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研究課題名（和文）生体活性ジルコニアインプラントの創製

研究課題名（英文）Fabrication of bioactive zirconia implant

研究代表者
バラネザハド　有礼左（Valanzhad, Alireza）

長崎大学・医歯薬学総合研究科（歯学系）・助教

研究者番号：００６０８８７０

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研究成果の概要（和文）：セラミック 45S5 BGは、その生体活性表面と骨結合能力により、機能性生体材料として研究者の注目を集めている。本研究では1 回の真空ゾルディッピング法でBGを溶融コーティングを行った。BG コーティング後に、形態、接着テスト、粗さなどの特性を解明し、フラクトグラフィの結果から、コーティング層の厚さは 5 um から 45 um の間で制御可能であることが解った。XRD の結果では、非結晶性および結晶性の BG 相構造が示された。

研究成果の学術的意義や社会的意義
ジルコニアへの 45S5 生体活性ガラスコーティングの研究は、科学と社会の両面で大きな前進を意味する。バイオメディカルエンジニアリングの強化: ジルコニア基板への BG コーティングの適用が成功したことで、さまざまな材料を組み合わせて優れた機械的特性と生物学的特性を実現できる可能性が示唆された。このハイブリッドアプローチは、さまざまな医療用途向けの新しい複合材料を生み出す可能性がある。アクセス性の向上: スケーラブルなゾルゲル調製技術により、製造コストが削減され、高度な生体材料をより幅広い医療施設や患者が利用しやすくなり、最先端の医療処置を公表することで、健康の公平性が向上する可能性がある。

研究成果の概要（英文）：Ceramic 45S5 BG (45%SiO2, 24.5%Na2O, 24.5%CaO, and 6%P2O5 by weight) have gained researchers attention as a functional biomaterial due to its bioactive surface and osseointegration ability. It has been approved that BG 45S5 can heal and repair bone damages. Therefore, The sol-gel technique preparation protocol followed to prepare 45S5 BG, by mixing the tetraethylorthosilicate (TEOS), nitric acid, triethylphosphate (TEP), calcium nitrate tetrahydrate, sodium nitrate (Wako). The zirconia substrates coated by one-time vacuum sol-dipping method. The characterizations such as morphology, adhesion test and roughness investigated after BG coating. The SEM images confirmed that 900 °C is not enough to melt and cover entire surface therefore 1200 °C was applied. Also the Fractography result showed that the thickness of the coating layer is controllable between 5 um to 45 um. The XRD result mentioned the non-crystallin and crystalline BG phase structures.

研究分野：生体材料学

キーワード：implant　zirconia　bioactivity

１．研究開始当初の背景 Background of the research

Recently, the importance of aesthetics has driven the search for metal-free implant materials. Zirconia, specifically yttria-doped zirconia tetragonal polycrystals, is suggested as a suitable alternative to titanium. Zirconia is widely used for dental implants due to its exceptional properties. Zirconia's aesthetic qualities, such as tooth-like or pink color, make it a preferable substitute for metallic implants.

Despite its benefits, zirconia is a bio-inert ceramic, requiring surface treatments to acquire bio-active properties. Coating bio-inert implants with bioactive ceramics can reduce healing time. Bioactive Glass (BG) is a degradable, well-known bioactive ceramic material. Coating zirconia with BG combines zirconia's mechanical properties with BG's biological advantages. The 45S5 BG (composed of 45% SiO2, 24.5% Na2O, 24.5% CaO, and 6% P2O5 by weight) has attracted researchers' interest due to its bioactive surface and osseointegration capability. It is proven that BG 45S5 can heal and repair bone damage, and it can be produced using techniques like melt-cast and sol-gel methods.

Studies confirm that cells prefer rougher surfaces, which control cell response to surface roughness. Porous surfaces enhance bone ingrowth and stress shielding, as evidenced by cobalt-based alloy stems with sintered beads, known as porous-coated anatomic (PCA) hip prostheses. However, bead shedding is a significant issue in PCA hip and knee implants. Previous studies on metallic implants indicate that bead shedding is primarily influenced by the bead and bulk contact area or the level of bead fusion to the substrate. Heat treatment after sintering may enhance bead and substrate adhesion. For ceramics like zirconia, high temperatures are needed for bead diffusion. Previous research on bead-coated alumina (Al2O3) using a binder revealed no interlocking or fusion between the substrate and beads. This study proposes a new method to fabricate bead-interlocked zirconia substrates.

２．研究の目的 Purpose of research

The objective of this study is the fabrication and assessment of a new zirconia implant with embedded and fused zirconia beads on the surface, followed by Bioactive Glass (BG) covering.

３．研究の方法 Research method

(1) Bead-fused zirconia substrate

Zirconia powder, TZ-3Y-E grade (TOSOH Corp., Tokyo, Japan), with the composition of 97 mol% ZrO_2stabilized with 3 mol% Y_2O_3 and two kinds of zirconia beads (TOSOH Corp., Tokyo, Japan), TZ-B53 (38～75μm in diameter), TZ-B125 (106～150μm in diameter), named B53 and B125 respectively in this research, were used for making zirconia discs with a rough surface.

