SCRP

令和3年度

日本代表選抜大会

2021 JDA Student Clinician Research Program

研究発表抄録集

公益社団法人　日本歯科医師会
スチューデント・クリニシャン・リサーチ・プログラム

CONTENTS

*SCADAは、米国で開催される本プログラムのコンペティション名（Student Competition for Advancing Dental Research and its Application）の略称ならびにSCRP参加経験者で構成される同窓会の名称として使われています。

令和3年度 日本歯科医師会
スチューデント・クリニシャン・リサーチ・プログラム
日本代表選抜大会

歯科医師会館（定礎：昭和63年）

ごあいさつ

公益社団法人 日本歯科医師会
会長　堀　憲郎　Kenro HORI

1959年に米国歯科医師会の主催によりスタートしたスチューデント・クリニシャン・リサーチ・プログラム（SCRP）は、世界各国を結ぶ歯学教育支援プログラムとして60年以上に亘り発展し続けています。米国内のコンペティションならびに各国代表による研究発表を通じて、歯科学生の国際学術交流が継続されていることを喜ばしく思います。

SCRP日本代表選抜大会は、新型コロナウイルス感染症の影響が続く中、本年度もWEBを活用した開催となりましたが、昨年度を上回る21校の歯科大学・歯学部から参加をいただき、感謝申し上げます。大学においても引き続き制約が多い中で研究指導に当たられた先生方、そして日頃の勉学に加えて研究活動にチャレンジし、立派な研究成果を発表されたスチューデント・クリニシャンの皆様方に心より敬意を表します。

日本歯科医師会は現在約65,000名の会員を有し、「医道の高揚、国民歯科医療の確立、公衆衛生・歯科保健の啓発、並びに歯科医学の進歩発達を図り、もって国民の健康と福祉を増進すること」という目的に沿い、多くの事業を展開しています。本会のそれらの取り組みの中で、国際戦略・国際貢献に関する分野については、歯科の国際組織であるFDI（世界歯科連盟）に1969年より加盟し、毎年開催される世界大会に参加しながら、国際社会が共有する重要課題を積極的に議論し、また財政的にも支援しています。本年度は9月にオーストラリア歯科医師会主催により初めてバーチャル形式で大会が開催されました。

また、これまでは世界大会の場で対面で行ってきたアメリカ、ドイツ、フランス、豪州、ニュージーランドの各歯科医師会との個別会議もWEB開催し、新型コロナウイルス感染症が歯科医療専門職に及ぼす影響等について活発な意見交換を行いました。

このような国際対応のひとつとして、本会は1995年からこのSCRPに参画しているところですが、我が国が今後益々国際感覚に優れた人材の育成を目指していく中、このSCRPへの参加者の多くが卒業後に歯科界の各分野で主導的な役割を果たされています。本年度参加されたスチューデント・クリニシャンの皆様には、本大会に参加したことで得られた貴重な経験をもとに、卒業後は臨床・研究・教育等の分野においてそれぞれご活躍されることを期待しています。

今後ともこのプログラムが日本の歯学教育と歯科の国際分野での発展に貢献することを祈念し、挨拶といたします。

公益社団法人 日本歯科医師会
常務理事　尾松 素樹　Motoki OMATSU

令和3年度の第27回SCRP日本代表選抜大会は、21校の歯科大学・歯学部からの応募があり、新型コロナウイルス感染症の影響で昨年と同様のオンラインでの審査となりましたが、関係各位のご協力によって開催され、日本代表を含む上位入賞者4名を決定いたしました。

今年度参加されたスチューデント・クリニシャンそして共同研究者の皆さまは、本年度もコロナ禍にもかかわらず研究を継続され、創意工夫により立派に研究成果をまとめ上げられました。プレゼンテーションの収録・ライブ発表に至るまでに多大なる努力を重ねられたことと思います。井上孝副審査員長の審査講評にあるようにどの研究発表も優れたものでした。この経験を糧とし、卒業後の進路においても活かされることを期待しています。また、研究指導に当たられたファカルティー・アドバイザーならびに研究指導協力者の先生方のご指導に厚く御礼申し上げます。

日本代表として選ばれたスチューデント・クリニシャンは、2022年3月に米国ジョージア州アトランタ市にて開催予定の国際歯科研究学会米国部会（AADOCR）学術大会に招待される予定です。この米国でのSCRP大会審査員に第3回大会のスチューデント・クリニシャンであった駒林卓先生が2008年から就任されています。その先生のSCRP日本代表選抜大会に寄せる熱い思いが込められたメッセージも本誌に掲載しましたのでお読みください。

本大会で日本代表を選抜することが一つの目的ですが、先輩スチューデント・クリニシャンとしてもう一方メッセージお寄せいただいた小林良喜先生が書かれているように、リサーチマインドを持った同世代がこの大会を通じて交流して頂くことも目的の一つです。これまでの大会では審査後、お互いの研究について意見交換を行い、さらにその後の懇親会でも情報交換を行っていました。昨年と今年はこのような場が設定できなかったことが残念で、今後、この交流をどのようにしていくかも課題だと考えています。

来年度もコロナの状況を注視しながらの開催となりますが、スチューデント・クリニシャンの皆さんが学業の妨げにならず、安心して発表できる大会運営をしていきたいと考えています。次年度も多くの参加を期待しています。

なお、この研究発表抄録集は本会ホームページでも閲覧できますので、来年度以降本大会にチャレンジするスチューデント・クリニシャンならびに指導関係者の参考としてご活用ください。

研究テーマ一覧

SC NO.	大学	氏名	研究テーマ（和文）	研究テーマ（英文）	ページ
1	昭和大学歯学部	四宮 寛大	口腔内に存在する組織幹細胞の同定法の開発	Development of method for identifying tissue stem cells present in oral cavity	5
2	長崎大学歯学部	長野 敏樹	周産期産物由来間葉系幹細胞に対する骨誘導性付与の試み	Bone tissue engineering with perinatal product-derived mesenchymal stem cells	8
3	新潟大学歯学部	安藤 まな	口腔乾燥がもたらす嚥下機能への影響を食品条件の違いから考察する	Effect of oral dryness and bolus property on swallowing function	11
4	東京医科歯科大学歯学部	髙村 彩	光造形 3Dプリンタによるフェイスガードコア材の造形検討 –積層方向と材料について–	Evaluation of face guard cores made by stereolithographic three-dimensional printing: build orientation and material	14
5	徳島大学歯学部	深田 有希	創傷治癒に対する低周波パルス磁場の効果	Effect of ELF pulsed magnetic field on wound healing	17
6	鶴見大学歯学部	田﨑 智也	*Candida albicans* 感染予防に対する*Probiotics* 候補 *Lactobacillus* の効果	Effect of probiotics candidates *Lactobacilli* on infection prevention of *Candida albicans*	20
7	岡山大学歯学部	棚井 あいり	歯周病と胎児の成長障害:*Porphyromonas gingivalis* はマクロファージの細胞外小胞を介して胎盤の血管形成を阻害する	Periodontitis & abnormal pregnancies: *Porphyromonas gingivalis* utilizes macrophage extracellular vesicles and inhibits placental angiogenesis	23
8	九州歯科大学	赤司 妃咲	パラミロンはCRP値ではなく血中中性脂肪値を改善する（二重盲検無作為化比較試験）	Paramylon intake improves blood triglyceride level, but not C-reactive protein level: double-blinded randomized control trial	26
9	日本歯科大学新潟生命歯学部	田邊 由佳	SPECT/CT を用いた MRONJ の定量分析	Volumetric analysis of medication-related osteonecrosis of the jaw with SPECT/CT	29
10	日本大学歯学部	髙田 紋花	口腔内細菌代謝産物がドライソケットにおける歯槽骨石灰化亢進に関与する可能性	Additive alveolar bone mineralization in the dry socket may be induced by oral bacterial metabolites	32
11	岩手医科大学歯学部	増田 彩	*Abiotrophia defectiva* のHsp70ホモログDnaKはヒト臍帯静脈内皮細胞に炎症応答を誘発する	The induction of proinflammatory response in human umbilical vein endothelial cells by Hsp70-homolog DnaK of *Abiotrophia defectiva*	35
12	九州大学歯学部	平田 薫子	高齢者の口腔機能低下症と栄養摂取状態の関連	Relation between oral hypofunction and nutrient intake condition in the elderly	38
13	日本大学松戸歯学部	熨斗 優樹	*Fusobacterium nucleatum* の口腔内接種によるマクロファージの小腸への誘導作用の検討	Induction of macrophages into the small intestine by oral inoculation with *Fusobacterium nucleatum*	41
14	鹿児島大学歯学部	福島 慎	異所性疼痛とラット三叉神経節体部位局在との関連についての研究	Analysis of relationship between ectopic pain and somatotopy of rat trigeminal ganglion neurons	44
15	北海道医療大学歯学部	呂 令凱	慢性拘束ストレスラットモデルにおける口腔および腸内細菌叢のメタゲノム解析	Metagenomic analysis of oral and intestinal microflora in a rat model of chronic restraint stress	47
16	東北大学歯学部	岡田 嘉奈子	新型コロナウイルス感染症に対する恐怖心の歯科受診控えに対する影響:インターネット全国調査による検討	Impact of fear of contracting COVID-19 on refraining from the dental visits: A nationwide internet survey	50
17	朝日大学歯学部	西口 真矢	プロポリスおよびPPAR-γ阻害因子（GW9662）が活性化脾細胞や未分化間葉系細胞の生物学的活性におよぼす影響	Effects of Propolis and a PPAR-γ inhibitor, GW9662, upon the biological activities of stimulated spleen cells and undifferentiated mesenchymal cells	53
18	北海道大学歯学部	中嶋 悠斐	副甲状腺ホルモン間歇投与マウス大腿骨における podoplanin/PHOSPHO1 陽性骨芽細胞の免疫局在	Immunolocalization of podoplanin-reactive/PHOSPHO1-positive osteoblasts in murine femora with intermittent administration of parathyroid hormone	56
19	大阪歯科大学	栗山 実久	高齢者の口腔ケアのための過酢酸を含む新しい歯科用消毒剤の開発	Development of a new dental disinfectant containing peracetic acid for oral care of the elderly	59
20	大阪大学歯学部	小山 愛結	シングルセル解析によって得られた口腔細菌のゲノム情報を用いた耐性遺伝子と病原因子の探索	Single-cell genomics approach for identification of antimicrobial resistance genes and virulence factors in oral bacteria	62
21	広島大学歯学部	安田 雅空斗	歯原性角化嚢胞の臨床病理学的検討	Clinicopathological study on odontogenic keratocysts	65

Development of method for identifying tissue stem cells present in oral cavity

昭和大学歯学部　5年生　Showa University School of Dentistry　Class of 2022

四宮 寛大　Kanta SHINOMIYA

ファカルティー・アドバイザー：口腔生化学講座 講師　山田 篤

This study focused on neural crest-derived cells as a new resource for bone regeneration. Neural crests are formed on both sides by invagination of the neural plate in the early embryonic period, after which some neural crest cells then migrate throughout the embryo as neural crest-derived cells. It is also known that other neural crest-derived cells remain as somatic stem cells in colonization destination tissue and have pluripotency. In order to collect tissue stem cells as a resource for bone regeneration from neural crest-derived cells with high efficiency, it is important to identify the properties of the target cells and determine which are suitable as tissue stem cells. A new analysis method termed single cell RNA-sequence (scRNA-seq) has been developed and shown capable of detecting factors involved in transcriptional regulation of individual cells. Based on results of scRNA-seq analysis, a total of 13 clusters were observed. Among those, trajectory analysis was performed to reveal tissue stem cells that served as the origin of those populations and the findings suggested that a model starting from a cluster expressing Sox2 would be appropriate. In a future study, cell surface proteins specifically expressed in clusters expressing Sox2 will be identified, then purified and applied for bone regenerative medicine.

口腔内に存在する組織幹細胞の同定法の開発

私たちは骨再生の新しい細胞リソースとして神経堤由来細胞に着眼した。胎生期初期に形成される神経堤から、生体内の各組織に遊走された神経堤由来細胞の一部は、組織幹細胞として定着先の組織内に残り、多分化能を維持することが知られている。神経堤由来細胞から骨再生のリソースとなる組織幹細胞を高純度に採取するためには、採取した細胞の性質を1細胞ごとに同定し、どの細胞集団が組織幹細胞としての性質を有するか検討しなければならない。私たちは個々の細胞における遺伝子発現様式を検出することが可能であるシングルセルRNAシークエンス解析を行うことで、神経堤由来細胞から骨再生のリソースとなる組織幹細胞を選定できるのではないかと考えた。その結果、神経堤由来細胞は13個のクラスターに分類することができた。また、トラジェクトリー解析から、Sox2を特異的発現するクラスターに組織幹細胞としての性質を有する細胞集団が存在する可能性が示唆された。今後、このクラスターに存在する細胞に特異的に発現している細胞表面タンパク質を同定し、そのタンパク質を基にクラスター細胞の精製およびそれらの細胞を用いた骨再生医療への応用を検討する。

研究発表内容の紹介

私たちは多分化能を持つ神経堤由来細胞を再生医療の新しい細胞ソースとして着想した。顎顔面領域において神経堤細胞は象牙質や歯髄へと分化する歯乳頭、セメント質や歯根膜、固有歯槽骨へと分化する歯小嚢の由来となる。この細胞は成体から得られ、生命倫理的観点や癌化リスクの低さから再生医療への応用が期待できる。私たちは再生医療に有用な神経堤由来細胞の分離・増殖及び選択方法の検討のために、転写制御機構の解明が必要と考え、解析を行った。（ファカルティー・アドバイザー：山田 篤）

Development of method for identifying tissue stem cells present in oral cavity

(Problem) This study focused on neural crest-derived cells as a new resource for bone regenerative medicine. In vertebrates, neural crests are formed on both sides by invagination of the neural plate in the early embryonic period, after which neural tubes are formed by connection between the left and right neural crests. At that time, some neural crest cells are left behind and migrate throughout the embryo as neural crest-derived cells (Fig. 1). These migrating neural crest-derived cells differentiate into various cell types, such as osteoblasts, neurons, and hair follicle cells, based on the colonization destination. In adults, it is also known that some neural crest-derived cells remain as somatic stem cells in colonization destination tissue and have pluripotency. Therefore, neural crest-derived cells, considered to be somatic stem cells, can be obtained from adult tissues, thus avoiding bioethical issues, different than with ES cells obtained from fertilized egg embryos. In addition, unlike iPS cells, the risk of cancer development with neural crest-derived cells is considered to be low, thus they are anticipated to become a new cell resource for regenerative medicine. For investigations of neural crest-derived cells, we have used P0-Cre/GFP mice, which express green fluorescent protein (GFP) under the control of the myelin protein 0 (P0) gene promoter, to collect neural crest-derived cells (Fig. 2). However, when using adult neural crest-derived cells as a regeneration source, the number of cells obtained is small and an efficient separation/proliferation method that offers a high level of purity has yet to be established. In addition, even should separation and proliferation be possible, somatic stem cell proliferation and differentiation abilities differ depending on the individual cells, thus it is necessary to develop an effective method for selecting cells useful for regenerative medicine.

