Chemical Modification of Metallic Implant Surfaces with Biofunctional Proteins (Part 1) Molecular Structure and Biological Activity of a Modified NiTi Alloy Surface

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Received July 31, 1995/Accepted October 27, 1995

Human plasma fibronectin (pFN), an adhesive protein, was covalently immobilized onto an alkylaminosilane derivative of a NiTi substrate with glutaraldehyde through Schiff's base formation. The surface at different stages of the modification was characterized by X-ray photoelectron spectroscopy (XPS), and the amount of immobilized pFN was determined by a fluorometric method. The spreading behavior of human gingival fibroblasts was examined on the modified surface. The XPS spectra suggested that γ -aminopropyltriethoxysilane (γ -APS) was bonded to the surface through metallosiloxane bonds (Ti-O-Si) formed via a condensation reaction between the silanol end of γ -APS and the surface hydroxyl group, with a highly cross-linked siloxane network formed after heat treatment of the silanized surface at 100°C. The pFN was immobilized at the surface density of $1.1 \mu\text{g}\cdot\text{cm}^{-2}$, and significantly promoted fibroblast spreading, suggesting that this chemical modification offers an effective means of controlling metal/cell interactions. These results may contribute to the development of bioactive metallic implants.

Key words: Implant, Surface treatment, Biocompatibility

INTRODUCTION

When a metallic implant comes into contact with tissue, the first reaction at the material-tissue interface is the adsorption of proteins from the biofluid¹⁾. It is widely accepted that these adsorbed proteins influence the subsequent development of the biological response.

A major consideration in designing implants is the production of surfaces that promote desirable responses in the cells and tissues that contact with the implant. Precoating the metal surface with specific proteins seems to offer a promising method for producing biocompatible implants which combine sufficient bulk mechanical properties with surface biological activity. Simple preadsorption of proteins, however, is insufficient to produce a biologically active metallic implant because of the dynamic exchange between the preadsorbed proteins with those circulating *in vivo*. Williams *et al.*²⁾ examined the adsorption and desorption behavior of albumin and fibrinogen on 17 different metal surfaces using ¹²⁵I-labeled proteins, and found that *in vivo* substantial desorption of the preadsorbed proteins occurred within 106 hours for most metals. The immobilization of biological macromolecules or bioinert hydrophilic polymer chains has been extensively studied to prevent the physical removal of the molecules from the surface, especially in relation to blood compatibility^{3,4)} and tissue integration^{5,6)}. In contrast, these chemical surface modifications have not been applied to metallic implants, presumably due to their relatively unreactive surfaces.

In the present study, a chemical modification technique for metal surfaces, which has previously been applied to biosensors⁷⁻⁹, was employed for the covalent immobilization of biofunctional proteins on a metallic implant surface. Human plasma fibronectin (pFN), an adhesive glycoprotein of blood, was covalently immobilized onto a NiTi shape memory implant through Schiff's base formation to enhance cell attachment and spreading on the surface. The surfaces at different stages of the modification process were characterized by X-ray photoelectron spectroscopy (XPS). The stability of the covalent bonds formed between the substrate and pFN via coupling agents was assessed, and the fibroblast response on the pFN-modified surface was examined *in vitro*.

MATERIALS AND METHODS

Covalent immobilization of protein

A NiTi alloy plate (10 mm dia. and 1 mm thick) was employed as a substrate. The alloy was polished mechanically to a mirror finish using emery papers followed by 0.05 μm alumina paste. Prior to the surface modification, the polished substrates were cleaned ultrasonically in distilled deionized water for 1 min and then dried with flowing air. The human plasma fibronectin* (pFN) was immobilized by coupling it to an alkylaminosilane derivative of the substrate with glutaraldehyde through Schiff's base formation⁷⁻⁹. pFN was used because its molecular structure and function as an adhesive protein have been well established^{10,11}.

Firstly, γ -APS (γ -aminopropyltriethoxysilane) was applied to the NiTi substrate to introduce amino groups onto the surface.

The silanation was performed by dipping the substrate in a 1 % (v/v) aqueous solution of γ -APS (pH=10.4). After 1 min, the substrates were removed from the solution and excess solution was blown off the surface using a stream of air. Silane-coated substrates were allowed to cure in an electric furnace at 100°C for 60 min, cooled to room temperature in a desiccator, and then placed in 5 % (v/v) aqueous glutaraldehyde solution (pH 7.0) for 90 min. After rinsing with 0.1M borate-buffered saline, pH 8.5 (BBS), pFN was immobilized on the substrates by dipping in the protein solution (1 mg/ml, pH 8.5).

