped beta-tricalcium phosphate ceramics (β-TCP) could direct the orientation and morphology of osteogenesis and vascular formation within the tunnel structures.

Materials and Methods.
We used the honeycomb-shaped artificial ECM with a long cylinder-form (4 or 10 mm in length and 3 mm in diameter), equipped with numerous straight tunnels, which were made by β-TCP. They were combined with BMP-7 and implanted subcutaneously or intramuscularly into rats. Parts of the β-TCP were implanted without BMP-7. Implanted materials were extracted and examined histologically with HE staining and partly immunochemical-staining.

Results.
After 2 weeks, it was clearly shown that new bone and capillary developed straightly side by side, throughout the entire length of the tunnel (4 mm) of honeycomb-shaped β-TCP. Osteoblasts were clearly lined up on the surfaces of newly formed bone. Adjacently, the endothelial cells were clearly observed lining up along the capillaries. Between the newly formed bone and capillaries, numerous unidentified cells, which were positively stained with endothelial cell markers. Interestingly, inside the tunnels of implanted long cylinder without BMP-7, there was clear development of capillaries, although bone formation was not recognized.

Discussions.
The results are the first demonstration of close relationship between the newly formed bone and capillaries developing side by side in the artificial ECM. Within these tunnels the relationships between activities of the osteoblasts and endothelial cells were clearly observed, due to their constant close vicinities, which had never been found in the other experimental systems. This artificial ECM is useful both for tissue engineering and analysis of the relationship between osteogenesis and vascularization.

References.
[1] Y. Kuboki, Q. Jin, and H. Takita: J Bone Joint Surg Am. 2001; 83-A Suppl 1(Pt 2): S105-15.
[2] R. Yoshimoto, H. Takita, K. Nemoto, and Y. Kuboki Archives of Bioceramics Research, Vol. 5: 404-407, 2005.
[3] E. Tsuruga, H. Takita, H. Itoh, Y. Wakisaka and Y. Kuboki: J. Biochem. Vol. 121 (1997), p. 317-1324. 105-115.
[4] Y. Kuboki, M. Kikuchi, H. Takita, R. Yoshimoto, Y. Nakayama, T. Matsuda, Y. Ikada: Connect Tissue Res. 2003; 44 Suppl 1:318-25.
[5] Kuboki Y, Takita H, Mizuno M and Fujisawa R: Dentistry in Japan 37: 42-50, 2001

JOURNAL OF THE ROYAL SOCIETY Interface

Material nanosizing effect on living organisms: non-specific, biointeractive, physical size effects

Fumio Watari, Noriyuki Takashi, Atsuro Yokoyama, Motohiro Uo, Tsukasa Akasaka, Yoshinori Sato, Shigeaki Abe, Yasunori Totsuka and Kazuyuki Tohji

J. R. Soc. Interface published online 8 April 2009
doi: 10.1098/rsif.2008.0488.focus

Interface focus

FirstCite e-publishing

J. R. Soc. Interface
doi:10.1098/rsif.2008.0488.focus
Published online

Material nanosizing effect on living organisms: non-specific, biointeractive, physical size effects

Fumio Watari[1,*], Noriyuki Takashi[1], Atsuro Yokoyama[1], Motohiro Uo[1], Tsukasa Akasaka[1], Yoshinori Sato[2], Shigeaki Abe[1], Yasunori Totsuka[1] and Kazuyuki Tohji[2]

[1]*Graduate School of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan*
[2]*Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan*