(Hypothesis) In order to collect tissue stem cells from neural crest-derived cells to be used as a resource for bone regeneration with high efficiency, it is important to identify the properties of the target cells and determine which are suitable. A new analysis method, termed single cell RNA (scRNA)-sequence, has been developed and shown capable of detecting factors involved in transcriptional regulation of individual cells. We speculated that this method would be suitable for determining cells expected to differentiate in a manner suitable for the transplant destination and thus effective for regenerative medicine.

(Methods) Mouse neural crest-derived cells were obtained from P0-Cre/GFP mice expressing GFP under the control of the myelin protein 0 (P0) gene promoter, which is specifically expressed in neural crest cells. These mouse cells have been shown effective for tracing neural crest-derived cells by visualizing GFP (Fig. 2).

(1) Cells collected from mouse inferior turbinate differentiate into osteoblasts

Cells collected from the inferior turbinate of mice were cultured for 14 days, then placed in calcified medium containing BMP-2 (100 ng/ml) for three days to determine the expression and activity of alkaline phosphatase (ALP) and osteocalcin (OCN), known osteoblast differentiation markers.

(2) Neural crest-derived cells collected from mouse inferior turbinate

Mouse inferior turbinate cells (n=100,000) were labeled with propidium iodide (PI), then using a FACS cell sorter PI-negative live cells were designated (n=38,083). Next, GFP-positive cells, neural crest-derived cells, were isolated (n=10,114). After sorting, GFP-positive cells with a high proportion of mitochondrial genes (>10%) were considered to have a poor condition and excluded from scRNA-seq analysis, which was subsequently performed with 1056 cells.

(3) scRNA sequence analysis

scRNA sequence analysis of cells obtained by sorting was performed using a chromium single-cell gene solution. The workflow steps used were as follows: 1) bar coding of each cell and transcript library preparation, 2) sequence analysis with a next-generation sequencer, and 3) bioinformatics analysis of obtained data.

(Results) (1) Differentiation of cells collected from mouse inferior turbinate into osteoblasts

After culturing cells collected from mouse inferior turbinate specimens for 14 days, nearly all were found to be GFP-positive neural crest-derived cells (Fig. 3A). Next, treatment with mineralization medium containing BMP-2 increased the gene expression of ALP and OCN, markers of osteoblast differentiation, and promoted differentiation into osteoblasts, resulting in increased alkaline phosphatase activity (Fig. 3B, C).

(2) scRNA sequence analysis of neural crest-derived cells from mouse inferior turbinate

Based on the gene expression of 1085 cells, two-dimensional mapping using uniform manifold approximation and projection (UMAP) analysis, as well as cell clustering analysis were performed, with a total of 13 clusters observed (Fig. 4).

Each cluster was classified according to the gene expression mode of the cells. Wnt/β-catenin signals and Lef1, a transcription factor involved in maintenance and proliferation of ES cells, were specifically expressed in cluster No. 8. In addition, Klf2 and Klf4, which regulate expression of pluripotent stem cell-specific genes and are involved in maintenance of pluripotency, were widely expressed, though centered on cluster No. 0, while Sox2 was specifically expressed in cluster No. 7 (Fig. 4). Trajectory analysis was performed to determine which tissue stem cells were the origin of these populations, which indicated that a model starting from cluster No. 7 expressing Sox2 would be appropriate.

(Conclusion) Among cells collected from the inferior turbinate of mice, GFP-positive cells, which are neural crest-derived cells, were induced to differentiate into osteoblast-like cells. Furthermore, by scRNA sequence analysis, it was possible to suggest that the expression of genes characteristic of tissue stem cells is particularly present in cluster No. 7 expressing Sox2. In a future study, we will identify cell surface proteins that are specifically expressed in cluster No. 7 to purify and apply them for bone regenerative medicine.

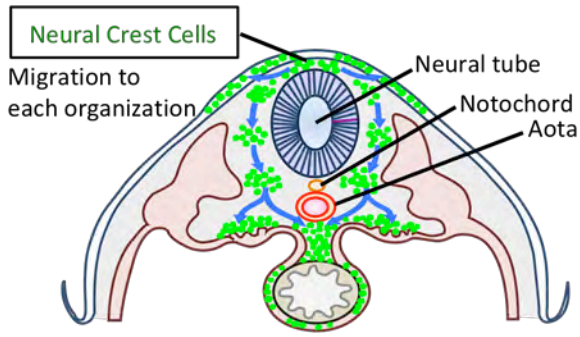


Fig.1 Migration of neural crest cells in an early embryo

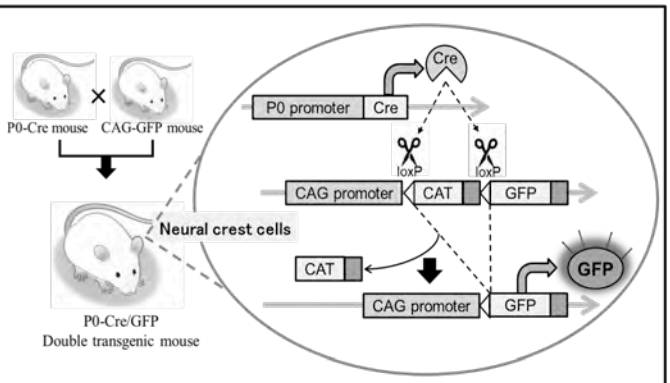


Fig.2 P0-cre/GFP transgenic mice

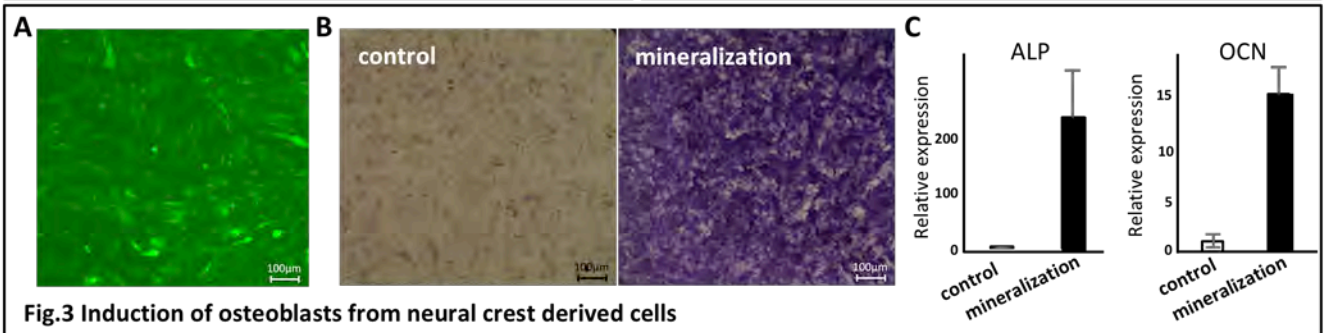


Fig.3 Induction of osteoblasts from neural crest derived cells

(A) Cells collected from mouse inferior turbinate specimens for 14 days, nearly all were found to be GFP-positive neural crest-derived cells (B, C) After 14 days' culture, cells were treated with osteoblast mineralization medium containing BMP-2 (mineralization) for three days to determine alkaline phosphatase (ALP) activity staining and ALP and osteocalcin (OCN) gene expressions.

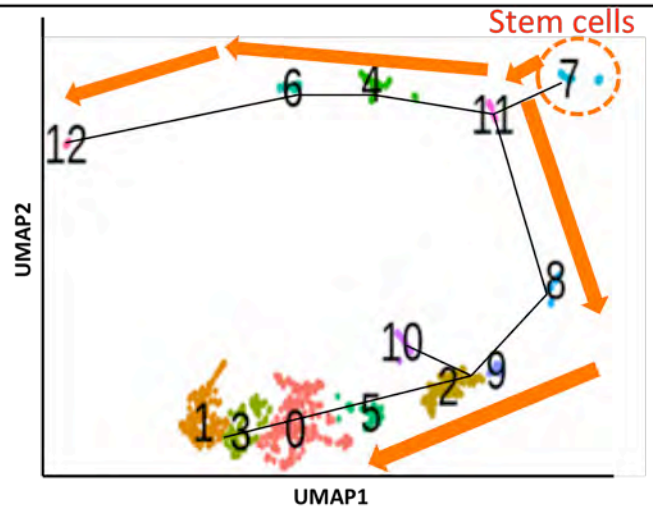


Fig.4 Estimating tissue stem cell clusters in neural crest derived cells by single-cell RNA sequence analysis

Neural crest-derived cells (GFP positive) and live cells (PI negative) were collected from the inferior turbinate of P0-Cre/GFP transgenic mice using a FACS cell sorter. After collection, scRNA sequence analysis was performed on 1056 cells, excluding cells with a high proportion of mitochondrial genes (> 10%).

Neural crest-derived cells in mouse inferior turbinate were classified into 13 clusters based on the correlation of gene expression patterns by UMAP analysis. From the trajectory analysis, it was presumed that the cells existing in the 7th cluster (*dotted circle;* Sox2 expressed) has high properties as tissue stem cells.

Bone tissue engineering with perinatal product-derived mesenchymal stem cells

長崎大学歯学部 6年生 Nagasaki University School of Dentistry Class of 2021

長野 敏樹 Toshiki NAGANO

ファカルティー・アドバイザー：硬組織疾患基盤研究センター 准教授 住田 吉慶
研究指導協力者：硬組織疾患基盤研究センター 教務職員 岩竹 真弓

Tissue engineering has been considered a potential alternative strategy to bone reconstruction, and growth factors and stem cells are receiving significant attention as key elements in tissue engineering that can confer osteo-inducibility to alloplastic bone substitutes. Particularly, for large bone reconstruction, delivering mesenchymal stem cells (MSCs) or osteoblastic cells is considered to be very promising. In such a strategy, we have focused on the perinatal products-derived MSCs, such as umbilical cord (UC) - and amniotic membrane (AM) -MSCs, in an allogeneic setting. Human UC-MSCs and AM-MSCs have a higher proliferative potential, however osteoblast differentiation ability is quite limited when compared to bone marrow-MSCs (BM-MSCs). Therefore, these MSCs usually require the long-term culture for differentiation but even differentiated cells cannot show sufficient *in vivo* osteo-inducibility. In this study, when examined various 2D and 3D matrix cultures on UC-MSCs, we found a culture on surface of type I collagen (Col1) gel matrix induced the depolymerization of F-actin to monomeric G-actin in UC-MSCs, and then promoted osteoblast differentiation and *in vivo* osteo-inducibility. However, Col1 gel culture could not affect the osteoblastic differentiation of AM-MSCs. Therefore, Col1 gel culture must be useful to enhance osteoblastic differentiation ability of human UC-MSCs, and changes of cell morphology determined by dynamics of the actin cytoskeleton may associate with the inducibility of osteoblastic differentiation of UC-MSCs. Meanwhile, different feature was recognized on osteoblastic differentiation among UC- and AM-MSCs

周産期産物由来間葉系幹細胞に対する骨誘導性付与の試み

われわれは、顎骨・歯槽骨再生治療に応用する同種骨芽細胞製品の開発に取り組んでいます。その同種骨芽細胞源として、非侵襲的に採取できる周産期産物由来の間葉系幹細胞（MSC）は、他の組織由来MSCと比較して強い免疫寛容能と組織修復能を発揮します。そのため、MSC製品の安定供給原として有用性が高いと考えられます。しかしながら、高い未分化性と組織の特性から、周産期産物由来MSCは、骨芽細胞への分化に時間を要し、分化誘導後も生体内での骨誘導性が低いという問題点があります。そのため、周産期産物由来MSCから骨髄由来MSCと同等以上に骨誘導性を発揮する骨芽細胞を効率的に、且つ簡便に作製するための培養条件を検討してきました。本研究では、一定の厚みで調整したタイプⅠコラーゲン（Co1）のゲル上培養を応用することで、臍帯由来MSCの骨芽細胞分化と生体での骨誘導性を飛躍的に促進させる条件を見出しました。これは、Col1上で細胞骨格の可変性が亢進されることによるものと考えられますが、その一方で、同一患者から採取した羊膜由来MSCでは同様の影響は認められなかったことから、組織の特性に応じた分化誘導条件をさらに検討する必要性があることが分かりました。

