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研究課題名（和文）ジルコニア生体親和性の制御機構解明におけるメカノバイオロジカルアプローチ

研究課題名（英文）A Mechanobiological Approach to Elucidate the Control Mechanism of Zirconia Biocompatibility

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交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：本研究では、ジルコニアが有する生体親和性の分子メカニズムをメカノバイオロジーとセルバイオロジーの観点から解明する。申請者が取り組んできたRNAiスクリーニングを共焦点顕微鏡解析系にて応用構築し、ジルコニア上における骨芽細胞のメカノバイオロジカルな形態形成および細胞付着に関与する遺伝子を特定する。次に、対象遺伝子の変異体導入解析により骨芽細胞の形態制御機構とシグナル伝達系との関連を明らかにする。さらに、対象遺伝子のノックアウトマウスを作出し、ジルコニアインプラントのOsseointegrationに関する表現型解析を行う。

研究成果の学術的意義や社会的意義
Zirconia is a potential material for dental implants, but still exist shortcuts such as high toughness, low-temperature degradation and long-term stability. In this study, we intend to investigate the cell ability on zirconia, detect the mechanism behind, and aiming to improve zirconia materials.

研究成果の概要（英文）：We introduced two osteoblastic cell lines-- MC3T3-E1 and Saos-2 cells into this research. We seed the cells respectively on the control plate, CpTi, 3Y-TZP and NanoZr for 14 days. Then the cell proliferation ability and ALP ability was detected The result shows that both cell lines can attach and culture well on all materials and Saos2 cells show a better osteogenesis ability on zirconia and titanium than the control group. However, MC3T3-E1 cells could not conduct the osteogenesis ability well on all materials. Interestingly, in PCR tests of Saos-2 cells group, syndecan shows an increased expression on CpTi but a low expression on 3Y-TZP and NanoZr. The different expression levels of syndecans indicate that HSPGs might induce different mechanisms on titanium and zirconia. We pre-treate the cells with RDG (Arg-Fly-Asp-Ser) and nagstive control reagent RGE (Arg-Fly-Glu-Ser) and intend to detect the expression of syndecan family on different materials.

研究分野：Prosthodontics

キーワード：osteoblast　zirconia　cell adhesion

１．研究開始当初の背景

Titanium is currently the most used material for manufacturing dental implants. However, its potentially toxic effects and the gray color have resulted in increasing requests for metal-free treatment options. The typical metal-free zirconia dental implant has excellent biocompatibility, which means fewer inflammations or allergic reactions around it, and it provides a stable metal-free environment for us to attach an abutment and crown to replace the missing tooth. Zirconia ceramics gradually become the most promising materials applied for dental implants. However, the bioinertness of untreated implants impaired the initial cell adhesion and bone integration, resulting in delayed osseointegration and even implant failure. In order to solve these problems, surface modification becomes an effective way to promote osseointegration on implants. In the quest to achieve more predictable osseointegration, researchers have attempted to modify the zirconia surface to make it desirable for better cell attachment, growth, proliferation, and differentiation. But until now, the investigation of zirconia surface modification has not been fully understood.

２．研究の目的

The aim of this study is to explore a proper zirconia material and investigate the surface modification methods on zirconia.

３．研究の方法

(1) We screened the zirconia surface treatment-related articles to date and published a review discussing and summarizing the surface modifications of zirconia implants classified as physical treatment, chemical treatment, and surface coating.[1] Based on our previous findings and current knowledge of modification methods, we have made two research directions to investigate the modification of the zirconia surface.

①We keep on searching for the HSPG's (Heparan Sulfate Proteoglycan) effect on zirconia. We select two osteoblastic cell lines as candidates for checking the cell proliferation and osteogenesis ability: mouse derived MC3T3-E1 cells and homo sapiens-derived Saos2 cell lines. We seed the two kinds of cells at a density of 1x10*4 cells/ml and 2x10*4 cells/ml, respectively, on the control plate, CpTi, 3Y-TZP, and NanoZr surfaces for 7 days.

② For the ALP activity assay, we seed the two kinds of cells at a density of 1x10*4 cells/ml and 2x10*4 cells/ml respectively on the control plate, CpTi, 3Y-TZP and NanoZr surfaces for 14 days. The ALP ability was detected on Day 7, and Day 14.

③ To detect the original expression of HSPGs-riches syndecan members in MC3T3-E1 and Saos2 cell lines. MC3T3-E1 and Saos2 cells were seeded in a density of 5x10*4 cells/well onto CpTi, 3Y-TZP, NanoZr, and the control plate for 24H.

