modulus thereof was somewhat lower, statistically. These results suggest that the difference of the elasticity may depend on the crystallinity on the surface. By means of the graft-polymerization on the SF, the crystallinity on the surface of SF decreases. The elasticity of polymer-grafted SF, therefore, decreases. After the coupling between HAp and the modified SF, it seems that the crystallinity is compensated by the covering with the inorganic substrate on the surface of SF and the elasticity of the HAp/SF composite is apparently equal to that of the original SF. These results show that this HAp/SF composite maintains flexibility equivalent to the original SF.

3.5 Cell Interaction

Mouse fibroblast cell lines of L929 cells were cultured in a complete α-minimum essential medium (α-MEM, Invitrogen Corporation, Tokyo, Japan), supplemented with heat-inactivated 10% fetal bovine serum (FBS, Gibco), 50 IU/ml of penicillin, 50 μg/ml of streptomycin, and 2.5 μg/ml of amphotericin B (ICN Biomedicals Inc. CA, USA). To detect cell adhesiveness on the samples, cell morphologies were observed by SEM. L929 cells (1×10^5 cells/ml) were plated onto

24-well multiplates with fabric samples of 1.5 cm in diameter in an α-MEM with 10% FCS, and incubated at 37 °C for 24 h. As for the button-shaped samples, on the other hand, after the samples were immersed into 1.5-ml micro-tubes with 1.0 ml of the culture medium, an ultrasonic treatment was conducted via the tubes to remove gases from the gaps of the fibers for 1 min. L929 cells were subsequently plated in the micro-tubes with the samples at 1 x 10^6 cells/tube in an α-MEM with 10% FCS and incubated at 37 °C for 48 h. After being washed with phosphate-buffered saline (PBS(-)) three times, the samples were fixed with 2.5% buffered-glutaraldehyde for 20 min at 30 °C. The cells were dehydrated with aqueous ethanol (30-100%) and 100% *n*-butanol for 5 min at room temperature step by step. The samples were lyophilized and coated with gold.

To evaluate the cell adhesiveness on the HAp-coated SF, the morphologies of L929 fibroblast cells incubated on sample fabrics or devices were observed by SEM. **Figure 17** shows SEM observations of the surfaces of sample fabrics --- gelatin-coated glass as a positive control (a), untreated SF (b), hydrolyzed poly(MPTS)-grafted SF (c) and HAp-coated SF (d) --- incubated with L929 cells for 24 h. The cells hardly

adhered on the hydrolyzed poly(MPTS)-grafted SF as well as the untreated SF in **Figure 17 (b, c)**. Although it has been known that the initial cell adhesion on intact SF is actually not good **[19, 20]**, the reason has not been thoroughly manifested. It is presumed to depend on high surface wettability due to containing many hydrophilic amino acid residues[17] --- the hydroxyl group: Ser 10.63 mol%, Tyr 4.97 mol%, Thr 0.89 mol%; the carboxyl group: Asp 1.65 mol%, Glu 1.21 mol%; the amino groups: Lys 0.33 mol%, His 0.18 mol%, Arg 0.49 mol% --- and peptide bonds without an arrangement of Arg-Gly-Asp (RGD) as a cell-adhesion molecule, or probably the existence of a microdomain structure **[64]** consisting of crystalline and amorphous regions attributed to an arrangement of $(Gly\text{-}Ala\text{-}Ser)_n$ and the other residues in SF . The cells indicated weak interaction with the hydrolyzed poly(MPTS)-grafted surface during the initial incubation period because of the surface hydrophilicity belonging to Si-OH moieties on Si-O-Si cross-linking networks produced by hydrolysis of the poly(MPTS)-grafted SF substrate demonstrated by ATR FT-IR analysis in **Figure 11**. Meanwhile, the cells adhered well on HAp-coated SF similarly to the previous reports **[19, 20, 26]** and as shown in **Figure 17(d)**. The cross sectional view of the composite with a cell stained

shown in **Figure 18**. The needle-like microspikes from a cell incubated for 24h were elongated to a calcined HAp nanoparticle chemical bonded on the polymer substrate. It is clear that cells, thus, favorably adhere only on the HAp surface of the composite but not on the dehydrated grafted-surface without HAp particles on the SF substrate. It is estimated that cell-adhesion proteins in serum, such as fibronectin, vitronectin, bFGF, etc., prior to cell adhesion, adsorb on a HAp surface much better than on an area of dehydrated graft-polymer (**Scheme 4**). We previously reported that L929 cells restrictedly elongate their needle-like microspikes against an amino-group-modified TiO_2 (TiO_2-NH_2) surface but that poly(acrylic acid(AAc))-grafted regions on the composite of TiO_2-NH_2 nano-particles covalently linked onto a polyAAc-grafted silicone substrate [**65**]. A similar phenomenon takes place in the interface between a cell and a HAp composite on a nano-scaled HAp-coated SF substrate. That is to say, sintered HAp nanoparticles can provide bioactivity to a polymer substrate.