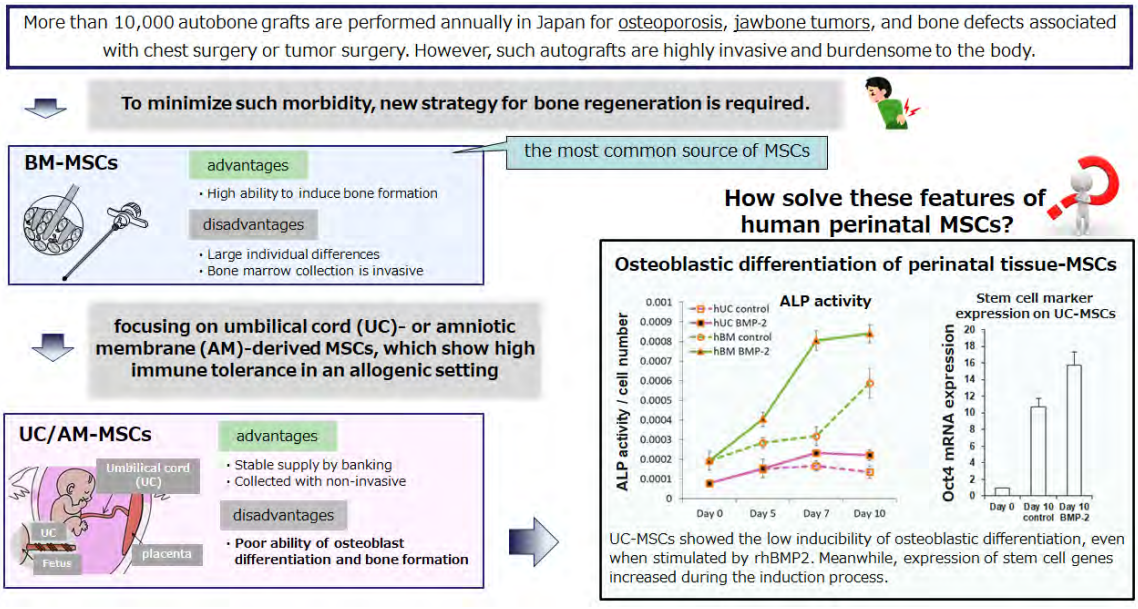
研究発表内容の紹介

私どもでは、顎骨腫瘍や口蓋裂顎裂などによる顎骨・歯槽骨欠損を対象として、その骨再生治療に応用する同種骨芽細胞製品の開発研究を実施しています。本研究は、その研究開発の一部として実施されたものです。組織採取の侵襲がなく、安定供給と大量生産が可能である周産期産物由来MSCによる細胞製品が開発されれば、即納性と有効性が担保された治療を提供できるようになります。その結果、適切な咀嚼機能の回復がより簡便になされるようになれば、健康寿命の延伸にも大きく貢献できると考えています。
（ファカルティー・アドバイザー：住田 吉慶）

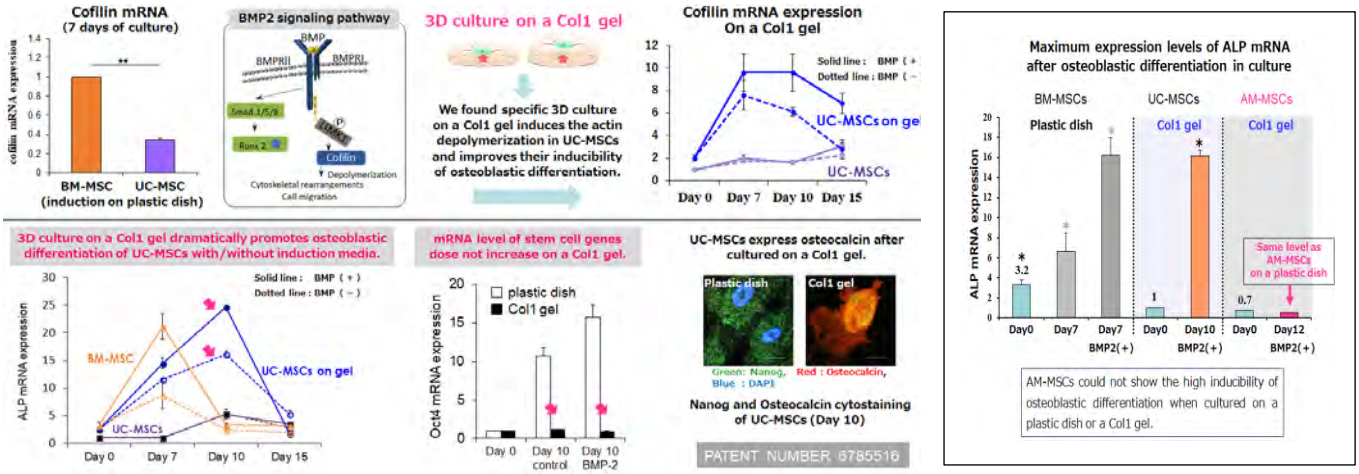
Bone tissue engineering with perinatal product-derived mesenchymal stem cells

(Problem) Current surgical strategies for healing bone defects arising from trauma or disease employ either autogenous bone grafts or alloplastic bone substitutes. While autograft procedures can involve donor site morbidity, this strategy can be a realistic option for patients with severe bone defects. Meanwhile, because alloplastic materials lack osteogenic potential, their application remains limited, and the results of these strategies have been inconsistent to date. Therefore, tissue engineering has recently been considered a potential alternative strategy to bone reconstruction since it is thought to be less invasive and safer than conventional methods. For this reason, growth factors and stem cells are now receiving significant attention as key elements in tissue engineering that can confer osteoinducibility to alloplastic bone substitutes. In particular, for large bone reconstruction, delivering mesenchymal stem cells (MSCs) or osteoblastic cells is considered to be very promising. However, obtaining therapeutic cells from autologous MSCs have many issues such as the time required cell expansion and differentiation, and individual differences among patients in osteogenic ability. Therefore, we have focused on the perinatal products-derived MSCs such as umbilical cord (UC) - and amniotic membrane (AM) -MSCs. Human UC-MSCs and AM-MSCs have a higher proliferative potential and an ability to differentiate into osteoblasts. Moreover, these cells are thought to be able to be applied in an allogenic setting without eliciting host immune responses. However, the osteoblast differentiation ability is limited when compared with that of bone marrow-derived MSCs (BM-MSCs). Therefore, UC-MSCs usually require the long-term culture for differentiation but even differentiated cells cannot show sufficient *in vivo* osteo-inducibility.

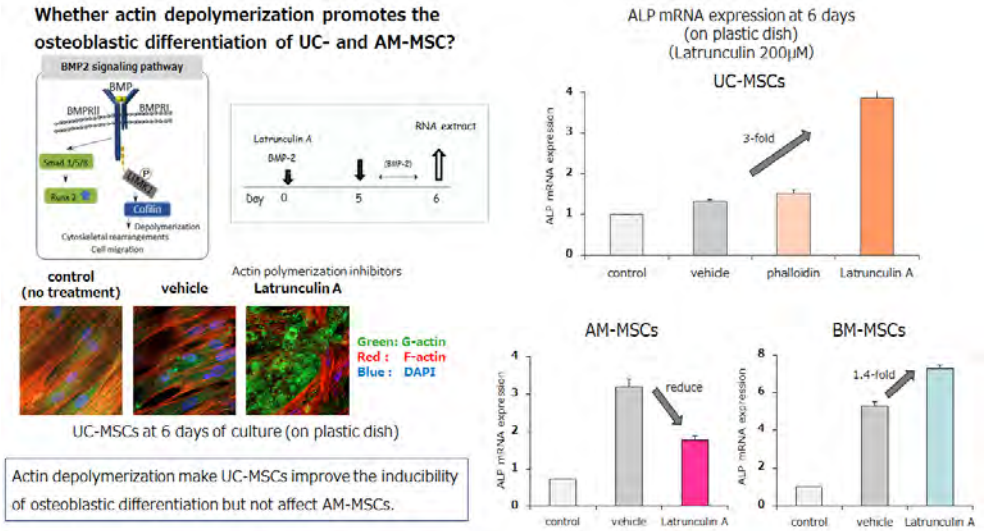
(Hypothesis) When investigated the downstream signaling pathways of BMPs in UC-MSCs during the osteoblastic differentiation in culture, we found the inhibition of cofilin, an actin-depolymerizing factor, gene expression. Furthermore, upregulation of pluripotency transcription factors such as Oct4 or Nanog was shown. Therefore, to promote the differentiation ability of cultured UC-MSCs and AM-MSCs for future clinical setting, we should investigate the ideal culture conditions by focusing on these features.



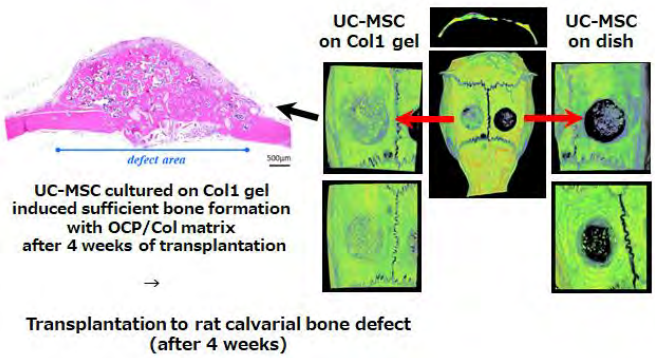
(Methods and Results) We firstly examined various 2D and 3D matrix cultures on UC-MSCs by focusing on cofilin gene expression. As results, we found a culture on the surface of Col1 gel matrix lead the up-regulation of cofilin gene significantly with/without rhBMP2. Therefore, when explored the localization of G-actin in UC-MSCs cultured on Col1 gel, we detected F-actin was rapidly depolymerized to monomeric G-actin and subsequently cellular G-actin had been accumulated in UC-MSCs. Then, after 7-10 days of culture, the expression level of alkaline phosphatase (ALP) mRNA and its activity in UC-MSCs were obviously enhanced up to the same level as BM-MSCs. In addition, differentiated UC-MSCs on day 10 significantly decreased Oct4 or Nanog mRNA expressions, and clearly enhanced osteocalcin expression. However, surface culture on Col1 gel could not affect the osteoblastic differentiation of AM-MSCs.



Taken together of these phenomena, Col1 gel culture must have promoted the osteoblastic differentiation ability of UC-MSCs *via* induction of actin-depolymerization on type I collagen matrix. Therefore, we have then investigated whether actin polymerization inhibitor affect the osteoblastic differentiation of UC-MSCs. As an experiment, UC-MSCs, AM-MSCs and BM-MSCs from the same patient were cultured with Latrunculin A (a actin polymerization inhibitor) and rhBMP2 on a plastic dish. After that, when evaluated the osteoblastic differentiation, UC-MSCs markedly increased ALP mRNA expression after 6 days of culture but AM-MSCs did not show significant changes.



Finally, to examine in vivo osteo-inducibility of UC-MSCs cultured on Col1 gel, we transplanted cultured UC-MSCs on Col1 gel or plastic dish to mouse cranium surface (bone augmentation model) and rat calvarial defect (defect model). As results, UC-MSCs cultured on Col1 gel could induce the new bone tissues remarkably compared with that on plastic dish. In addition, Latrunculin A treated UC-MSCs also showed enhanced osteo-inducibility in mice.



(Conclusion) Col-1 gel culture must be useful to enhance the osteoblastic differentiation ability of human UC-MSCs, and changes of cell morphology that determined by dynamics of the actin cytoskeleton may associate with the inducibility of osteoblastic differentiation of UC-MSCs. However, different feature was recognized on osteoblastic differentiation among UC- and AM-MSCs.

Effect of oral dryness and bolus property on swallowing function

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In this study, I investigated the effect of oral dryness on the swallowing movements. Electromyograms (EMGs) were recorded from supra and infra-hyoid muscles during swallowing of jelly, paste, ene-ice (unique crystalline oil which has a melting point at 28 degrees Celsius) and vanilla-ice before and 30 and 60 min after 1-mg atropine sulfate administration. Subjective feeling of oral/pharyngeal dryness continued to increase and salivary flow continued to decrease over the time. After atropine administration, a total number of swallows tended to increase during paste and vanilla-ice swallowing, which was a significant difference from jelly. Ease to swallow gradually decreased, particularly in jelly and paste while thermal sensation did not change in all foods. Comparing EMG burst activity (area under the curve of rectified EMG burst) and duration at the oral stage of swallowing among the conditions, they were both significantly larger in vanilla-ice as compared to any other food while there was no difference among the time points. Those at the pharyngeal stage were significantly larger in paste, which was gradually increased with time. We can suggest that ene-ice was less affected by oral/pharyngeal dryness probably due to activation of thermo-sensitive receptors.

口腔乾燥がもたらす嚥下機能への影響を食品条件の違いから考察する

本研究では、口腔乾燥が種々の食品摂取時の嚥下機能に与える影響を調べることを目的としたヒト実験を行った。15名の若年健常者を対象として、ゼリー、ペースト、エネアイス（28℃の融点をもつ結晶性油脂）、バニラアイスを嚥下した際の舌骨上下筋群筋電図記録を、アトロピン硫酸塩水和物1 mg投与前、投与30分、60分後に行い、嚥下困難感や温度感覚に関する主観的評価も行った。アトロピン投与後口腔・咽頭乾燥感は経時的に増加し、唾液分泌量は低下し続けた。ゼリー、ペーストでは主観的飲み込みやすさが有意に低下したが、温度感覚の経時的変化はいずれの食品にも認められなかった。総嚥下回数はペースト、バニラアイスで増加傾向にあり、ゼリーとの間で有意差を認めた。嚥下口腔期舌骨上筋群筋電図を比較したところ、活動量、活動時間ともにバニラアイスが有意に大きかったが、いずれの食品も経時的な変化は認められなかった。嚥下咽頭期では筋活動量、活動時間ともにペーストで高く、さらに経時的な増加を認めた。口腔乾燥下で温度感受性受容体を活性化するエネアイス摂取の経時的変化がなかったことは、温度感受性受容体の効果は口腔乾燥に影響を受けにくいことを示唆していた。

研究発表内容の紹介

嚥下運動が種々の食品条件や生体条件にどのような影響を与えるかについては、未だ不明な点が多い。本研究で用いたエネアイスは、その融解点が28℃（28度？）であり、これは温度感受性受容体であるTRPM8を活性化することから、室温で溶けた時に冷感を感じるだけでなく、TRPM8がもつ嚥下運動誘発の促進効果をもつことが期待される。本研究の結果をもとに、高齢者に多い口腔乾燥にも対抗できる食品研究やその生理機能評価を確定することで、将来的には新たな介護食品開発のヒントや臨床推進につながるという期待をもち、超高齢社会における高齢者歯科医療にもたらす貢献は計り知れない。（ファカルティー・アドバイザー：井上 誠）

Effect of oral dryness and bolus property on swallowing function

（問題点-Problem）

Japan has the highest life expectancy in the world; in 2020, the rate of aging was 28.7%, which was also the highest in the world. With aging, functional decline may occur not only in the body but also in the oral region. Of these changes, oral dryness, eating problem or difficulty in speaking due to hyposalivation is reported in many older people. However, few previous studies have clarified how oral dryness affects eating function depending on the bolus property (1st problem).