Nanosizing effects of materials on biological organisms was investigated by biochemical cell functional tests, cell proliferation and animal implantation testing. The increase in specific surface area causes the enhancement of ionic dissolution and serious toxicity for soluble, stimulative materials. This effect originates solely from materials and enhances the same functions as those in a macroscopic size as a catalyst. There are other effects that become prominent, especially for non-soluble, biocompatible materials such as Ti. Particle size dependence showed the critical size for the transition of behaviour is at approximately 100 μm, 10 μm and 200 nm. This effect has its origin in the biological interaction process between both particles and cells/tissue. Expression of superoxide anions, cytokines tumour necrosis factor-α and interleukin-1β from neutrophils was increased with the decrease in particle size and especially pronounced below 10 μm, inducing phagocytosis to cells and inflammation of tissue, although inductively coupled plasma chemical analysis showed no dissolution from Ti particles. Below 200 nm, stimulus decreases, then particles invade into the internal body through the respiratory or digestive systems and diffuse inside the body. Although macroscopic hydroxyapatite, which exhibits excellent osteoconductivity, is not replaced with natural bone, nanoapatite composites induce both phagocytosis of composites by osteoclasts and new bone formation by osteoblasts when implanted in bone defects. The progress of this bioreaction results in the conversion of functions to bone substitution. Although macroscopic graphite is non-cell adhesive, carbon nanotubes (CNTs) are cell adhesive. The adsorption of proteins and nano-meshwork structure contribute to the excellent cell adhesion and growth on CNTs. Non-actuation of the immune system except for a few innate immunity processes gives the non-specific nature to the particle bioreaction and restricts reaction to the size-sensitive phagocytosis. Materials larger than cell size, approximately 10 μm, behave inertly, but those smaller become biointeractive and induce the intrinsic functions of living organisms. This bioreaction process causes the conversion of functions such as from biocompatibility to stimulus in Ti-abraded particles, from non-bone substitutional to bone substitutional in nanoapatite and from non-cell adhesive to cell adhesive CNTs. The insensitive nature permits nanoparticles that are less than 200 nm to slip through body defence systems and invade directly into the internal body.

Keywords: nanoparticle; phagocytosis; inflammation; internal diffusion; non-specificity; immune system

1. INTRODUCTION

1.1. Nanotechnology and biological organisms

The development of nanotechnology has a large influence on human life. Completion of genome analysis has brought the post-genome era, enabling applications for biological and medical purposes. The reactions of biological organisms to proteins and saccharides including viruses, bacteria, enzymes and pharmacological agents have been investigated in biology and medicine. For materials, the reaction to the usual cases, i.e. at the macroscopic size, is well investigated.

However, the effect of micro/nanosizing of materials onto biological organisms remains unclear. Nanosizing of materials brings in a quantum effect at less than approximately 1.5 nm and the formation of activity sites such are seen in some catalysts (figure 1). The most unambiguous and influential effect is the surface

*Author for correspondence (watari@den.hokudai.ac.jp).

One contribution to a Theme Supplement 'Japanese biomaterials'.

Received 14 November 2008
Accepted 13 March 2009

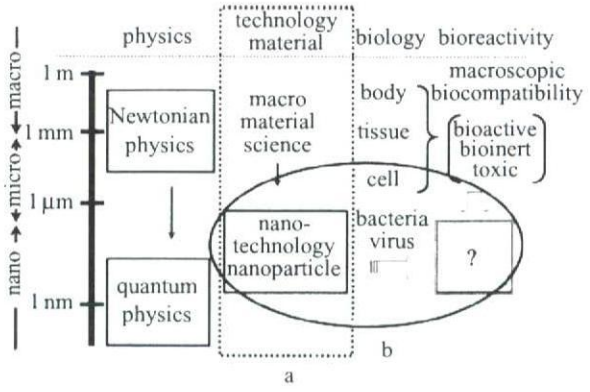

Figure 1. Correlation of the nanosizing effect with bioreactivity in relational map of nanotechnology with physics and biology. a, Specific surface area effect originated solely from material properties; b, physical size effect arising through particle–cell/tissue interaction.

area effect. It is well known that the specific surface area, which is defined as surface area for unit volume, is increased reciprocally with a decrease in particle size and chemical reactivity is pronounced. Therefore, high throughput is expected in the functions and performances of material properties and devices.

1.2. Specific surface area effect

One of the most important factors that affects the biocompatibility of materials is ionic dissolution, which is closely related to the specific surface area. This is also true for micro- and nanosizes, and very often becomes apparent as stimulus and toxicity. The nanosizing effect that affects biocompatibility is usually interpreted from this aspect.