After 90 min, 0.5 mg of NaBH_4 was added to the solution twice at 20 min intervals to reduce the Schiff's bases formed between the aldehyde and the amino groups¹².

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At 130 min, the substrates were removed from the solution and rinsed with phosphate-buffered saline (pH 7.4, PBS), to remove protein molecules non-specifically adsorbed on the surface.

Characterization of modified surface

The molecular structures of films formed by silanization and protein-immobilization on NiTi substrates as well as the structure of the oxide film on the polished surface were characterized by XPS. The XPS spectra were obtained using an X-ray photoelectron spectrometer** with Al K α source run at 7 kV accelerating voltage and 30 mA current under a vacuum of 5×10^{-7} Pa. Each sample was analyzed at a 90° angle relative to the electron detector. The binding energy scale was calibrated by the Au 4f $_{7/2}$ and Cu 2p $_{3/2}$ peaks at 83.8 eV and 932.8 eV, respectively. Curve fittings of multiple peaks in the spectra were effected using the background subtraction and Gaussian shape fit program#.

Quantitation of immobilized protein

The fibronectin molecules immobilized on the substrate were hydrolyzed in an alkaline solution and the resulting amino acids were determined by the fluorometric method¹³⁾. The specimen was placed in a test tube (12 mm dia. and 40 mm high) containing 0.5 ml of 0.5N NaOH, and heated at 120°C for 20 min in an autoclave. After the sample solution was neutralized with 0.5 N HCl (0.4 ml) and BBS (1.0 ml), 0.15 ml of fluorescamine in acetone (30mg in 100 ml) were added, and immediately the test tube was vigorously shaken for 30 s. A spectrofluorometer## was used to measure the fluorescence intensity of the sample solution with the excitation wavelength at 370 nm and emission at 475 nm.

The pFN-immobilized substrates were immersed in PBS (pH 7.4) or a phthalate buffer solution (pH 4.0) for 1 week at 37°C to examine the stability of the covalent bonds formed between the proteins and a passive oxide film on the substrate. The buffer solutions were renewed daily. The amount of fibronectin remaining on the substrate was determined as described above.

Electron microscopic observation of cell morphology

Human gingival fibroblasts were obtained from young, healthy patient before tooth extraction for orthodontics procedures. Dulbecco's modified eagle medium (DMEM®) supplemented with antibiotics (penicilline G 100 μ g/ml ; gentamycin 50 μ g/ml ; amphotericin B 3 μ g/ml) and 18% fetal bovine serum was used for the culture. When the outgrown cells from the tissue explant became confluent, they were treated with a trypsin solution [0.25% trypsin and 0.1% glucose in citrate-saline (pH 7.8)], and resuspended in DMEM free of serum. The cell suspension was plated on the substrates placed in 35 mm culture dishes at a cell population density of 4×10^4 cells/ml. At 30 min or 120 min after cell plating, the cells attached on the substrates were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 120 min, rinsed three times with PBS, and dehydrated in a graded series of ethanols from 35 to 100%. The samples were then critical-point dried with CO $_2$ and sputter-coated with gold. The

* * ESCA-850, Shimadzu Co. Ltd., Kyoto, Japan

ESPAC-200, Shimadzu Co. Ltd., Kyoto, Japan

F-2000 Fluorescence Spectrophotometer. Hitachi, Tokyo, Japan

@ Nissui, Pharmaceutical Co., Ltd., Tokyo, Japan

samples were examined through a scanning electron microscope (SEM) operated at an accelerating voltage of 25 kV.

RESULTS

Surface characterization by XPS

Figs. 1 (a) to (e) show the Ti 2p, Ni 2p, O 1s, N 1s, and Si 2p spectra obtained from the polished (PL), the silanized (SL), and the pFN-immobilized (FN) NiTi substrates. In Fig. 1 (a), the Ti 2p spectral peaks at 458.7 eV and 464.7 eV corresponded to the Ti 2p_{3/2} and Ti 2p_{1/2} sublevels in TiO₂^{14,15}. A small peak at 454.3 eV for PL was attributed to the Ti 2p_{3/2} sublevel in metallic titanium under an oxide film, implying that the thickness of the film on the NiTi substrate was less than the escape depth of photoelectrons (< 2 nm)¹⁶. There were no changes in Ti 2p spectral peak positions on the surfaces at different stages of the modification process, but the spectral intensity decreased when γ -APS was coupled, and then pFN was immobilized on the NiTi substrates.