My senior presented the effect of crystalized oil and fat (COF) on swallowing function in SCRP 2019. COF is a special material, in that it composes in a crystal form in a room temperature and has a melting point at 28 degrees Celsius. One cold sensitive receptor, Transient Receptor Potential Melastatin 8 (TRPM8) is activated at this temperature. When COF is melted, the heat is absorbed and TRPM8 is activated so that the we are given the cold sensation. Based on the fact that TRPM8 is one of the receptors which activate/facilitate swallowing neural network, she demonstrated that COF application onto the tongue resulted in facilitation of swallowing initiation. Although oral dryness inhibits taste sensation, we do not know how oral dryness affects thermal sensation and hence swallowing initiation or movements (2nd problem).

（仮説-Hypothesis）

Under oral dryness condition, it may be difficult to perform bolus formation and propulsion during swallowing, which depends on the bolus property such as viscosity or adhesiveness. However, thermal sensation and following swallowing initiation are less affected by the oral dryness.

（方法-Methods）

Subject: 15 young healthy volunteers (8 females, average age 24.5±3.9 y/o). Recordings: surface electromyographic (EMG) activity was recorded from right suprahyoid and left infrahyoid muscles during freely swallowing of test foods (4 g of jelly, paste, ene-ice and vanilla-ice) (Fig. 1). Ene-ice includes COF. Next, atropine sulfate was orally given at a dose of 1.0 mg and subjective feeling of oral and pharyngeal dryness and unstimulated salivary flow per 30 sec were recorded immediately and every 10 min after administration for 60 min. Heartbeat, respiratory rate and blood pressure were also recorded before and after atropine administration.

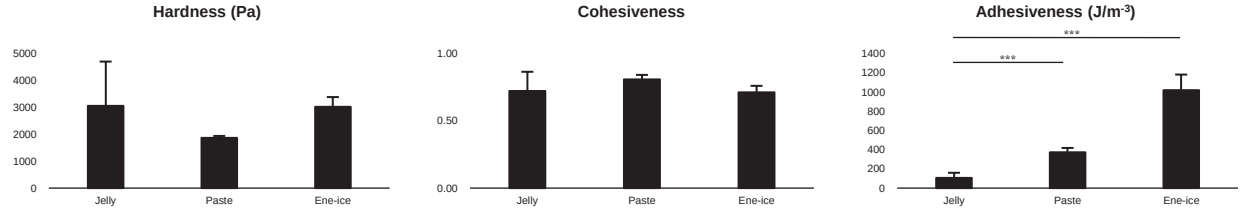

Fig. 1. Bolus property of test foods at 20℃. Vanilla-ice could not be measured at this temperature. The data was obtained by averaging three values. There was a significant difference only in viscosity among the foods (one way ANOVA followed by Tukey's test).

Analysis: subjective feeling of oral/pharyngeal dryness, vital signs, subjective evaluation of easy to swallow and thermal sensation were compared between before and after atropine administration. Using EMG data, a total number of swallows, EMG activity (area under the curve of rectified EMG waveform during swallowing) and EMG burst duration were also compared among the conditions.

This study was approved from the Ethics Committee of Ethics Committee of Niigata University (2020-0125)

（結果-Results）

1. After atropine administration, oral/pharyngeal feeling of dryness continued to increase and salivary flow continued to decrease (Fig. 2). There was no difference in vital signs (data not shown).

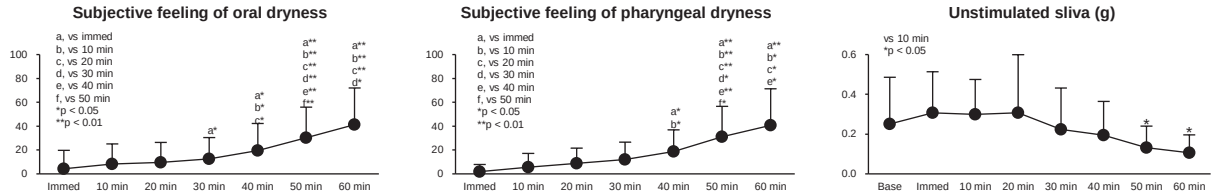

Fig. 2 Changes of visual analogue scales (VAS) of oral (left)/pharyngeal (middle) dryness and unstimulated salivary flow (right). Immed, immediately after atropine administration; Base, before administration.

2. Evaluation of swallowing (Fig. 3)

(1) Ease to swallow gradually decreased with time, particularly in jelly and paste. This was not the case of thermal sensation during swallowing, in that there was no significant difference in thermal sensation among the time points in all foods. (2) A total number of swallows was less affected, but it tended to increase during paste and vanilla-ice swallowing.

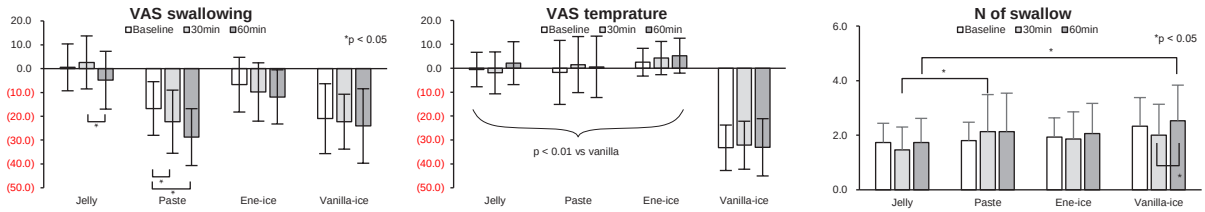

Fig. 3 Changes of ease to swallow (left), cold sensation (middle) and total number of swallows (right).

(3) Effects on oral stage of swallowing (Fig. 4): EMG activity and duration of suprahyoid muscles were significantly larger in vanilla-ice than any other food. There was no significant difference in these values among the time points.

(4) Effects on pharyngeal stage of swallowing (Fig. 4): Only paste swallowing was affected by atropine administration, in that EMG duration gradually increased with time and EMG activity was significantly larger than that during jelly or ene-ice swallowing.

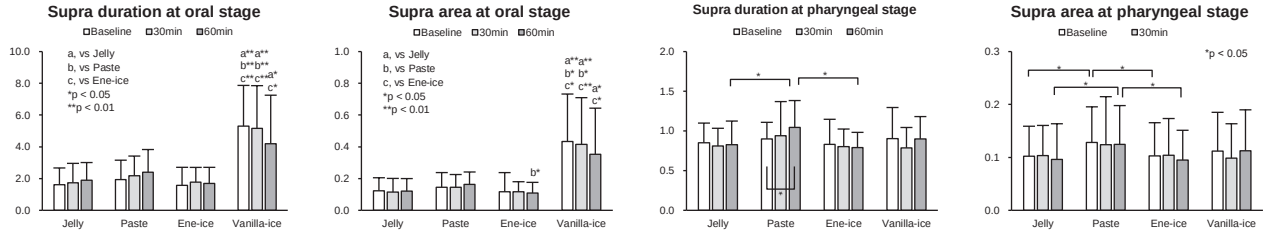

Fig. 4 Changes of EMG duration (supra duration) and EMG area (supra area) with time.

（結論-Conclusion）

Under oral/pharyngeal dryness condition, swallowing movements in all foods were affected. In particular, impacts on subjective feeling and pharyngeal stage were high in jelly or paste. On the other hand, effect on oral stage was the highest in vanilla-ice. Although we can suggest that ene-ice was less affected by oral/pharyngeal dryness probably due to activation of thermo-sensitive receptors, any positive evidence to support this suggestion were not obtained from EMG data. In our future study, we should collect the data from older people or patients with oral dryness or using different foods.

Evaluation of face guard cores made by stereolithographic three-dimensional printing: build orientation and material

東京医科歯科大学歯学部 5年生 Tokyo Medical and Dental University Faculty of Dentistry Class of 2022

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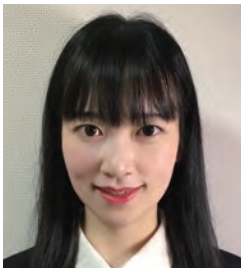
研究指導協力者：先端材料評価学分野 助教 和田 敬広



[Problem・Hypothesis] A face guard (FG) is a protection device that is worn by athletes having maxillofacial injuries. It consists of a thermoplastic core and cushion covering materials. Conventional FG is a time-consuming and sensitive technique. Recent progress in digital dentistry has enabled accurate construction of FG using a 3D printer; however, its mechanical properties have not been evaluated. In this study, the appropriate building conditions and resin resource for FG core preparation using a stereolithography 3D printer was estimated by bending test and impact test, then, the application of 3D printer for FG preparation was considered.
[Methods] The most rigid build orientation was decided from the flexural modulus by the bending test. The impact absorption and pressure dispersion properties of four kinds of resin built in above orientation were evaluated.
[Results] The highest flexural modulus was obtained in which built in lateral to force orientation. In the impact test, FG core prepared by an impact resistant resin of 3 mm thick or more did not show macroscopic fracture. In addition, its impact force absorption and dispersion were same or better than a conventional FG core.
[Conclusion] An FG's core, 3D printed impact resistant resin achieved same or better impact absorption and dispersion properties compared to a conventional FG core. Therefore, the feasibility of practical FG core production by a 3D printer.

光造形 3D プリンタによるフェイスガードコア材の造形検討 -積層方向と材料について-

【問題点・仮説】 フェイスガード（FG）は、顎顔面外傷を持つ競技者用保護具であり、熱可塑性樹脂のコアとクッション材からなる。従来のFG製作には多くの手間と時間が必要であり、デジタルデンティストリーの応用が検討されているが、最適な製作法やFG素材として必要な物性について不明な点も多い。本研究では光造形3Dプリンタを用いて、FGに適する造形条件やレジン素材の検討を機械的特性を指標として行い、同法のFGコア材製作への応用を検討した。
【方法】 3つの積層方向で製作した試験片の曲げ強さから最も剛直な積層方向を調査し、その条件で積層した種々のレジン素材をコア材としたFGの衝撃試験を行って衝撃吸収・分散能を評価した.
【結果】 3点曲げ試験から荷重方向に積層した試料が最大の弾性率を示し、FGに適していると判明した。衝撃試験では厚み3mm以上の高耐衝撃性レジンのコア材は巨視的破壊が生じることなく、従来素材と同等かそれ以上の衝撃吸収・分散能が得られた。
【結論】 高耐衝撃性レジンで作製したコア材は、臨床で使用されている従来素材と同等以上の衝撃吸収・分散性を有していた。光造形3Dプリンタで実用可能なFGコア材を作製できることが示唆された。

研究発表内容の紹介

スポーツ競技者の安全確保でフェイスガード（FG）の果たす役割は大きい。応募者は近年、発展の著しい3DプリンタをFG製作に活用し、簡便に高強度で競技者保護効果が大きいFG素材の開発に必要な基礎検討を行った。同手法では従来法に比べて高強度の素材を簡便かつ正確に成形可能で、強い衝撃を十分に低減・拡散し競技者を保護し得ることを示し、この知見は今後のスポーツ医歯学臨床に十分に寄与すると考えられた。（ファカルティー・アドバイザー：宇尾 基弘）

Evaluation of face guard cores made by stereolithographic three-dimensional printing: build orientation and material

(Problem)

A face guard (FG) is a protection device that is worn by athletes having maxillofacial injuries. It consists of a thermoplastic core and cushion covering materials. Conventional FG is a time-consuming and sensitive technique that involves impression-making and heat pressing. Recent progress in digital dentistry has enabled accurate construction of FG using a 3D printer (Fig. 1); however, its mechanical properties (e.g., flexural modulus, impact absorption, and impact dispersion) have not yet been evaluated.

(Hypothesis)

In this study, core materials for FG were created using a stereolithography (SLA) 3D printer. The four types of resin resources used for this 3D printer had three build orientations. Afterwards, their application potential was evaluated using three-point bending and impact tests.

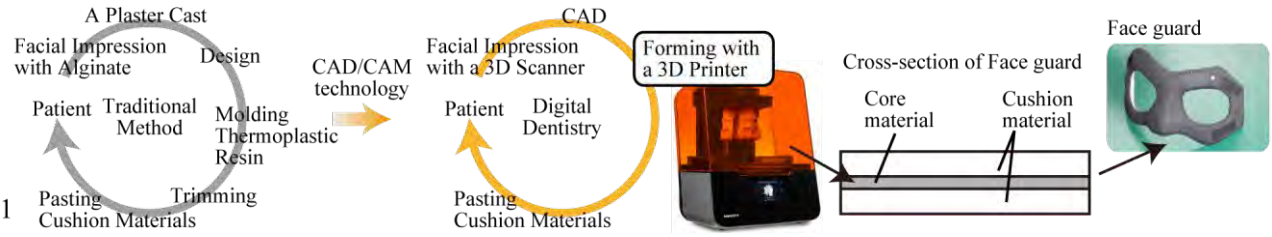


Fig. 1

(Methods)

An SLA 3D printer (Form3, Formlabs, Inc.) was used. Four types of resins (Durable, Draft, Rigid 10 K, and Tough 1500, Formlabs, Inc.) were used as materials.