4. Fabrication of Medical Devices

4.1 A Percutaneous Device

A polymer substrate for a button form was molded using a silicone compound (KE153-U, Shin-Etsu Chemical Co. Ltd., Tokyo, Japan) to fix the pattern (**Figure 19**). The internal diameter was based on the external diameter of a catheter for intravenous hyperalimentation (IVH). Silicone buttons covered with a silicone adhesive (TSE399, GE Toshiba Silicones Co., Ltd., Tokyo, Japan) which were then attached to a rotator on a motor which revolved at 450 r.p.m., by which HAp/SF fibers of about 100 mm in length thoroughly coated the buttons. The HAp/SF fiber-coated samples were heated at 120 °C for 2 h in vacuum and were then washed in distilled water by using an ultrasonic generator for 3 min (output: 20kHz, 35 W) to remove the fibers which adhered incompletely.

To fabricate a prototype for a percutaneous device, the HAp-coated SF fibers were transplanted onto a button-shaped substrate made of silicone via an adhesive agent. The weight ratio of three-dimensional coating consisting of composite fibers versus the total weight of the device was 14.3 ± 0.4 wt% (n=4). The prototype of a button was fabricated by a silicone compound cast into a mold according to the illustration shown in **Figure 19**. The form was designed to install it a catheter for IVH whose

outer-diameter of tube showed 3.0 mm. **Figure 20** shows the external appearances of the button allowing the catheter tube through. The device is white and very flexible. **Figure 21** shows a magnified view of the edge of the button coated with HAp-modified SF fibers. The HAp-coated SF fibers of 100 μm in length were uniformly transplanted individually in random directions on the button.

A cell adhesion experiment using fibroblast cells was also conducted via the sample device. After incubation of untreated SF or HAp-coated SF buttons in a micro-tube for 48 h, the morphologies of the cells on both substrates were observed by SEM (**Figure 22**). On HAp-coated SF buttons, the cells adhered and spread widely on the side surface. More spherical cells accumulated on other cells spread on the upper side of the button than on the side. This phenomenon shows that adhering cells accumulated in a multiple way on the substrate according to gravity in a three-dimensional incubation. On the other hand, few spherical cells were observed on untreated SF fibers transplanted on the button. These phenomena were equally consistent with the tendency of cell-adhesiveness on the fabric samples. The three-dimensional composite is, therefore, useful as a cell scaffold. The percutaneous

device was implanted in back of a rabbit for 3 months according to the Guideline for Animal Experimentation National Cardiovascular Center. The skin tissue was adhered on the device without a gap between the tissue and the material surface, and severe inflammation and abscess was not observed from the external view (**Figure 23**).

4.2 An Artificial Blood Vessel

We developed a novel inorganic-organic composite consisting of calcined HAp nanoparticles chemically bonded on polymer substrate. HAp nanoparticles were covalently linked onto a poly(ethylene terephthalate) (PET) fabric substrate chemically modified by graft polymerization with MPTS for development of artificial blood vessel. **Figures 24** shows SEM images of human umbilical vein endothelial cells (HUVEC) morphologies and fluorescence images of stained nuclei of HUVEC on sample substrates after 4 h of incubation. The initial interaction of HUVEC on substrates was evaluated after 4h of incubation, according to several reports [**66, 67**]. In the SEM images, it seemed that many cells adhered on HAp/PET fabric as well as collagen-coated PET, while only a few cells adhered on the original fabric. The difference in the number of

cells which adhered could not be distinguished by SEM observation, since HUVEC were flattened and spread over the substrate. The cells adhered were then stained by fluorescent dye and observed by a fluorescence microscope. It was found that the number of cells adhered on HAp/PET was qualitatively the same as that of collagen-coated PET, although the cells seldom adhered on the original PET for such a short period of incubation. This phenomenon can be explained because cell adhesion proteins such as fibronectin or vitronectin in a culture medium may be favorably adsorbed on the HAp surface [**22**]. In other words, it is clear that calcined HAp coating on popular biomedical substrates is effective to obtain the affinity of cells without using biological scaffold proteins such as collagen or gelatin. It can be said that the HAp/PET composite is very meaningful for biological safety in medical fields today when the danger of BSE infection by using proteins derived from a bovine animal is trumpeted loudly. A prototype of artificial blood vessel made of the HAp/PET composite was fabricated (**Figure 25**). The calcined HAp nanoparticles were thoroughly coated on PET fibers of inside and outside of an artificial blood vessel. The effect of HAp nanocrystals on it through animal implantation experiments *in vivo* are evaluating

now.