Fig. 1 (a) Ti 2p spectra obtained from surfaces at different stages of the modification, PL: polished surface, SL: silanized surface, FN: pFN-immobilized surface.

Fig. 1 (b) Ni 2p spectra obtained from surfaces at different stages of the modification, PL: polished surface, SL: silanized surface, FN: pFN-immobilized surface.

The major Ni $2p_{3/2}$ peaks at 852.8 eV in Fig. 1 (b) were assigned to metallic nickel in the alloy substrate under the oxide film. These spectra were asymmetric with two small peaks on the higher binding energy side at 856.0 eV and 859.0 eV. The peak at 856.0 eV could be assigned to the Ni^{2+} state in the oxide film. This chemical state was expected to be similar to $Ni(OH)_2$ from reported binding energy value¹⁷⁾. The small intensity of the peak at 856.0 eV, however, suggested that the oxide film on the NiTi substrate mainly consisted of TiO_2 , almost identical to an oxide film formed on pure titanium. The other small peak at 859.0 eV was the shake-up satellite associated with the major peak at 852.8 eV. There was no difference in the peak positions of Ni $2p_{3/2}$ spectra obtained from the three surfaces.

The O 1s spectrum for the polished substrate (Fig. 1 (c), PL) exhibited a shoulder on the higher binding energy side of the major peak. The major peak at 530.2 eV with 1.6 eV full width at half maximum (FWHM) corresponded to the bulk oxygen of TiO_2 ¹⁴⁾. This shoulder could be attributed to the surface hydroxyl groups and the adsorbed water molecules on the oxide film¹⁴⁾. A striking feature of the O 1s spectrum for the silanized substrate was the presence of a peak at 532.6 eV with 2.2 eV FWHM (Fig. 1 (c), SL), which could be attributed

Fig. 1 (c) O 1s spectra obtained from surfaces at different stages of the modification, PL: polished surface, SL: silanized surface, FN: pFN-immobilized surface.

Fig. 1 (d) N 1s spectra obtained from surfaces at different stages of the modification, PL: polished surface, SL: silanized surface, FN: pFN-immobilized surface.

to the oxygen atoms in the γ -APS film. On the pFN-immobilized surface, an O 1s peak centered at 532.1 eV with 2.6 eV FWHM was observed (Fig. 1 (c), FN). This broad spectrum consisted of several component spectra arising from oxygen atoms in different chemical states present in the coupled γ -APS and the immobilized pFN as well as the surface hydroxyl groups, as will be discussed later.

The N 1s major peak at 399.6 eV for the silanized surface (Fig. 1 (d), SL) was attributed to the free amine introduced to the substrate^{18,19}. The major peak at 400.7 eV for the pFN-immobilized surface (Fig. 1 (d), FN) could be assigned to the imino groups in the peptide bonds of pFN. Surface nitrogen contaminants were not observed on the polished sample (Fig. 1 (d), PL).

The spectral peak of Si 2p exhibited no change in binding energy upon immobilization of pFN but its intensity was lowered by the overlying pFN (Fig. 1 (e)). No silicon contaminants were observed on the polished surface (Fig. 1 (e), PL).

Fig. 1 (e) Si 2p spectra obtained from surfaces at different stages of the modification, PL: polished surface, SL: silanized surface, FN: pFN-immobilized surface.

Table 1 The amount of fibronectin ($\mu\text{g}\cdot\text{cm}^{-2}$) on the NiTi substrates

As-immobilized	After immersion in PBS (pH 7.4) for 7 days	After immersion in phthalate buffer (pH 4.0) for 7 days
1.08 (0.12)	0.94 (0.18)	1.04 (0.13)

Averages of determinations on three specimens, () S.D.

Fig. 2 Spreading responses of fibroblasts on the NiTi substrate. Human gingival fibroblasts were inoculated onto (a, b) polished or (c, d) pFN-immobilized substrates. (a, c): 30 min spreading, (b, d): 120 min spreading.