1) Build orientation and flexural modulus

Specimens (80 mm in length, 10 mm in width, and 4 mm in thickness) were constructed in three build orientations (Fig. 2) for three point bending tests. These tests were performed using a universal test machine (EZ-LX, Shimadzu Co.) with a support span width of 64 mm, crosshead speed of 2.0 mm/min, 5 mm indenter radius, and 5 mm support radius. The difference in the flexural modulus was investigated (n=5).

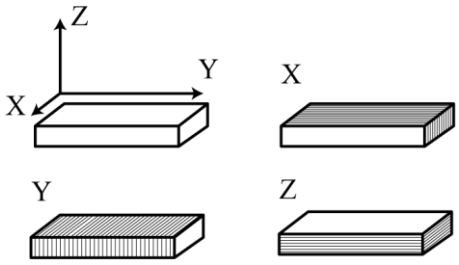


Fig. 2

2) Shock absorption capacity

The FG core materials were constructed with thickness ranging from 1 to 4 mm (100×100 mm). The control was a thermoplastic resin, AP3 (3 mm thickness, Aquaplast, Sakai Medical), that was used as a conventional FG core material. Both the sides of the core material were covered with cushion materials (neoprene, Sakai Medical) that were commonly used for current FG using adhesive.

The specimen was placed on a modified DuPont impact tester (IM-201, Tester Sangyo, Fig. 3). A 500 g weight was dropped from a height of 240 mm (the diameter of the tip was 6.34 mm) that collided over the specimen. Impact force (approx. 4920 N) was considered sufficient for frontal bone fractures. The cracks in the specimen were inspected visually. The pressure distribution under the specimen, which was corresponding to the surface of the living body, was checked with the pressure measurement. The maximum impact force was measured using a load cell under the specimen (n=5).

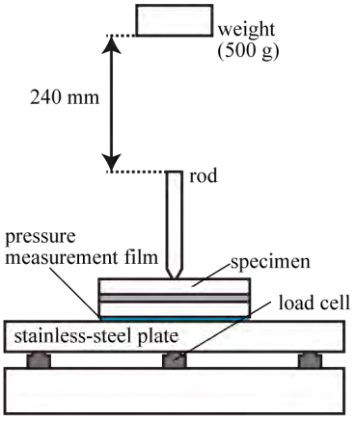


Fig. 3

(Results)

1) The best build orientation

The flexural modulus of all the materials, estimated using the three-point bending tests, was highest when the specimen had a built in thickness in the direction of the Z axis (Table 1). A high flexural modulus revealed less deformation following impact. The following impact tests were carried out for the specimens built in the Z direction.

Table 1

Material	Build Orientation		
	X	Y	Z
Durable	499 ± 33 [a]	625 ± 30 [b]	711 ± 88 [c]
Tough 1500	1030 ± 59 [a]	1030 ± 41 [a]	1150 ± 63 [b]
Rigid 10K	7380 ± 890 [a]	8870 ± 160 [b]	8630 ± 210 [b]
Draft	942 ± 31 [a]	1090 ± 23 [b]	1250 ± 40 [c]

2) Evaluate the shock absorption and dispersion

The fracture ratio of the core material is listen in Table 2. Thinner core materials (≤ 2 mm) fractured after a single impact. The fracture ratio of the specimens having dimensions of 3 mm or more varied with the type of a resin. Durable resin, having dimensions of 3 mm and 4 mm (Du3 and Du4), did not reveal macroscopic fracture, therefore it is most suitable as a core material.

Table 2

Material	Thickness (mm)			
	4	3	2	1
Durable	0	0	100	100
Tough 1500	20	40	100	100
Rigid 10K	40	100	100	N/A
Draft	20	80	100	100

Fig. 4 shows the maximum force under the specimen. The impact force was reduced to approximately 10% when the specimen was inserted. Impact force was significantly decreased for AP3 and Du3, compared to Du4 ($p < 0.05$; Tukey-Kramer multiple comparisons). Therefore, Du3 had sufficient impact absorption capacity.

Fig 5 shows the pressure distribution images of the specimen. The pressure was concentrated at the impact when specimen was not used. After inserting AP3, the pressure was dispersed to the surrounding area (20 mm in diameter). Upon inserting Du3 and Du4, the pressure drastically decreased to an unmeasurable range. The pressure distribution measurement suggested that Du3 and Du4 had sufficient impact dispersibility.

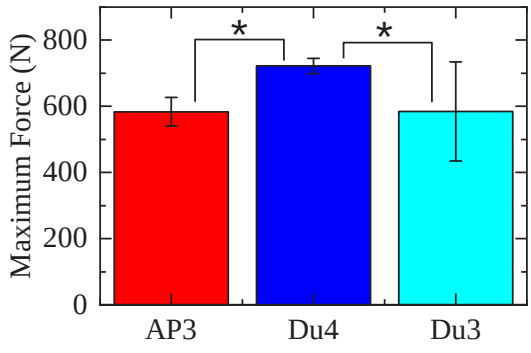


Fig. 4

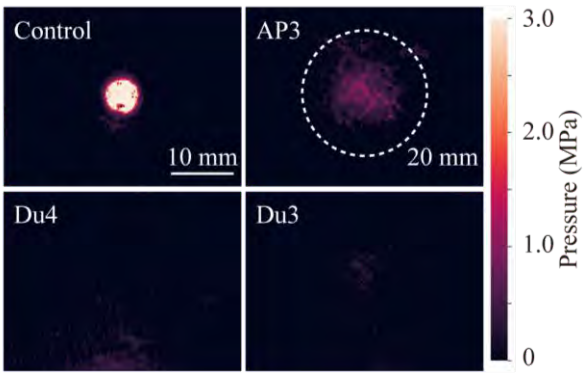


Fig. 5

(Conclusion)

In this study, core materials for the FGs were created using various resins with a 3D printer. Compared with the conventional FG core (AP3), Du3 as well as Du4 showed sufficient impact absorption properties for the maximum impact force and pressure distribution tests. Therefore, it is expected that creating the core material with a Durable resin and using a 3D printer will offer better protection.

Effect of ELF pulsed magnetic field on wound healing

徳島大学歯学部　6年生　Faculty of Dentistry Tokushima University　Class of 2021

深田 有希　Yuki FUKADA

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研究指導協力者：口腔顎顔面補綴学分野 講師　渡邉 恵

Dental treatments involve invasion of the epithelium and bone and the sooner and normal healing process is strongly required. Low-frequency pulsed magnetic fields have been used in orthopedic field to treat bone fractures, and their safety has been recognized. In this study, we investigate whether our extremely low-frequency pulsed magnetic field can be applied to wound healing by *in vitro* experiments using cultured cells. The keratinocyte cell lines HaCaT and PAM2.12 were irradiated with 7000 mG (0.7 mT) 6 Hz extremely low-frequency pulsed magnetic fields, and sooner wound healing in the magnetic field irradiation group were observed by wound healing assay. Next, cell proliferation analysis showed no significant difference between the magnetic field irradiation group and the control group, but cytoskeleton observation showed increased actin stress fiber formation in the magnetic field irradiation group. Finally, the intracellular signal transduction after magnetic field irradiation showed activation of RhoA and ERK1/2 in the magnetic field irradiation group.
These results suggest that low-frequency pulsed magnetic field irradiation promotes wound healing by inducing cell migration, especially in the early stage, through signaling from ERK1/2 to RhoA.

創傷治癒に対する低周波パルス磁場の効果

歯科治療の過程では上皮や骨に侵襲が与えられることがあり、その侵襲に対して治癒が正常に早く進む方がよいことは自明である。低周波パルス磁場は整形外科領域で骨折の治療などに用いられており、その安全性は認められている。本研究では、我々が開発した低周波パルス磁場装置を創傷治癒に応用できるかどうか、培養細胞を用いて検討することにした。
ケラチノサイト細胞株HaCaTおよびPAM2.12に7000 mG (0.7 mT) 6 Hz低周波パルス磁場を照射して、創傷治癒アッセイを行ったところ、磁場照射群で創傷治癒の有意な促進を認めた。次に細胞増殖を解析したところ、磁場照射群と対照群に有意な差はなかったが、細胞骨格の観察では、磁場照射群でアクチンストレスファイバー形成の増加がみられた。最後に、磁場照射後の細胞内シグナル伝達を調べたところ、磁場照射群でRhoAおよびERK1/2の活性化を認めた。
これらの結果から、低周波パルス磁場照射はERK1/2からRhoAへのシグナル伝達により、特に初期の細胞遊走を誘導することで創傷治癒を促進しすることが示唆された。

研究発表内容の紹介

歯科診療では、病態として、あるいは治療行為に伴って口腔粘膜に創傷が生じることがあるが、その早期の治癒は患者、術者双方に有益である。この研究は低周波パルス磁場を創傷治癒促進に応用することを目的にしたもので、現段階では細胞を用いた基礎的研究である。しかしながら、培養細胞における創傷治癒の促進とそのメカニズムが明らかになり、さらに細胞への為害性も確認されなかったことから、今後は動物を用いた実験へ進み、近い将来臨床で使用することも可能であると考える。
（ファカルティー・アドバイザー：市川 哲雄）

Effect of extremely low-frequency pulsed magnetic field on wound healing

Background and Problems

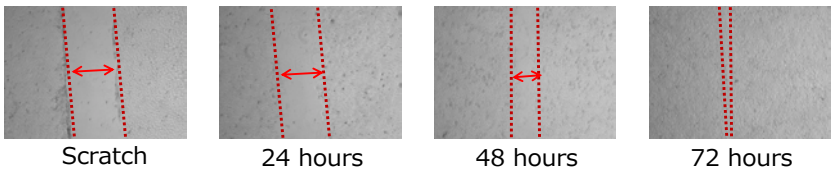
Periodontitis and periodontal surgery, stomatitis, denture ulcers, drilling for the placement of dental implants and tooth extraction involve invasions of the epithelium and bone. The sooner healing with normal process is expected. Basic research on the promotion of tissue regeneration and healing, especially the bone formation,
has been actively conducted, and many studies have utilized the effects of growth factors and cytokines for bone formation. In recent years, in addition to these methods, it has become clear that physical factors play an important role in wound healing and bone growth. In particular, physical/mechanical stimuli such as the exposures of ultrasound and magnetic fields are known to be effective in promoting bone formation. In the field of orthopedics, low-frequency pulsed magnetic fields have been used as an adjunct therapy for fracture healing. Many studies have been conducted on the effects of magnetic field stimulation on living organisms. However, the results have not always been consistent due to the lack of uniform conditions such as the type and frequency of magnetic fields (static, high-frequency, and low-frequency magnetic fields), the large variation in the target cells and individuals, and the inconsistent cell culture conditions. In the case of low-frequency magnetic fields, many studies have shown that magnetic fields have some effects on living organisms, and that these effects are not particularly negative.
In this study, we investigate how the extremely low-frequency (ELF) pulsed magnetic stimulation, which has a potential to align water molecules, affects wound healing process through in vitro experiments using cultured cells.

Methods

The stimulation intensity of the ELF pulsed magnetic field
was 7000 mG (0.7 mT) at 6 Hz. The human keratinocyte cell line HaCaT and the mouse keratinocyte cell line PAM2.12 were used as epithelial cells.


ELF pulsed magnetic field

1. Wound healing assay: The cultured cells were scratched and irradiated with a magnetic field for 24 to 72 hours to observe the closing of the scratch marks, and examined whether the magnetic field affected the wound healing.


Scratch　24 hours　48 hours　72 hours

2. Cell proliferation assay: Assay was examined after the magnetic field irradiation to determine whether the magnetic field-induced wound healing was due to cell proliferation or cell migration.

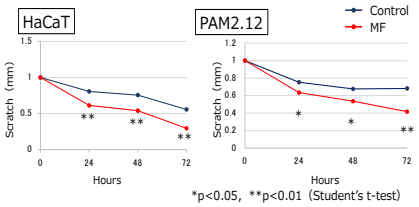
3. Observation of cytoskeleton: Actin fibers were stained to observe the cell skeleton after magnetic field irradiation.

4. Observation of signal transduction: The activation of the MAP kinase ERK and the low molecular weight G proteins RhoA, Rac1, and Cdc42 were analyzed by Western blotting and GLISA.

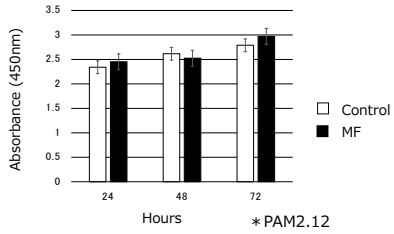
Results

1. Results of wound healing assay

A significant acceleration of wound healing was observed in the magnetic field irradiation group (MF) for both HaCaT and PAM2.12.

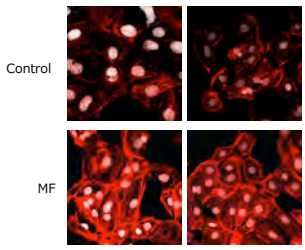


2. Results of cell proliferation assay



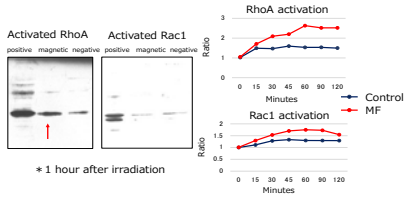
There was no significant difference in cell proliferation between both groups.

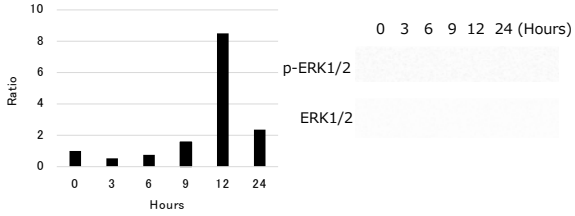
3. Cytoskeletal changes



The formation of actin stress fibers was observed in the MF group compared with the control group.