④ Moreover, from our pervious study, we know that HSPGs-riches syndecan plays a role in cell adhesion and cell spreading. Before investigating this effect, we checked the cell mobility and cell migration by SEM (Scanning Electron Microscopy). A cell-free gap around 500um was set when seeding cells.

⑤ Integrin is the main cell surface adhesion receptor which can detect the exposure of RGD motif of ECM proteins specifically. It has been widely known that integrin is the main cell surface adhesion receptor that can detect the exposure of RGD motif of ECM proteins specifically. For detecting the relationship between integrin and Heparan sulfate proteoglycans, we pre-treated the cells with RDG (Arg-Fly-Asp-Ser) and negative control reagent RGE (Arg-Fly-Glu-Ser) and intend to detect the expression of syndecan family on different materials. However, according to the results, we did not find any difference between these two treatments.

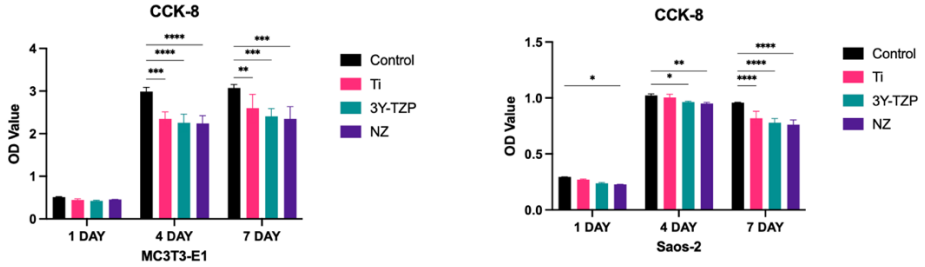
(2) Meanwhile, nanotubes, as one of the treatment methods achieved by anodic oxidation have been attracting increasing attention. The nanotubular structure was an ideal model for investigating size effects on the living matter or biologically relevant species in biomedical applications.[2] Moreover, TiO_2 nanotubes generated by an electrochemical anodization technique have drawn wide attention compared with conventional implant and potential

drug delivery applications, especially in titanium.[3] The nanotube structures on zirconia could also promote its bioactive through electrochemical anodization fabrication. We use the electrochemical method to fabricate the nanotube on the three different zirconia surfaces (Nano-Zr, 3Y-TZP), and check the proliferation and differentiation of osteoblast cells. At the first step, we intend to create the nanotubes. On zirconia surface. In previous work, we find that some research articles focused on the zirconia nanotube to improve bone formation. But there is no article applied this technic to new zirconia implants. We thought we find a new research topic and used some different anodization methods. But unfortunately, there are no well results in our experiments.

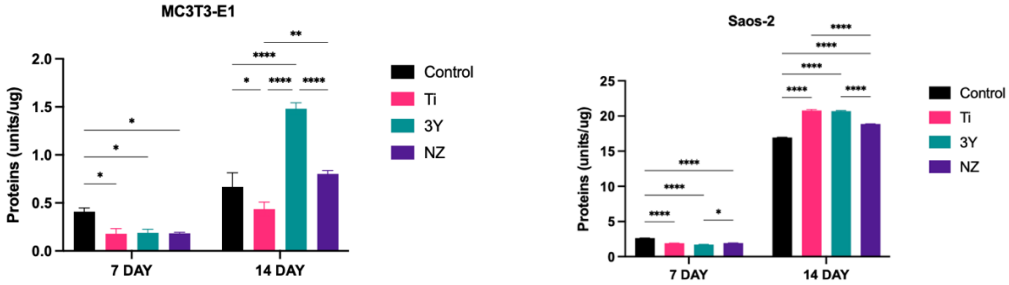
At this moment, the concept of exosomes secreted by cells which cultured in different topography can influence other cells behavior was put forward. Based on this concept, we have a hypothesis is using exosome secreted by cell in Ti nanotubes to stimulate cell cultured in zirconia implant surface can simulate similar cell behavior cultured in Ti nanotubes surface. At first, we searched for the most efficient condition for nanotube fabrication. Based on current technology and equipment, we changed the voltage selection. Then, we checked the wettability of different nanotubes as well as the cell proliferation.