5. Conclusions

Well–crystallized and calcined HAp nanoparticles with free impurities were synthesized through the modified emulsion process. The morphology and size of the HAp nanoparticles were drastically changed by the altering of reaction temperature. Calcinaed HAp nanoparitcles with spherical or lod-like morphologies well-disperspersed in a liquid media were also successfully prepared by calcinations using an anti-sintering agent interspersed between or surrounding the particles, followed by removal of the agent. The inorganic-organic composite consisting of calcined HAp nanoparticles and polymer substrates were prepared through chemical bonding, such as covalent or ionic bonding. The elasticity of the composite was not change compared to the original polymer substrates. The cell-adhesion test shows that the HAp/polymer substrate improves bioactivity compared to original substrates. Cells were also able to penetrate into the gaps between the inorganic-oroganic compsite in a three-dimensional tangle. A percutaneous device and an artificial blood vessel were fabricated from the

HAp composite polymer fibers have been examined by animal implant experiments.

Preparation of composites consisting of calcined HAp nanoparticles and polymer substrates, for examples, mixture, *in situ* or nano-chemical bonding methods, is necessary for nano-scaled observation from the points of view of the bulk structures, surface properties, biological interactions, etc. For our composite, especially, the size, coverage ratio or strength of chemical bonding of sintered HAp nanoparticles assumed to be very important to know interactions with biomolecules such as proteins, cells, tissues to develop medical devices. This composite material is expected to establish a novel concept for fabrication of an inorganic-organic composite as biocompatible materials for hard and soft tissue.

Acknowledgements

The authors are grateful to Dr. Junzo TANAKA, Biomaterials Center, National Institute for Materials Science, and Prof. Akio KISIDA, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, for their helpful advices and supports. A series of the study about the development of the

nanocompoiste was financially supported in part by research programs of Japan Science and Technology Corporation (JST) -- CREST, PRESTO and Innovation Plaza Osaka --, and promoting projects on Comprehensive Research on Cardiovascular Diseases.

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Scheme and Figure Captions

Scheme 1 Schematic presentation of an inorganic-organic composite material by chemical bonding between the interface.

Scheme 2 Chemical structures of (a) 2-(o-[1'-methylproplidenamino] carboxyamino) ethyl methacrylate (MOI-oxime), (b) 2-methacryloxyethyl isocyanate (MOI), (c) γ-methacryloxyethyl trimethoxysilane (MPTS), and (d) 4-methacryloyloxyethyl trimellitate anhydride (4-META).

Scheme 3 Schematic presentation of the synthesis of the HAp/SF composite by ionic bonding.

Scheme 4 Schematic presentations of the preparation of the composite and the expected mechanism of cell adhesion on calcined HAp nanoparticles coated SF.

Figure 1 XRD profiles of the calcined HAp nanoparticles synthesized at (a) 25 and (b) 50 °C.

Figure 2 FT-IR spectra of the calcined HAp nanoparticles synthesized at (a) 25 and (b) 50 °C.

Figure 3 TEM micrographs of the calcined HAp nanoparticles ynthesized at (a) 25 and (b) 50 °C.

Figure 4 TEM micrographs of prepared HAp nanoparitcles obtained after calcining; (a) prepared at 50 °C showing truncated faces and elongation along c axis; (b) associated ED pattern corresponding to [0 1 0] zone.

Figure 5 Size distributions of HAp particles calcined at 800°C for 1 h with (a) and without (b) an anti-sintering agent, PAA-Ca, surrounding the HAp particles followed by washing with water to remove the thermal decomposed product of PAA-Ca, CaO. The size distribution was measured in ethanol as a medium by using dynamic light scattering.

Figure 6 SEM photograph (a), TEM photograph (b) and the associated electron diffraction pattern (c) of spherical HAp crystals calcined at 800°C for 1 h