4. Signal transduction inside the cell





Activation of RhoA and Rac1 and phosphorylation of ERK1/2 were enhanced in the MF group compared with the control group.

Discussion

In this experiment, the ELF pulsed magnetic fields accelerated wound healing in both human and mouse keratinocyte cell lines. Since cell proliferation or cell migration is a possible mechanism for wound closure, we investigated this mechanism and found that the accelerated healing caused by the magnetic field was not due to cell proliferation. When cells migrate, actin stress fibers are formed in the early stage by signaling from ERK to RhoA behind the direction of movement, and foliated pseudopodia are formed in the late stage in front of the direction of movement. In this experiment, we found that ELF pulsed magnetic field promoted the formation of actin stress fibers and the activation of ERK and RhoA, suggesting that ELF pulsed magnetic field promotes the early phase of cell migration in wound healing.

Conclusion

The ELF pulsed magnetic field accelerated wound healing in both human and mouse cells *in vitro*. In addition, the ELF pulsed magnetic field activated RhoA and Rac1 without affecting cell proliferation. These results suggest that the ELF pulsed magnetic fields may accelerate wound healing by promoting early cell migration via ERK to RhoA signaling.

Effect of probiotics candidates *Lactobacilli* on infection prevention of *Candida albicans*

鶴見大学歯学部 4年生 Tsurumi University School of Dental Medicine Class of 2023

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研究指導協力者：口腔微生物学講座 学部助手 溝辺 智美
口腔微生物学講座 ポストドクター 向井 陽子

With aging, immunity declines and morbidity rises leading to an increasing incidence rate and number of carriers of *Candida*. I hypothesized that probiotics using *Lactobacillus* would be effective in improving the balance of indigenous oral flora and activating immunity in order to prevent overgrowth and pathogenicity of *Candida*. However, since lactic acid bacteria produce organic acids implying the risk of caries and hypersensitivity, it was necessary to searched for and verify strains that produce effective metabolites under neutral pH conditions.
The *Candida albicans* type strain was used as test strain and the culture supernatants (CS) of 5 *Lactobacillus* strains were used as test samples. I performed antibacterial tests, a hyphal formation suppression test of *C. albicans*, a protease activity inhibition test and a cytokine production test from human epithelial cultured cells. As a result, fungicidal activity against *C. albicans*, inhibition of protease activity, suppression of hyphal formation, immune-activation and an immune-modulatory effect of CS were confirmed in most of the 5 strains. Therefore, I conclude that the combined use of effective substances produced by these strains used as probiotics might prevent the spread of *Candida*.

*Candida albicans*感染予防に対する*Probiotics*候補*Lactobacillus*の効果

高齢化による免疫低下と有病率の上昇伴い、口腔*Candida*菌保有率と発症率が増加している。*Candida*の過剰増殖及び病原性の発揮を防ぐために、*Lactobacillus*を用いたプロバイオティクスによる口腔常在菌叢のバランス改善と免疫活性化が効果的であるという仮説を立てた。しかし、乳酸菌は有機酸を産生するためう蝕や知覚過敏症のリスクも考えられたので、pH中性条件で有効な代謝産物を産生する菌株を探索し、検証した。
被検菌株は*Candida albicans*基準株に対し、5株の*Lactobacillus*培養上清を用いて、*C. albicans*の抗菌試験と菌糸形成抑制試験、プロテアーゼ活性阻害試験、ヒト上皮培養細胞からのサイトカイン産生の確認を行った。その結果、ほとんどの菌株で*C. albicans*に対する抗菌性、プロテアーゼ活性阻害、菌糸形成抑制、免疫活性化および免疫調整効果が確認された。従って、有効なこれらの菌株の産生物質を組み合わせてプロバイオティクスとして用いることにより、*Candida*の拡大防止につなげることができると考えられる。

研究発表内容の紹介

口腔カンジダ症は痛みや不快感でQOLを大きく低下させるが、未だに予防法が確立されていない。そこで、人類が長い食歴をもつプロバイオティクスに着目したことは、導入しやすいだけでなく、薬剤と異なり耐性化や副作用のリスクが回避される点でも優れている。本研究は、プロバイオティクス候補を中性条件で機能的に評価した基礎研究であるが、実用化も期待され、齲蝕や歯周病を含む口腔疾患全体の予防に適応を広げられる可能性を含んでいる。（ファカルティー・アドバイザー：大島 朋子）

Effect of probiotics candidate *Lactobacilli* on infection prevention of *Candida albicans*

(Problems)

With increasing age, oral *Candida* prevails in inverse proportion to the decrease in immunity. Problems and diseases related to *Candida* rise, such as denture stomatitis due to increasing denture usage and aspiration pneumonia due to decreasing reflexes. With the aging of the population, the prevalence of underlying diseases grows. Due to plenty of medication, xerostomia and microbial substitution surge, and the number of *Candida*-carriers tends to increase dramatically. However, specific measures to prevent candidiasis have not yet been established.

(Hypothesis)

Probiotics is effective in improving the balance of the indigenous oral flora and in activating immunity. It prevents overgrowth and pathogenicity of the oral indigenous microorganism *Candida*.

(Methods)

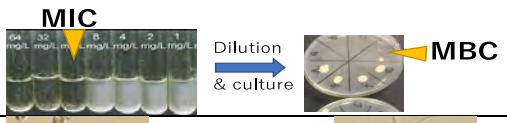
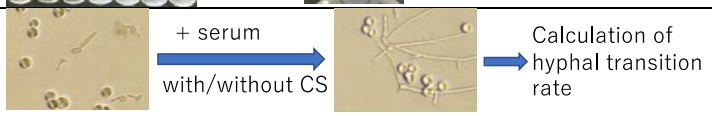
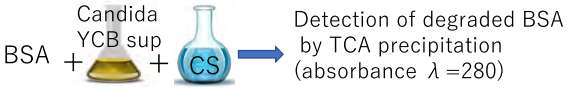
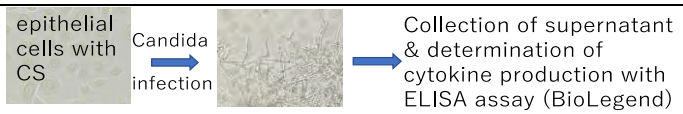
From preliminary tests with 42 Lactobacillus strains stored in the laboratory, 5 strains were selected as inhibitor candidates against *C. albicans* growth. They are:

Lactobacillus plantarum LP103, Lactobacillus plantarum LP108

Lactobacillus plantrum LP122, Lactobacillus negelii FL1, Lactobacillus plantarum FL2

Candida albicans (ATCC:18804) was the fungus to be tested.

Cultured supernatant (CS) of *Lactobacilli* in MRS medium was neutralized to pH 7.0 and concentrated 20-fold to be used as CS sample. Since the Lactobacilli culture supernatant is originally acidic and contains lactic acid, its application to the oral cavity is not desirable due to the risk of caries and hypersensitivity. Therefore, the CS was neutralized before use. The following tests were performed:

1. Growth inhibitory test of CS with yeast or hyphal type of *C. albicans*.	
2. Hyphal formation inhibitory test of CS by microscopic observation.	
3. Protease activity inhibitory test of CS by detection of BSA degradation.	
4. Cytokine production test (TNF-α, IL-6, IL-8) in infected human epithelial cells (HEK-a, ThermoFisher) with CS using ELISA.	

(Results)

1. Since a growth inhibitory effect was observed at 50% CS for both yeast and hyphal type of *C. albicans* (Fig.1&1'), the minimum inhibitory concentration (MIC) was judged to be 50%, and the minimum fungicidal concentration (MBC) was also the same concentration, therefore CS is considered fungicidal.
2. When the amount of inoculated fungi was increased 10 times, the antimicrobial property decreased, but a significant hyphal inhibitory effect of CS was observed (Fig.2). In oral candidiasis, the hyphal type of *C. albicans* is said to be pathogenic and invade tissue, therefore low hyphal formation is desirable.

3. CS had an inhibitory effect on protease activity, which was significantly different from the control (Fig.3). The protease activity is one of the virulence factors of *C. albicans*, then inhibition was desirable. Especially for FL1 and FL2 the quantity of degraded protein was reduced.
4. *C. albicans* infection resulted in increased TNF-α production, but CS had a suppressing effect (Fig.4), indicating an anti-inflammatory effect. IL-6 and IL-8 were significantly promoted with CS (Fig.4) showing immunity activation.

Fig. 1. Growth inhibition test (yeast type MIC)

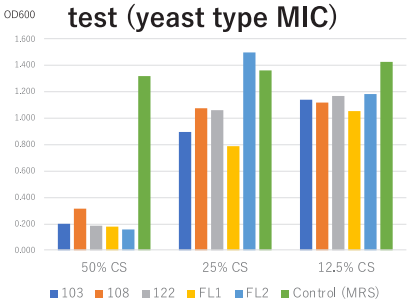


Fig. 1'. Growth inhibition test (hyphal type MIC)

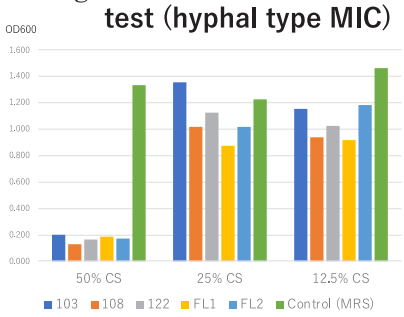


Fig. 2. Hyphal formation inhibitory test

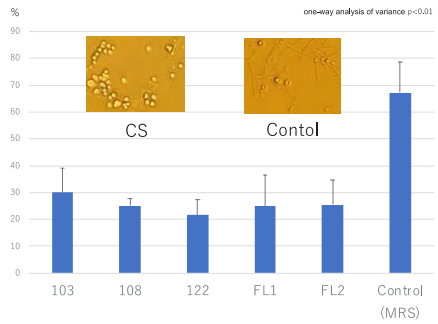


Fig. 3. Protease (SAP) activity inhibitory test

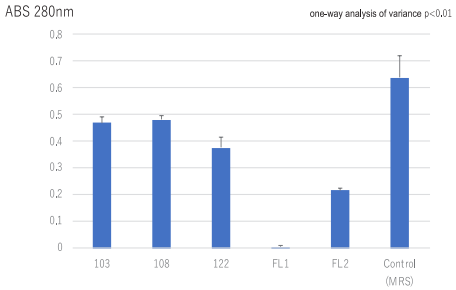
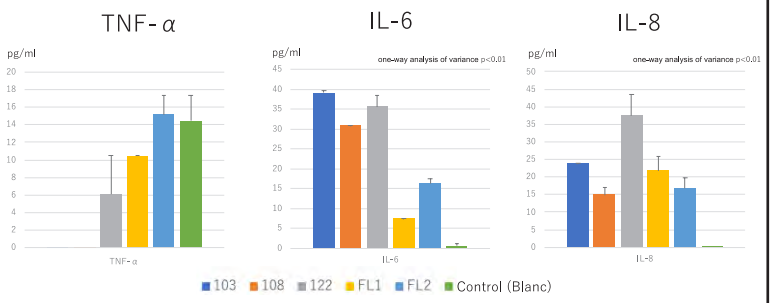


Fig4. Cytokine production test in the infected epithelial cells

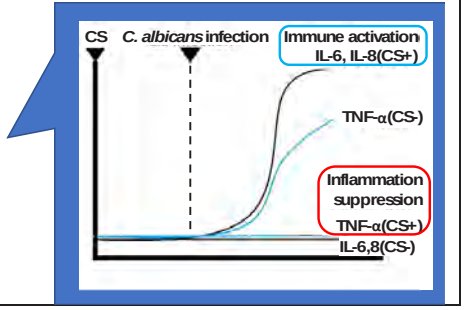


(Conclusion)

- It was shown that CS has an inhibitory effect on the overgrowth and pathogenicity of *C. albicans*, indicating the possibility of improved balance of the oral flora.
- Further, it was shown, that following the increased immune activity of infected epithelial cells by CS, a moderate suppression due to TNF-α could be observed.
- From the above, it is possible, that CS can be applied to food, mouthwash, dentifrice and else as oral probiotics. To be effective, it might be necessary to combine all 5 test strains (as the summary table), because they were not all at the same level.

Summary table

Test systems		Lp103	Lp108	Lp122	FL1	FL2
Growth prevention and fungicidal effect		+	+	+	+	+
Inhibition of hyphal formation		+	+	+	+	+
Inhibition of protease (SAP) activity		+	+	+	++	+
Immuno-modulatory effect of infected epithelial cells	Inflammation suppression (TNF-α)	++	++	+	+	-
	Immune activation (IL-6)	++	++	++	+	+
	Immune activation (IL-8)	+	+	++	+	+



Periodontitis & abnormal pregnancies: *Porphyromonas gingivalis* utilizes macrophage extracellular vesicles and inhibits placental angiogenesis

岡山大学歯学部　4年生　Okayama University Dental School　Class of 2023

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Problem: The placenta connects the mother and fetus through a sophisticated vascular structure. Placental dysfunction is associated to fetal abnormalities. *Porphyromonas gingivalis* (Pg) affects fetal growth, but the detailed mechanism is not clarified. I examined the mechanism of how Pg utilizes macrophage extracellular vesicles (Mφ EVs) and affects fetal growth through the inhibition of placental angiogenesis.
Method: Pg-infected Mφ EVs (Pg-inf Mφ EVs) were collected and injected into pregnant mice. The extracted placenta and fetus were analyzed by *in vivo* imaging, bioinformatics, and histological methods. Human Umbilical Vein Endothelial Cells (HUVEC) were used *in vitro* experiments. All animal studies were approved by the Ethics Committee.
Results: Pg-inf Mφ EVs translocated and inhibited the development of the placenta and fetus. The amount of translocated EVs correlated with the level of abnormality of the placenta and fetus. The placenta of Pg-inf Mφ EVs-injected group exhibited decreased blood vessel area and expression of Vascular Endothelial Growth Factor Receptor 1 (VEGFR1). Decreased VEGFR1 expression and migration were also observed in HUVEC.
Discussion: Pg-inf Mφ EVs downregulate VEGFR1 and delay the migration ability in endothelial cells, inhibiting the process of angiogenesis and decreasing blood circulation. The placenta is unable to provide sufficient nutritions to the fetus, causing fetal abnormalities.
Conclusion: Pg utilizes Mφ EVs and inhibits placental angiogenesis through the downregulation of VEGFR1, which results in fetal abnormalities.