Amount of immobilized protein

Table 1 shows the amount of pFN immobilized and the amount remaining on the NiTi substrate after immersion in PBS (pH 7.4) or a phthalate buffer solution (pH 4.0) for 7 days. With the chemical modification technique employed, approximately 1 μg of pFN was immobilized on the unit area (1 cm^2) of the NiTi substrate. There were no significant differences in the amounts of pFN on the substrate before and after immersion, indicating that the covalently immobilized pFN remained on the substrate surface in neutral solutions and in acid solutions at pH 4.0.

Spreading of fibroblasts on the modified surface

Fig. 2 shows the morphology of human gingival fibroblasts on the polished substrates (a, b) and on the pFN-immobilized substrates (c, d) after incubation for 30 min (a, c) and 120 min (b, d) in the serum-free medium. After 30 min of incubation, approximately 50 % of the attached cells on the polished substrate were still spherical in shape without prominent cytoplasmic webbing (Fig. 2 (a)). In contrast, all cells on the pFN-immobilized substrate initiated a cytoplasmic spreading peripherally in a centrifugal fashion from the base of the cell mass (Fig. 2 (c)). After 120 min of incubation, fibroblasts on the pFN-immobilized substrate flattened considerably and developed a spindle shape (Fig. 2 (d)), whereas cells on the polished substrate exhibited insufficient spreading (Fig. 2 (b)). These results demonstrated that fibroblast spreading was extensively promoted by the covalently immobilized pFN on the NiTi substrate.

DISCUSSION

Molecular structure of γ -APS film

γ -APS plays an essential role in binding protein molecules to the NiTi substrate, and the molecular structure of the γ -APS film is a critical factor in the subsequent immobilization of pFN. The O 1s and N 1s spectra obtained from the silanized surface indicate the interaction of γ -APS with the oxide film on the substrate and the chemical state of its organofunctional group ($-\text{NH}_2$), respectively.

In the O 1s spectrum (Fig. 1 (c), SL), the FWHM of the spectral peak centered at 532.6 eV is broad (2.2 eV) compared with that of the peak at 530.2 eV (1.6 eV) which is attributed to TiO_2 . This apparent broadening can be explained by the overlapping of two sub-peaks. The O 1s spectrum can thus be separated into three spectra as shown in Fig. 3, assuming that the component spectra I and III may be attributed to the O^{2-} in the surface oxide film (TiO_2) and the bridging oxygen in the siloxane bond (Si-O-Si), and that their peak positions and the FWHMs were consistent with those for bulk oxygen in TiO_2 and bridging oxygen in silica glass. Table 2 summarizes the peak positions and the FWHMs for the standard samples and the three separated component spectra. The O 1s peak II, which had an intermediate binding energy between the 530.2 eV for O^{2-} in TiO_2 and the 533.1 eV for the bridging oxygen, can be assigned to the oxygen in the metallosiloxane bond (Ti-O-Si) formed via the condensation reaction between the silanol-end of γ -APS and the surface hydroxyl group, although a contribution from adsorbed water is also possible. Moses *et al.*¹⁸⁾ also suggested the presence of a Ti-O-Si bond on the silanized surface of TiO_2 from the observed O 1s peak at 532.0 eV,

Table 2 O 1s peak position and FWHM of standard samples (silica glass, TiO₂) and three separated component spectra of the O 1s spectrum obtained from the silanized surface (Fig. 4)

Standard sample spectra		Separated component spectra	
TiO ₂ ¹ (Ti-O-Ti)	530.2±0.1eV (1.6eV)	Component I	530.2eV (1.6eV)
		Component II	532.0eV (1.6eV)
Silica glass ²	533.1±0.1eV (1.7eV)	Component III	533.1eV (1.7eV)

() : FWHM

¹ prepared by heating Ti metal at 500°C for 30 min in air

² purity better than 99.99%

which is consistent with the energy position of peak II. The hydroxyl groups present in the uppermost layer of the surface oxide (TiO₂) are responsible for this Ti-O-Si bond formation. Considering that the pK_a of silanol groups is about 3.0, much lower than the isoelectric point of the polished titanium substrate (12.0)²⁰, the surface hydroxyl groups could act as Brönsted bases and react with silanol groups of γ -APS to form the Ti-O-Si bond in 1 % γ -APS aqueous solution at pH 10.4.