1.3. Physical size and shape effect

On the other hand, corrosion-resistant and biocompatible Ti (Matsuno *et al.* 2001) causes inflammation, leading to osteolysis for the abraded fine particles (Tamura, Y. *et al.* 2002*a*,*b*; Uo *et al.* 2005*b*, 2007; Zhu & Watari 2007) which are produced from an artificial joint. Asbestos (Watari *et al.* 2006), a kind of clay mineral, induces mesothelioma after long-term, exposure to large quantities. These phenomena cannot be explained by the specific surface area effect and understood to be a different effect, i.e. physical size and shape effect, apart from the material properties of either toxicity or biocompatibility.

1.4. Nanoapatite

Meanwhile, hydroxyapatite (HAP), the main component of bone, shows different behaviour in synthetic apatite and bone. Synthetic HAP of a macroscopic size exhibits excellent osteoconductivity. However, it is not substituted for natural bone and remains permanently in the body. Therefore, it is used as an implant (Watari *et al.* 1997, 2004; Li *et al.* 2008*b*; Matsuo *et al.* 2001). Natural bone is composed of collagen and nanocrystallites of apatite of approximately 50 nm (Watari *et al.* 2001, 2005). Bone is continuously remodelled by resorption and new bone formation. Thus, there exist apatites with different behaviour, non-resorbable and resorbable apatite (Watari *et al.* 2007*b*). It is necessary to know the effect of nanosizing on this change of biofunctions using nanoapatite composites (Liao *et al.* 2005, 2007*a*–*c*; Gelinsky *et al.* 2007; Li *et al.* 2008*a*).

1.5. Carbon nanotubes

As another typical subject of nanomaterials, much attention has been paid to carbon nanotubes (CNTs) up to now owing to their unique structure (Asakura *et al.* 2005; Ushiro *et al.* 2006) and properties. The development of applications in the electronic and chemical fields such as field emission electron gun and hydrogen storage materials has been intensive. Recently, the derivatives of CNTs with different structures and compositions have also been discovered and synthesized (Sato *et al.* 2005*a*). Nanomaterials (Watari *et al.* 2007*a*,*b*, 2008*b*) and nanocomposites (Liao *et al.* 2007*d*) may have various effects on living organisms. There are arguments that CNTs may have serious toxicity due to their acicular or fibrous particle shape, associated with lung carcinogenicity of asbestos (Takagi *et al.* 2008). As an element, carbon (C) is bioinert. Graphite is used as an artificial heart valve due to its antithrombogenicity for biomedical application. There have not been many studies of CNTs and their biological effects (Kiura *et al.* 2005; Sato *et al.* 2005*a*,*b*; Yokoyama *et al.* 2005*a*) and biomedical applications (Fugetsu *et al.* 2004*a*,*b*; Akasaka & Watari 2005, 2008, 2009; Akasaka *et al.* 2005, in press *a*,*b*; Rosca *et al.* 2005; Uo *et al.* 2005*a*; Wang *et al.* 2005, 2007; Li *et al.* 2008*b*,*c*). From the viewpoints of both nanotechnology application and risk assessment of nanotoxicology, it is necessary to study the effect of nanosizing carbon by comparing isomorphs of macroscopic graphite and nanocrystalline CNTs.

1.6. Nanoparticle internal diffusion and drug delivery systems

Particles less than 10 μm can pass through the bronchial, and even smaller nanosize particles may reach the alveoli of lung. Then there is the possibility that the uptake of nanoparticles occurs through the respiratory system and the digestive system. Meanwhile drug delivery systems (DDS) are one of the most typical biomedical applications of nanoparticles being developed (Rosca *et al.* 2004). DDS is expected for the selective administration of anti-cancer agent and gene transfection to the desired organs. It is necessary to investigate the behaviour of nanoparticles in the internal body for the assessment of nanotoxicology and also essential to comprehend the diffusion paths of the DDS (Moller *et al.* 2008) to reach the diseased target (Chan *et al.* 2002; Moller *et al.* 2007; Smith *et al.* 2008). In this sense, internal diffusion is significant for both the demerit and merit aspects of nanotechnology.