歯周病と胎児の成長障害：*Porphyromonas gingivalis*はマクロファージの細胞外小胞を介して胎盤の血管形成を阻害する

問題：胎盤は精密な血管構造を介して母体と胎児を結び、胎児の成長に大きな影響を与える。歯周病原菌*Porphyromonas gingivalis*（Pg菌）が胎児の成長障害に関与することがわかってきたが、その詳しいメカニズムは明らかでない。
方法：Pg感染Mφから細胞外小胞（Pg-inf Mφ EVs）を回収し、妊娠マウスに投与した。摘出した胎盤・胎児とヒト血管内皮細胞（HUVEC）を用いて、*in vivo* imaging、細胞組織学的手法等により解析した。
結果：Pg-inf Mφ EVsは、胎盤・胎児に到達し、それの成長を著しく阻害した。投与群の胎盤では血管形成と血管内皮細胞増殖因子受容体1（VEGFR1）発現の低下が認められた。Pg-inf Mφ EVsはHUVECにおいてもVEGFR1の発現と細胞遊走能を抑制した。
考察：Pg-inf Mφ EVsはVEGFR1の低下を介して胎盤の血管形成を阻害する。その結果、胎児への栄養供給が低下し、胎児の成長障害を誘導すると考えられる。
結論：PgはMφ EVsを介して胎盤の血管形成を阻害し、胎児の成長障害を誘導する。

研究発表内容の紹介

歯周病による胎盤組織への障害性に関する分子生物学的研究は極めて少ない。本研究では、歯周病原菌がマクロファージ由来の細胞外分泌小胞を介して母体と胎児を繋ぐ胎盤組織に重大な影響を及ぼすことを明らかにした。これらの所見は、従来の概念をこえて、口腔環境が母体だけではなく、子孫の健康にまで影響を与えていることを示唆するものであり、妊娠時の母体の口腔ケアの重要性を広く社会に広め、国民の健康増進に寄与すると考えられる。（ファカルティー・アドバイザー：岡村 裕彦）

Periodontitis & abnormal pregnancies: *Porphyromonas gingivalis* utilizes macrophage extracellular vesicles and inhibits placental angiogenesis

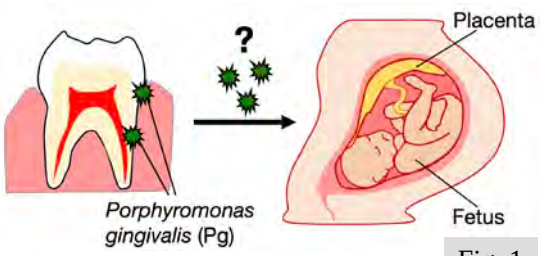


Fig. 1

【Problem】

The placenta is an important tissue with a sophisticated vascular structure, connecting the mother and the fetus for nutrients, metabolites, and gas exchange. Placental homeostasis strongly affects fetal development. Therefore, placental disorder is directly associated to fetal growth and development inhibition.

Periodontitis is a chronic inflammation caused by periodontal pathogens such as *Porphyromonas gingivalis* (Pg). It has been shown that periodontitis affects fetal growth, but the detailed mechanism is not clarified (Fig 1). I examined the mechanism of how Pg utilizes macrophages (Mφ) and affects fetal growth through the inhibition of placental angiogenesis.

【 Hypothesis 】

Pg can invade Mφ and transport its own pathogens into Mφ-derived extracellular vesicles (Mφ EVs) (Fig. 2). Pg-infected Mφ EVs (Pg-inf Mφ EVs) translocate to distant organs. In this study, I hypothesized that Pg-inf Mφ EVs reach the placenta and impair its tissue structure and function."

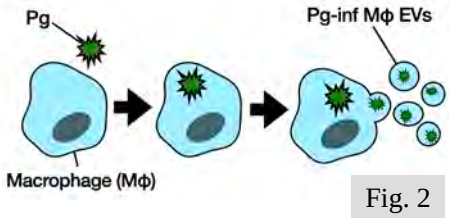


Fig. 2

【 Method】

***Collection of Pg-inf Mφ EVs*:** Monocytic cell line was differentiated into Mφ and treated with Pg for 4 h. After culturing for another 48 h, Pg-inf Mφ EVs were collected from the culture medium.

***Animal Experiments*:** Pregnant mice were injected with Pg-inf Mφ EVs and sacrificed at embryonic day 18. The localization of EVs was detected using In Vivo Imaging System (IVIS). The extracted placenta was used for HE staining, Western blot (WB), or Coomassie Brilliant Blue (CBB) staining. Placental EVs were analyzed by mass spectrometry and used for gene ontology analysis. All animal studies were approved by the Ethics Committee.

***In Vitro*:** Primary Human Umbilical Vein Endothelial Cells (HUVEC) treated with Pg-inf Mφ EVs were used for scratch assay, WB, or CBB staining.

【 Results】

1) Pg-inf Mφ EVs translocate and inhibit the growth of the placenta and fetus

The size and weight of the developed placenta and fetus in the Pg-inf Mφ EVs-injected group significantly decreased ($P < 0.0001$) to approximately half of that of the control group (Fig. 3A and 3C). In addition, many of the fetus in the Pg-inf Mφ EVs-injected group showed signs of delayed development at an early embryonic stage, resembling a pea-size cluster. Moreover, IVIS showed that Pg-inf Mφ EVs translocate to the placenta and fetus (Fig. 3B). Higher signals were observed in abnormal placenta and fetus. This indicates that Pg-inf Mφ EVs directly inhibit the growth of the placenta and the fetus and also that the level of inhibition is correlated with the amount of translocated EVs.

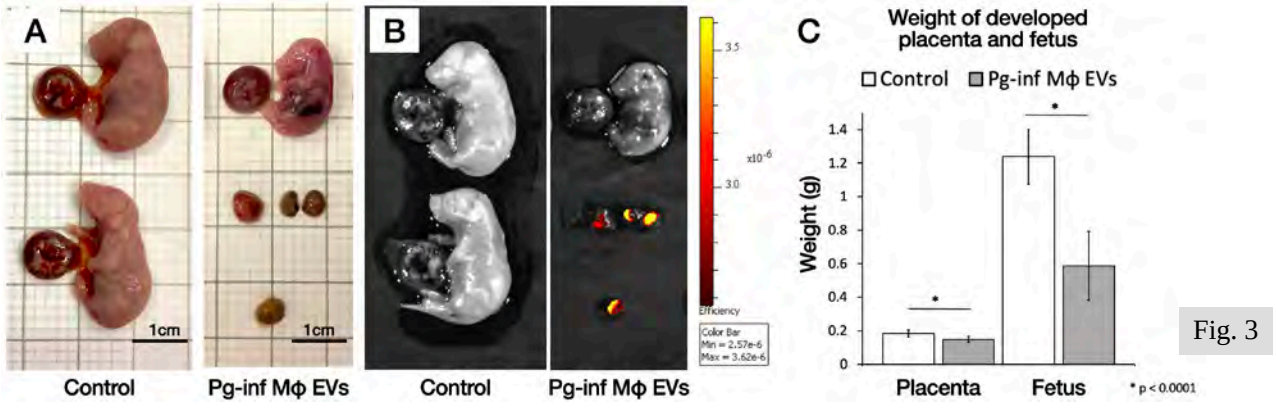
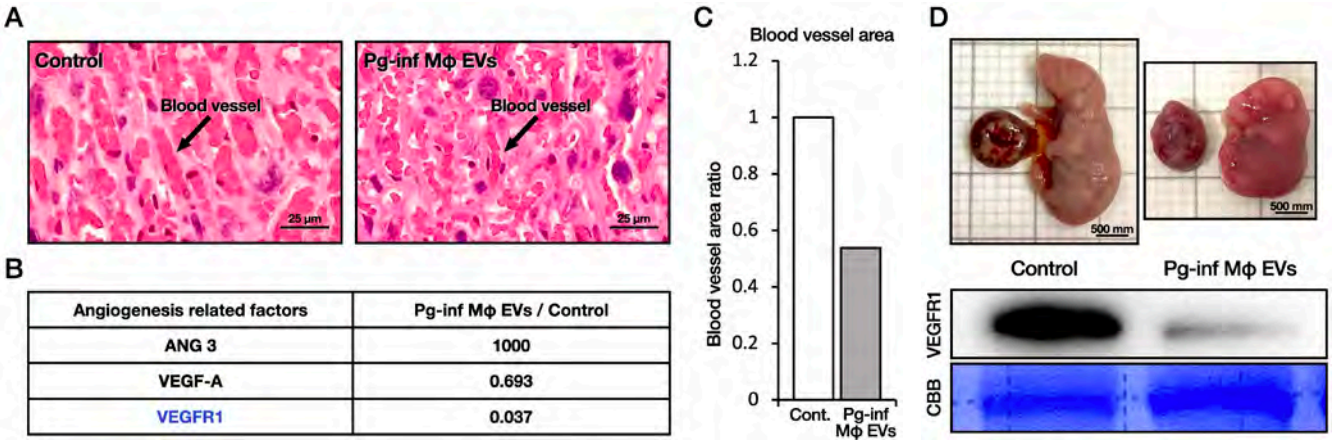


Fig. 3

2) Pg-inf Mφ EVs inhibit placental angiogenesis

The HE-staining of the Pg-inf Mφ EVs-injected group placenta exhibited decreased blood vessel area and width without inflammation (Fig. 4A and 4C). This suggests that the Pg-inf Mφ EVs can transmit to the placenta and cause damage to placental blood vessels while evading immune cells. The damaged placental blood vessels display lower blood circulation, hindering their ability to provide sufficient nutrients to the fetus.

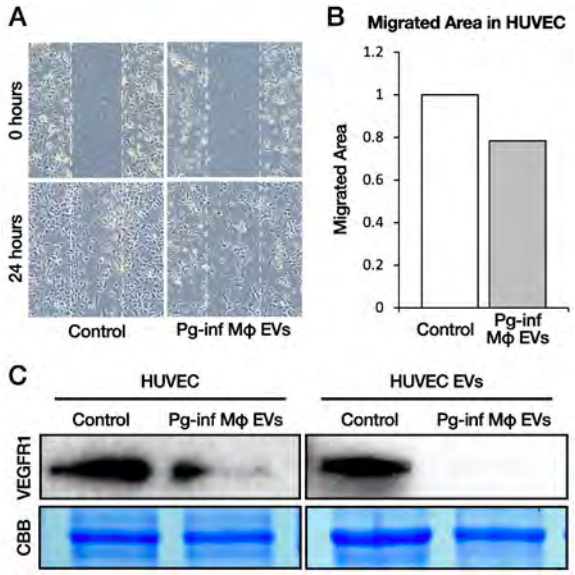
Mass spectrometry and gene ontology analysis of the placental EVs showed angiogenesis-related proteins with highest relevance: Angiogenin-3 (ANG3), Vascular Endothelial Growth Factor A (VEGF-A), and Vascular Endothelial Growth Factor Receptor 1 (VEGFR1) (Fig. 4B). In particular, the expression of VEGFR1 in placental EVs of Pg-inf Mφ EVs-injected group dramatically decreased. This was confirmed by WB of the placenta (Fig. 4D).



Angiogenesis related factors	Pg-inf Mφ EVs / Control
ANG 3	1000
VEGF-A	0.693
VEGFR1	0.037

Fig. 4

3) Pg-inf Mφ EVs inhibit migration and downregulate VEGFR1 in HUVEC



The observations from the vivo experiments were also examined in vitro. Scratch assay showed that the migrated area decreased in HUVEC treated with Pg-inf Mφ EVs (Fig. 5A and 5B). This suggests that Pg-inf Mφ EVs directly inhibit the migration ability in endothelial cells after they translocate to the placenta.

WB of Pg-inf Mφ EVs-treated HUVEC and their EVs showed decreased VEGFR1 expression. This indicates that Pg-inf Mφ EVs secondarily impend the function of endothelial cells through the downregulation of VEGFR1, further inhibiting placental angiogenesis (Fig. 5C).

Fig. 5

【 Conclusion】

In this study, I examined the mechanism of how Pg utilizes Mφ EVs and affects fetal growth through the inhibition of placental angiogenesis. Pg-inf Mφ EVs translocated and caused damage to the placenta, without inflammatory response. In the placental EVs of the Pg-inf Mφ EVs-injected group, the expression of VEGFR1 was downregulated and the migration ability in endothelial cells decreased. This delays angiogenesis, resulting in limited blood circulation. Therefore, the placenta is incapable of meeting the nutritional demands of the fetus, causing fetal abnormalities (Fig. 6).

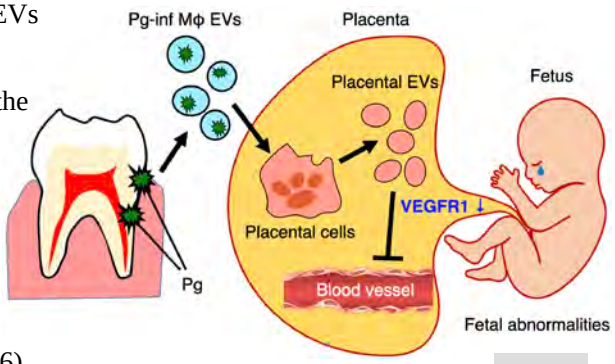


Fig. 6

Pg utilizes Mφ EVs and inhibits placental angiogenesis through the downregulation of VEGFR1, which results in fetal abnormalities.