Moses *et al.*¹⁸) performed silanization of a titanium sample under an anhydrous reaction condition (in benzene or xylene containing 1-10% alkylamine-silane) to prevent the growth of three-dimensional siloxane polymers, and no peaks were observed in the O 1s spectrum at binding energies higher than 532.0 eV for the Ti-O-Si bond. The O 1s peak at 533.1 eV in Fig. 3 indicates the presence of a high cross-linked siloxane network on the NiTi substrate. Silanization in an aqueous solution followed by heat treatment at 100°C for 60 min allowed the polymerization of silane to form a high molecular weight siloxane polymer²¹). This layer may contribute to the reduced permeation rate of water to the interface, resulting in an improved resistance to degradation of the substrate/silane interface after water immersion (Table 1).

The broad O 1s component peak at 532.1 eV for the pFN immobilized surface (Fig. 1 (c), FN) mainly arose from the immobilized pFN. The broadness of this peak indicates a mixture of various chemical states of oxygen including a peptide bond (-C (O) NH-) and several oxygen-containing groups present in pFN, such as -COOH, -OH, and -C-O-C-.

Curve fitting of the N 1s spectrum obtained from the silanized surface distinguished the two chemical states of nitrogen (Fig. 4). The major peak at 399.6 eV attributed to free amine (-NH₂) was coupled with a protonated amine (-NH₃⁺) peak at 401.2 eV. The relative intensities of the two components indicate that approximately 25 % of the amino groups were protonated. The protonated amine may be due to bicarbonate salt (-NH₃⁺HCO₃⁻)²²) or to the protonation achieved via surface hydroxyl (Ti-O⁻ +H₃N-)²²) and/or silanol groups (Si-O⁻ +H₃N-)²²). and/or silanol groups (Si-O⁻ +H₃N-)²³). The three possible forms of protonated amine are schematically illustrated in Fig. 5 (I-III). It was not possible, however, to distinguish between these three possibilities by XPS analysis alone. Approximately 75% of the amino groups introduced on the NiTi substrate were free amines (IV in Fig. 5) which took

Fig. 3 O 1s spectrum from the silanized surface of the NiTi substrate, Solid line: measured spectrum; dotted lines: separated component spectrum; chained line: sum of component spectra.

part in the immobilization of proteins. This implied that the majority of the γ -APS molecules bonded to the surface via the silanol groups; amino groups stayed away from the surface. This may support the idea that a metallosiloxane bond is present at the substrate/silane interface.

Characterization of immobilized pFN

The surface density of the immobilized pFN on the NiTi substrate was determined to be $1.1 \mu\text{g}\cdot\text{cm}^{-2}$ (1.5×10^{12} pFN molecules/ cm^2). Hughes *et al.*²⁴⁾ demonstrated that maximal spreading of a hamster fibroblast on a pFN-adsorbed plastic dish required a minimum surface density of ca. $0.015 \mu\text{g}\cdot\text{cm}^{-2}$ (1.8×10^{10} pFN molecules/ cm^2). With the chemical modification technique employed in this study, the pFN immobilized onto the NiTi substrate was approximately 84 times the minimum amount required to enhance cell spreading.

A major problem in protein immobilization is the significant reduction in biological activity due to a conformational change in protein molecules. The reported remaining enzymatic activities for various enzymes covalently immobilized onto the glass surface were

Fig. 4 N 1s spectrum from the silanized surface of the NiTi substrate. Solid line: measured spectrum; dotted lines: separated component spectrum; chained line: sum of component spectra.

Fig. 5 Schematic illustration of the molecular structure of γ -APS on the NiTi substrate. I-III: protonated amine, IV: free amine

37–74 % of the original values²⁵). In the present study, the immobilized pFN was found to significantly promote fibroblast spreading, indicating that pFN molecules retained biological activity after immobilization onto the NiTi substrate. It should be noted, however, that the activities of proteins covalently bound to the substrate must be separately examined for each protein employed.

Covalent bonds between the substrate and the immobilized pFN through γ -APS and a glutaraldehyde were resistant to hydrolysis in solutions at pH 4.0–7.0. The pH of the biofluid drops to as low as 5 when the implant is inserted into bone²⁶). From the present limited study it appears that the covalent bonds including the metallosiloxane bonds (Ti–O–Si) are stable in an acidic environment, but a longer-term, more comprehensive study is necessary to examine the stability of the modified layer, especially the stability of the immobilized proteins in the presence of lysosomal enzymes released by activated leukocytes at the site of injury²⁷).