The rough bead-fused zirconia substrates were made by two-step biaxial pressing of zirconia powder under 61 MPa in the first step to prepare zirconia tablets and followed by the biaxial pressing of zirconia beads on top of the prepared zirconia tablets under 74 MPa in the stainless steel mold. After drying at 60°C for 1 hour the pressed bead-embedded zirconia tablets were sintered at 1400°C for 12 hours. The zirconia substrate with partially embedded and fused zirconia beads were fabricated.

(2) Bioactive Glass coating: The sol-gel technique preparation protocol was followed to prepare 45S5 BG, by mixing the tetraethylorthosilicate, nitric acid, triethylphosphate, calcium nitrate tetrahydrate, sodium nitrate. The rough bead-fused zirconia substrates were coated by one-time vacuum sol-dipping method under 0.1 bar pressure dipped for 60 s and dried at 60°C for 1 hour followed by sintering at 1200°C for 1 hour to melt the BG on the surface in a programmable furnace with a heating rate of 10°C/min.

(3) Beads adhesive strength to zirconia: The zirconia substrates with embedded and fused zirconia beads on the surface were subjected to an adhesive strength test in order to evaluate the adhesive strength between the coating layer and the substrate. The stainless steel rods with 4ϕ mm diameter were bonded to the rough surface of the zirconia substrate with acrylic super glue. A substrate with $10\times10\times1 mm^3$ in size was used in this test. An adhesion test was performed at a cross-head speed of 2 mm/min by a universal testing machine with a universal joint to ensure axial loading. The adhesive strength of the beads layer was confirmed from the maximum recorded-load. The mean values of the adhesive strength and their standard deviation (SD) were measured for ten samples.

(4) Roughness: The assessment of the substrates surface roughness was done by confocal laser scanning microscopy and optical profiler. The illumination in the CLSM is accomplished by a laser of 408nm wavelength. A magnification of the objective was 125×. The surface roughness (Rz) was measured according to ISO 4287. An average value of Rz was calculated by linear roughness analyzing on the samples. The scanned line length and vertical resolution were 300 μm and 10 nm, respectively and the measurement was repeated 5 times.

(5) Cell response: Cell response of the bead-fused zirconia and BG coated substrates was investigated using osteoblast cell line MC3T3-E1. MEM Alpha Medium containing 10% fetal bovine serum was used for cells cultured in an incubator under a humidified 5% CO_2 air atmosphere at 37°C and the medium was changed every 2 days until the cells confluence. Disc-shaped bead-fused zirconia substrates, in the untreated and BG coated substrates were autoclave-sterilized for 15 min. The substrates placed in the 48-well polystyrene plates and disseminated 10000 cells each and cultured. One step MTT assay or MTS assay kit was used to evaluate viability of the substrates before and after coating. The cells' mitochondrial activity was detected 2, 4 and 6 days after cell culture (n=5/group). The cell cultured substrates were incubated for 3 hours with MTS assay at 37°C atmosphere of 5% CO_2 then the incubated medium taken from wells absorbance was measured using a plate reader at 490 nm.

４．研究成果 Research result

(1) Microstructure and elemental analysis

Figure 1 shows the FE-SEM images of surface morphology of the zirconia substrate coated with B53 and B125 beads before and after covering with BG layer. The B53 and B125 beads embedded in the zirconia substrates and fused by sintering at 1400°C for 12 hours (shown in Fig. 1-a, b). Fig. 1-A, B show the zirconia substrate coated with B53 and B125 zirconia beads after BG coating by applying vacuum sol-dipping method and sintering at 1200°C for 1 h.

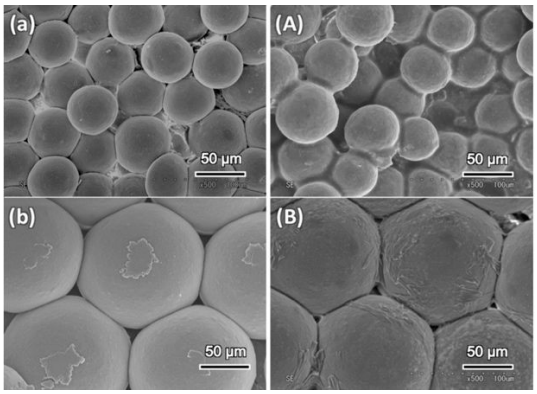

Fig. 1: SEM images of zirconia B53 beads coated (a), BG covered B53 (A), zirconia B125 beads coated (b) and BG covered B125 (B) on the zirconia substrates.