４．研究成果

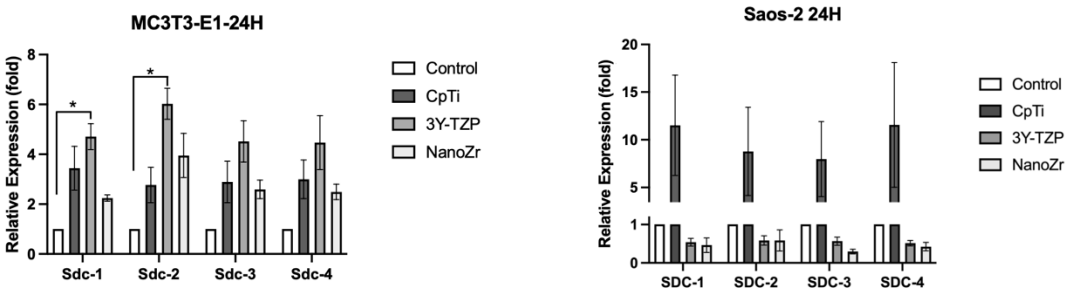
(1) The cell proliferation ability was detected on Day 1, Day 4, and Day 7. The results show that both MC3T3-E1 cells and Saos2 can be attached and cultivated well on four materials.



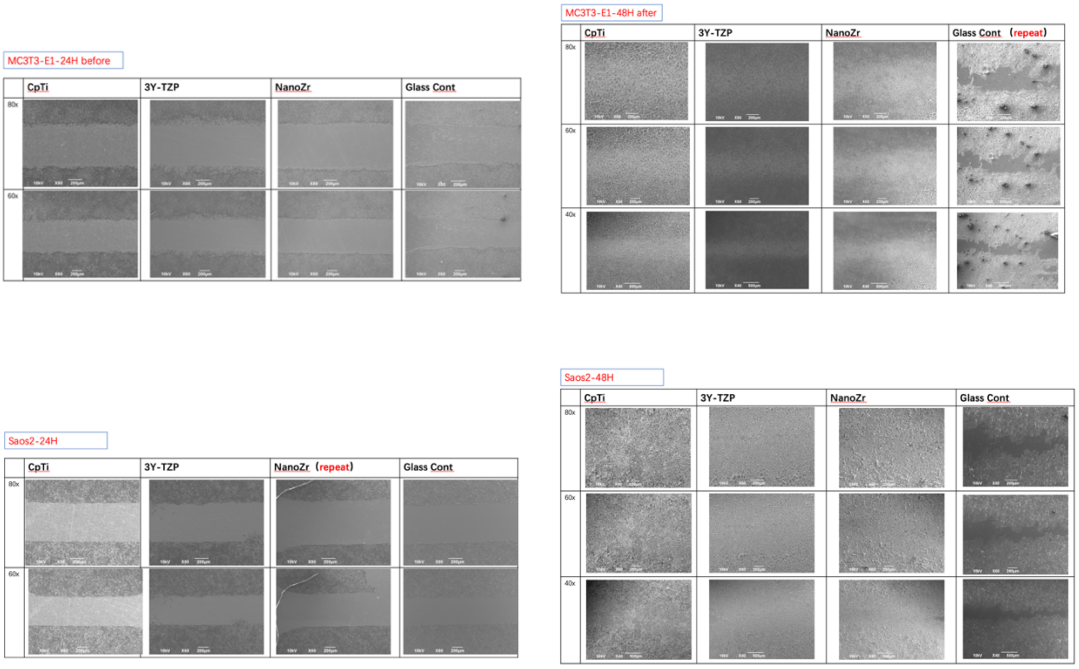
(2) The ALP results indicate that Saos2 cells show a better osteogenesis ability on zirconia and titanium than the control group. While MC3T3-E1 cells could not conduct the osteogenesis ability on all materials.



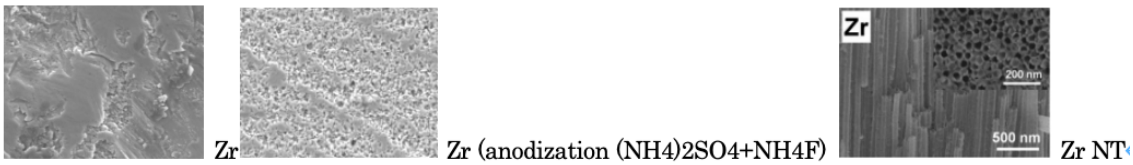
(3) According to the PCR data, all syndecan members (Syndecan 1, Syndecan 2, Syndecan 3, Syndecan 4) shows a relatively high expression on all three materials of MC3T3-E1 cells, compared with the control group. Interestingly, in Saos-2 cells, syndecan shows a high expression on CpTi but a low expression on two zirconia materials, compared with the control group. The different expression levels of syndecans indicate that HSPGs might induce different mechanisms on titanium and zirconia material.