Bioactive metallic implants with an immobilized protein

Fig. 6 illustrates the molecular structure of a bioactive metal surface with covalently immobilized biofunctional proteins obtained by chemical modification techniques using an aminosilane and a glutaraldehyde. The NiTi substrate possesses adequate mechanical properties to support heavy loads. The passive oxide layer on the surface, mainly composed of TiO_2 , is responsible for the good corrosion resistance of the NiTi substrate. A number of biological functions can be offered to the NiTi substrate with the immobilization of proteins. The coupling layer and the immobilized protein also provide additional corrosion resistance

Fig. 6 Schematic illustration of a surface structure of a bioactive metallic implant modified with a biofunctional protein. Each layer offers, (a): sufficient mechanical property, (b): high corrosion resistance, (c): additional corrosion resistance, (d): specific biological activity.

to the NiTi substrate. A complete discussion of the improved corrosion resistance of NiTi alloy chemically modified with pFN is found elsewhere²⁸⁾. The chemical modification of the metal surface with biofunctional proteins employed in this study provides an effective means to control the metal/tissue interactions. For an endosseous metallic implant, the immobilized pFN which promotes fibroblast spreading *in vitro* (Fig. 2) seems promising to enhance the epithelial and fibrous connective tissue adaption to a metallic implant neck. In addition, if the bone morphogenetic protein (BMP) with type I collagen can be immobilized onto a fixture surface while retaining the biological activity, the result would be an osteoinductive metallic implant.

The chemical modification is feasible for all materials which have hydroxyl groups (-OH) on the surface, including base metal alloys and ceramics. The same results can also be obtained for pure titanium, which has been widely used in endosseous implants, since the surface oxide film of TiO₂ is almost identical to the film on NiTi alloy. Prior to the application of γ -APS, precious metal alloys require the precoating of a glass layer to introduce the -OH groups on the surface. The glass layer can be formed using the silicoater system, which has been used for enhancing adhesion between precious metal alloys and resin.

CONCLUSION

Fibronectin was covalently immobilized onto a NiTi substrate by chemical modification using an aminosilane and a glutaraldehyde. Fibroblast spreading was significantly promoted by the immobilized fibronectin. Such chemical modification of metal surfaces can provide an effective means to control metal/tissue interactions and can lead to bioactive metallic implants.

ACKNOWLEDGMENTS

This research was partly supported by a Grant-in-Aid for Scientific Research of the Ministry of Education, Science and Culture of Japan (No. 06771843). The author wishes to thank Dr. H. Ohno, Health Sciences University of Hokkaido, and Dr. A. Sate, Tokyo Medical and Dental University for their advice and encouragement, Drs. Y. Abiko and T. Kaku for help with cell culture experiments.

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アルギン酸ナトリウムを用いて合成した繊維状リン酸カルシウム塩の結晶相

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リン酸塩を混合したアルギン酸ナトリウム水溶液をノズルを装着したシリンジを用いて, 塩化カルシウムと酢酸カルシウムを混合し酢酸で pH7 に調整した紡糸液中に吐出し, イオン交換させ, ゲル状繊維を合成した。乾燥後, 大気中焼成し繊維状リン酸カルシウム塩を得た。焼成温度の上昇に伴い, 繊維状リン酸カルシウム塩の結晶性は増加し, 900°C焼成時にハイドロキシアパタイト ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, 以下 HA と略す) の単一結晶相を

呈した。ゲル状繊維の紡糸液中での熟成時間が5分以下の時, 焼成繊維は HA 及び NaCaPO_4 の混合結晶相であった。また, 焼成繊維は熟成後の蒸留水による水洗時間が3秒以下の時, HA, クロロアパタイト ($\text{Ca}_5\text{Cl}(\text{PO}_4)_3$) 及び酸化カルシウムの混合結晶相であり, 5分~1時間では HA の単一結晶相であった。しかし, 水洗時間が2週間を越えると, HA と $\beta\text{-Ca}_3(\text{PO}_4)_2$ の混合結晶相を呈した。