(2) Roughness

Figure 2 shows the 3D laser scanning microscopic images of bead-fused zirconia substrates. The mean and SD values of Rz for B125 and B53 beads coated zirconia substrates were 104.41±3.33 µm and 25.32±2.46 µm respectively. Also the measured Rz for the BG coated B125 and B53 beads were 99.76±4.97 µm and 22.30±2.03 µm respectively. Fig. 2 shows the 3D images for B53 and B125 bead-coated zirconia substrates before and after BG coating.

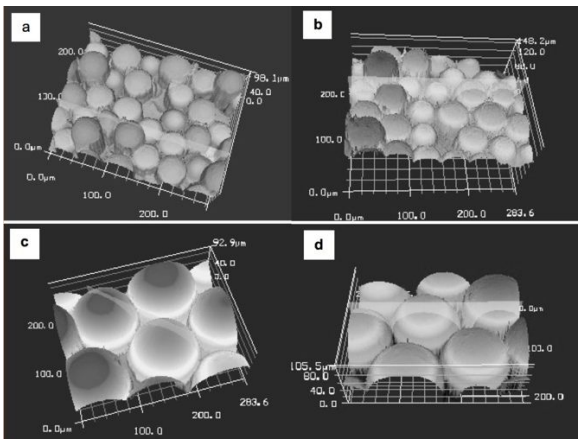

Fig. 2: 3D-Laser microscope images for the zirconia B53 beads coated zirconia substrate (a) before, (b) after BG coating and the zirconia B125 beads coated zirconia substrate (c) before and after BG coating (d).

(3) Beads adhesive strength

Adhesion test result revealed that the calculated average value for the adhesive strength for the zirconia B53 beads to the zirconia substrate surface before and after BG coating

were 18.5±3.1 and 18.2±8.1 MPa respectively. Also the adhesive strength for the B125 zirconia beads to the zirconia substrate surface before and after BG coating were 13.5±2.9 and 14.2±7.3 MPa respectively.

(4) Cell response

Figure 3 shows the MC3T3-E1 cells on the zirconia substrates coated with B53 and B125 zirconia beads before and after BG coating. The cells were cultured for 3 hours and 2, 4 and 6 days on the zirconia substrates coated with zirconia beads before and after BG cover. Cells were attached and extended after 3 hours and 2, 4 and 6 days incubation on the beads.

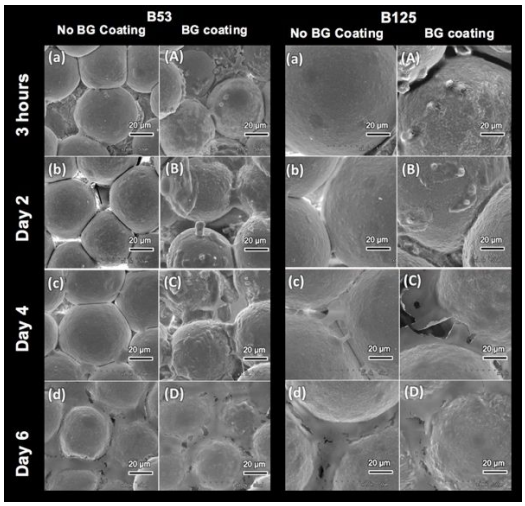

Fig. 3: SEM images of the B53 and B125 beads coated zirconia substrates with and without BG coating after cell culture for 3 hours (a, A), 2 (b, B), 4 (c, C) and 6 (d, D) days cell proliferation.

Figure 4 reveals the viability results histogram for MC3-T3-E1 cells cultured on the zirconia substrates coated with B53 and B125 zirconia beads before and after covering with BG layer. The histogram indicated that the viability of the substrate coated with B125 beads is slightly higher than substrates coated with B53 beads. On the other hand, the viability result after 6 days cell culture shows that the BG coated substrates has significantly higher compared to specimens BG coating.

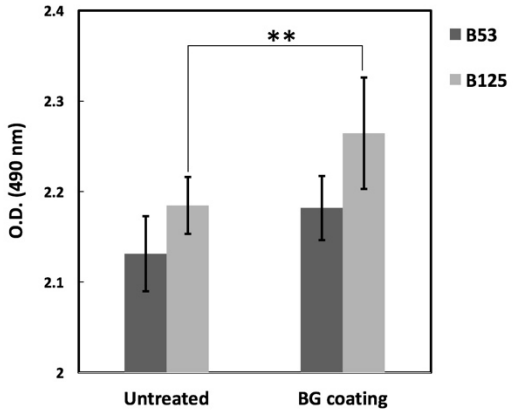

Fig. 4: The histogram of the cell viability for the B53 and B125 beads coated zirconia samples with and without BG coating after 6 days cell proliferation.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究分担者	渡邊　郁哉 (Watanabe Ikuya) (00274671)	長崎大学・医歯薬学総合研究科（歯学系）・教授 (17301)	
研究分担者	尾立　哲郎 (Odatsu Tetsuro) (70513167)	長崎大学・病院（歯学系）・講師 (17301)	

７．科研費を使用して開催した国際研究集会

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８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関