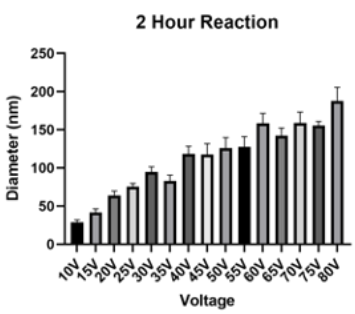
(4) After incubating for 24h for the cell migration test, the cell-free gap was covered with two cells without any space on CpTi. On 3Y-TZP and NanoZr surfaces, cells have almost covered the gap even though a tiny space can be seen. However, the gap in the control group was not fully covered but left a smaller space around 100um-200um.

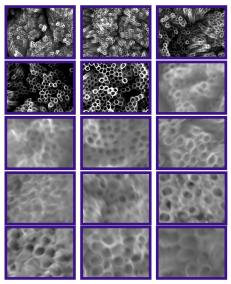


(5) Zirconia after anodization only formed some unregular holes like acid etching and not formed nanotube. After we checked some previous articles, we find that this is impossible formed nanotubes in zirconia by anodization. In other articles, they usually used zirconium to be a positive pole, and zirconium is metal, but zirconia (Nano-Zr or 3Y-TZP) is oxide which is main material in implants. Absolutely, there are no theoretical support for these experiments.

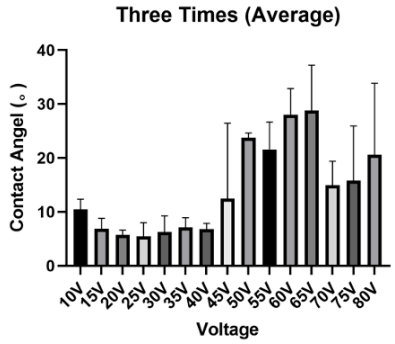


(6) This result shows the the diameters of nanotubes are from 30nm to 200nm. The diameter of nanotube was increased by the voltage; this result was the same as the previous study. But with the voltage increased, the otherness of diameter becomes more obvious. The reason why come out this phenomenon may be the electronic are not stable.

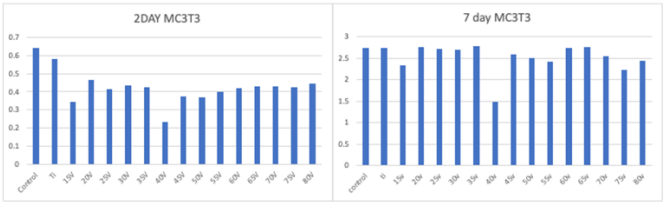




(7) The result of surface wettability is a little strange. But we can observe that before the 45v, all samples they have a well hydrophily we called super hydrophily. In previous study, we have never used the voltage above 40, but when I try to do that, a hard nut to crack the electrolyte become very hot. And after the 80v, the electrolyte was boiled basically.



(8) As for the cell proliferation in different nanotubes. It is necessary to increase the number of experiment and obtain stable data. And the ALP activity of cell in different nanotube were also be tested. The cell behavior of Ti and zirconia also be proved, and exosome secretion test was trying in these groups.



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[2] Park, S., Lee, C., & Kim, K. (2008). Size effect in nanotube-enhanced biological systems. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 84B(1), 72-79.
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５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　2件）

１．著者名 Hong Guang、Hung Chun-Cheng、Mayanagi Gen、Nishioka Takashi、Sun Lu、Lai Eddie Hsiang-Hua、Lan Ting-Hsun、Sasaki Keiichi、Takahashi Nobuhiro	４．巻 18
２．論文標題 Questionnaire survey on the satisfaction of SimEx dental education system	５．発行年 2023年
３．雑誌名 Journal of Dental Sciences	６．最初と最後の頁 840～847
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jds.2023.01.028	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Sun Lu、Hong Guang	４．巻 2
２．論文標題 Surface Modifications for Zirconia Dental Implants: A Review	５．発行年 2021年
３．雑誌名 Frontiers in Dental Medicine	６．最初と最後の頁 733242 (1-8)
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fdmed.2021.733242	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計3件（うち招待講演　2件／うち国際学会　1件）

１．発表者名 SUN LU
２．発表標題 Introduction of International Collaborative and Innovative Dentistry
３．学会等名 TU- FJMU International Workshop（招待講演）
４．発表年 2022年～2023年

１．発表者名 SUN LU
２．発表標題 The viability of ceramic dental implants
３．学会等名 2022 CA+inD Summer Short-term Exchange Program Multimodal Global Leaders Development through Asian-Model Dentistry Consortium（招待講演）
４．発表年 2022年～2023年

１．発表者名 SUN LU
２．発表標題 The role of syndecan on osteoblastic cell adhesion of nano-zirconia
３．学会等名 The International Oral Health Symposium 2022（国際学会）
４．発表年 2022年～2023年

〔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

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

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