臼歯部用光重合型コンポジットレジンの疲労強度

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近年, 歯科材料における疲労特性の重要性が指摘されている。そこで本研究では水中保管した2種類のコンポジットレジンの疲労特性について検討した。試験には Hybrid type と MFR type の2種類のコンポジットレジンを用い, ダンベル型の試片を作製し, 37°C水中に24時間, 1ヵ月間, 3ヵ月間, 6ヵ月間, 12ヵ月間保管してから疲労試験と引張試験を行った。疲労試験ではステアケース法を用いて 10^5 回における疲労強度を求めた。

24時間後の疲労強度は, Hybrid type で 54.7 MPa, MFR type で 28.1 MPa であり, ともに経時的に減小傾向がみられた。一方, 直接引張強さは Hybrid type で 77.0 MPa, MFR type で 53.5 MPa であり, Hybrid type では水中保管期間にかかわらず変化がみられなかったが, MFR type では経時的に小さくなる傾向が認められた。

機能性タンパク質薄膜によるインプラント用金属表面の化学修飾

(第1報) 修飾表面の分子構造と生体活性

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インプラント用金属に生体活性を付与するために, 金属表面を機能性タンパク質で化学修飾する方法を検討した。今回はシッフ塩基結合法を用い, NiTi 合金表面に接着性タンパク質として知られる血漿フィブロンネクチン

(pFN) を固定化し, 各処理段階における表面構造を X線光電子分析装置で調べた。さらに, 固定化した pFN をフルオレサミンを用いた蛍光法により定量した。その結果, pFN は合金表面に結合したシランカップリング剤に

より導入されたアミノ基及びグルタルアルデヒドにより表面に固定され、表面上での密度は約 $1 \mu\text{g} \cdot \text{cm}^{-2}$ であることが明らかとなった。また、pFN を表面に修飾した合金上では、研磨状態の試料と比較して、ヒト歯肉由来の線維芽細胞はより扁平に伸展しており、細胞の伸展が

促進されていることが確認された。以上の結果より、機能性タンパク質を金属表面に修飾し、金属-細胞間の相互作用を制御することにより、バイオアクティブな金属インプラントの開発が可能であることが示唆された。

機能性タンパク質薄膜によるインプラント用金属表面の化学修飾 (第2報) 化学修飾した NiTi 合金の耐食性

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機能性タンパク質を表面に化学修飾したインプラント用 NiTi 合金の耐食性を生理食塩水中ならびに細胞培養液中で評価した。アミノシランとグルタルアルデヒドを架橋剤として用い、ヒト血漿フィブロネクチン(pFN)を表面に固定化した試料のアノード分極挙動と腐食速度を電気化学的手法を用いて調べた。また、NiTi 合金上に生成した酸化被膜の組成、厚さ、ならびに表面に吸着したタンパク質をX線光電子分析装置を用いて調べた。不働態保持電流密度は、血清タンパク質の存在により増大した。これは、血清タンパク質が合金表面に速やかに吸着し、金属イオン・タンパク質複合体を形成して溶液中

に拡散することにより、酸化被膜の溶解速度が増大したためと考えられる。表面に化学修飾を施した試料の腐食速度は、いずれの溶液中においても、研磨状態の試料と比較して約 1/2 に減少することが明らかとなった。合金表面に形成されたポリシロキサン層により、腐食反応に関与する溶存酸素や血清タンパク質、腐食生成物である金属イオンや金属配位化合物の拡散が抑制されたためと考えられる。本表面処理法は、合金表面に生体活性を付与すると同時に耐食性を向上させるため、バイオアクティブな金属インプラントの開発に有用と考えられる。

コンポジットレジンの歯質接着性が辺縁の劣化に及ぼす影響

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コンポジットレジンの歯質接着性を変えることにより、繰り返し負荷後、窩洞辺縁部の劣化度にどのような影響がみられるのかを検討した。接着条件として、エッチングおよびボンディング処理の有無や種類を変えて1級窩洞にコンポジット充填を行い、40万回の繰り返し荷重負荷後、辺縁部の露出エナメル質の幅を劣化度として測定した。その結果、エッチングおよびボンディング処

理を併用した場合は、そのどちらかまたは両者を省略した場合と比べ、1種のコンポジットレジンを除いて露出エナメル質の幅は有為に小さかった。辺縁でのSEM観察でもほぼ同様の所見が得られた。いずれの接着条件においても、劣化度は最初の5万回の間が最も大きかったが、繰り返し回数とともに減少した。