Paramylon intake improves blood triglyceride level, but not C-reactive protein level: double-blinded randomized control trial

九州歯科大学 3年生 Kyushu Dental University Class of 2024

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Many diseases in dentistry involve chronic inflammation. Paramylon is a linear (1,3)-β-glucan derived from the cells of *Euglena gracilis Z*, and recent animal studies suggest anti-inflammatory and blood glucose and blood lipid-lowering effects. In this study, we conducted a double-blind, randomized controlled trial in humans to investigate changes in serum high-sensitivity C-reactive protein (CRP) and triglyceride levels by the intake of paramylon. Thirty-one subjects were randomly divided into placebo and paramylon groups. Each subject was given four capsules twice a day, before breakfast and dinner, for one week. The subjects were also asked to record their meals using a mobile application, and the average daily nutrient intake was calculated. The results of this study showed that serum high-sensitivity C-reactive protein levels did not differ significantly between before and after the study in both groups. In contrast, serum triglyceride levels decreased significantly in the paramylon group before and after the study. Energy intake during the study was comparable in both groups. On the other hand, only lipid intake was significantly higher in the paramylon group than in the placebo group among the three macronutrients. In conclusion, the results suggest that one-week intake of paramylon has the effect of lowering blood lipids.

パラミロンはCRP値ではなく血中中性脂肪値を改善する（二重盲検無作為化比較試験）

歯科臨床で遭遇する疾患の多くに慢性炎症が関与する。パラミロンはユーグレナ（一般名：ミドリムシ）の細胞に由来する直鎖状の（1,3）-β-グルカンであり、近年動物実験で抗炎症作用や血糖値・血中脂質低下作用が報告されている。本研究では、ヒトにおける二重盲検化ランダム化比較試験を行い、パラミロンの摂取による血清中高感度CRP値と中性脂肪値の変化を調査することを目的とした。31人の被験者を無作為にプラセボ群とパラミロン群の2つに分け、朝食前、夕食前の2回、4カプセルを1週間摂取させた。また被験者に携帯用アプリケーションを使って食事を記録してもらい、1日あたりの平均摂取栄養素を計算した。本研究の結果、血清高感度CRP値は研究前後で両グループとも有意な差を示さなかった。一方、血清中中性脂肪値はパラミロン群の研究前後で有意に減少した。研究中のエネルギー摂取量については、プラセボ群とパラミロン群で同等の結果となった。研究期間中に摂取した三大栄養素のうち、脂肪摂取量（%エネルギー）だけが、パラミロン群はプラセボ群よりも有意に高かった。結論として、1週間のパラミロン摂取は血中脂質を低下させる効果があることが示唆された。

研究発表内容の紹介

歯科臨床で遭遇する疾患には、歯周炎など慢性炎症が関与する場合が多くある。これらの疾患に対する治療法の一つとして、抗生物質の全身投与が考えられている。しかし、抗生物質の長期使用は口腔内細菌叢の耐性など欠点があり適応が限られるのが現状である。近年プロバイオティクスなど化合物に依存しない細菌叢へのアプローチが注目される中、生物由来のパラミロンの抗炎症作用による歯科疾患への効果が期待される。（ファカルティー・アドバイザー：向坊 太郎）

Paramylon intake improves blood triglyceride level, but not C-reactive protein level: double-blinded randomized control trial

【Problem】

Paramylon is a linear (1,3)-beta-glucan, which is derived from the cells of *Euglena gracilis Z*. Recent animal studies have reported that paramylon intake has a positive effect on health, including anti-inflammatory effects and reduction of blood lipids level. However, it is unknown whether the effects are similar in humans. In the present study, we aimed to investigate the effects of paramylon intake on serum high-sensitivity C-Reactive Protein (hs-CRP), a sensitive marker of inflammation, and serum Triglyceride (TG) level.

【Hypothesis】

We hypothesized that the paramylon intake would reduce the serum CRP level and improve lipid metabolism. To test the hypothesis, we set up the following study design.

【Methods】

1. Subjects

Thirty-eight subjects (19 males and 19 females, mean age 25.4 ± 0.8 years old) were enrolled, and seven subjects with hs-CRP levels below 50 ng/ml were excluded from the study. The thirty-one subjects were divided into a placebo group (16 subjects) and a paramylon group (15 subjects) in a double-blind randomized mannar.

2. Intervention

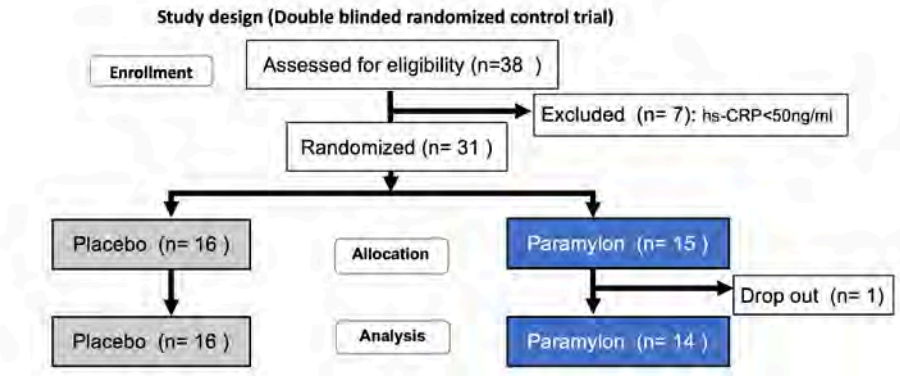
The placebo-containing and the paramylon-containing capsules 1000 mg/day (Euglena Co., Ltd., Tokyo, Japan) were each taken for one week.

3. Evaluation of blood samples and nutrient intakes

Blood samples were collected before and after the start of the study to measure the high-sensitivity CRP (hs-CRP) and Triglyceride (TG) in serum. In addition, each subject's daily diet was recorded using a smartphone during the study period, and nutritional intake was automatically analyzed using the AI of a mobile application (Calomael, Lifelog Technology, Tokyo, Japan).

4. Statistics

Statistic analysis were performed using the Willcoxson test or the Mann-Whitney test, and a p-value of less than 0.05 was considered as significant.



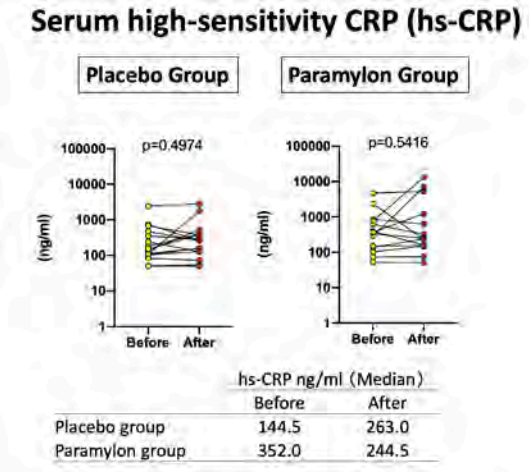
【Results】

1. Serum hs-CRP level was comparable before and after taking the capsules in both groups

The median serum hs-CRP in the placebo group was 144.5 ng/ml before the start of the study, and 263.0 ng/ml after the end of the study. In contrast, in the paramylon group, it was 352.0 ng/ml before the start of the study and 244.5 ng/ml after the end of the study. There was no significant difference in serum hs-CRP in both groups before and after the study.

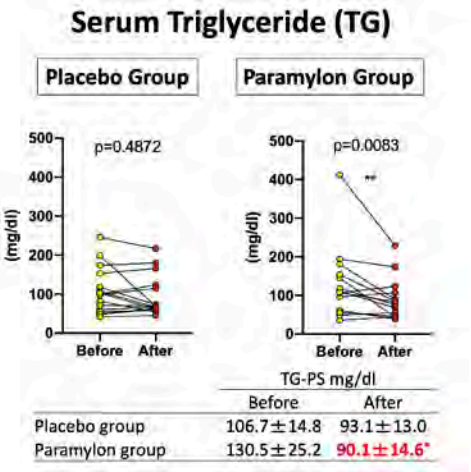
2. Serum TG declined after taking paramylon containing capsule

Serum TG in the placebo group was comparable between before and after the study, where 106.7±14.8mg/dl before and 93.1±13.0mg/dl after the study. On the other hand, in the paramylon group, the serum TG significantly declined from 130.5±25.2 mg/dl to 90.1±14.6 mg/dl after the study (p=0.0083).



hs-CRP ng/ml (Median)

	Before	After
Placebo group	144.5	263.0
Paramylon group	352.0	244.5



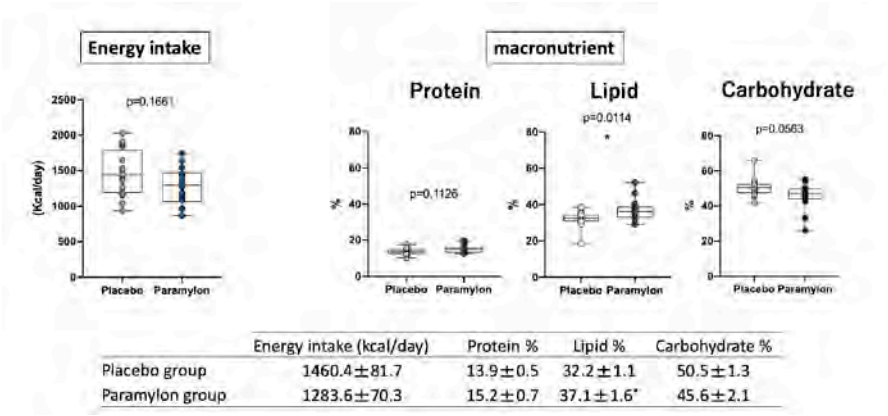
TG-PS mg/dl

	Before	After
Placebo group	106.7±14.8	93.1±13.0
Paramylon group	130.5±25.2	90.1±14.6*

3. Lipid energy ratio in nutrient intake was significantly different between the group

In the nutrient intake assessment, the placebo group had a caloric intake of 1460.4±81.7 kcal/day. Macronutrients' energy ratio of protein, lipid, and carbohydrate was 13.9±0.5%, 32.2±1.1%, and 50.5±1.3%, respectively. In contrast, the paramylon group had a caloric intake of 1283.6±70.3kcal/day, and the intake of protein, lipid, and carbohydrate was 15.2±0.7%, 37.1±1.6%, and 45.6±2.1%, respectively.

In comparing the two groups, no significant difference, except for the lipid energy ratio, was found among the macronutrient energy ratio.



	Energy intake (kcal/day)	Protein %	Lipid %	Carbohydrate %
Placebo group	1460.4±81.7	13.9±0.5	32.2±1.1	50.5±1.3
Paramylon group	1283.6±70.3	15.2±0.7	37.1±1.6*	45.6±2.1

【Conclusion】

- Contrary to the hypothesis, one-week paramylon intake did not show significant difference in serum hs-CRP level of both groups.
- The study demonstrated that 1week intake of paramylon reduced serum TG level, suggesting that the paramylon (1000 mg/day) intake likely has positive effect on reducing blood lipids.
- Energy ratio of lipid intake was significantly higher in paramylon group. In general, lipid intake leads to serum TG increase. Reduced serum TG in paramylon group despite high dietary lipid intake might support the evidence that paramylon is effective in lowering blood lipids.
- Within the limitation of the study, paramylon likely reduces the blood TG level.
- The relationship between lipid metabolism and chronic inflammation has been reported in recent years, therefore it is necessary to examine the effects of long-term administration of paramylon in the future.

Volumetric analysis of medication-related osteonecrosis of the jaw with SPECT/CT

日本歯科大学新潟生命歯学部　4年生　The Nippon Dental University School of Life Dentistry at Niigata Class of 2023

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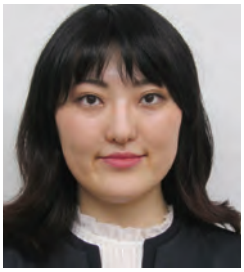
ファカルティー・アドバイザー：歯科放射線学講座 教授　小椋 一朗



Objective The aim of this study was to investigate quantitative SPECT/CT imaging for medication-related osteonecrosis of the jaw (MRONJ).
Methods Fifty-one patients with mandibular MRONJ underwent SPECT/CT after injection of Tc-99m hydroxymethylene diphosphonate. The maximum standardized uptake value (SUV) was obtained by using a software and workstation. The parameters of MRONJ patients with osteoporosis and bone metastases were compared by Pearson chi-square test and Mann-Whitney U test. A p value lower than 0.05 was considered as statistically significant.
Results MRONJ patients with osteoporosis and bone metastases had significant relation to age ($p = 0.001$), gender ($p = 0.002$), medication ($p = 0.001$). Then, the maximum SUV for osteoporosis (19.5 ± 9.0) was significantly higher than that for bone metastases (14.3 ± 7.6, $p = 0.019$).
Conclusion Volumetric analysis should be useful for the evaluation of MRONJ.

SPECT/CTを用いたMRONJの定量分析

目的　この研究の目的は薬剤関連顎骨壊死（MRONJ）のSPECT/CT像の定量分析である。
方法　下顎においてMRONJを患った51人の患者が当院で^{99m}Tc HMDPを静注した後、SPECT/CTで撮影した。最大値標準化取込値（SUV）はソフトウェアとワークステーションを使って得られた。骨粗鬆症と骨転移を患ったMRONJ患者の値はピアソンのカイ二乗検定とマン・ホイットニーのU検定で比較された。p値が0.05よりも小さいとき、有意であると判断した。
結果　骨粗鬆症と骨転移を患ったMRONJ患者には年齢（$p = 0.001$）、性別（$p = 0.002$）、薬剤（$p = 0.001$）において有意差が認められた。また、骨粗鬆症の最大値SUV（19.5 ± 9.0）は骨転移の最大値SUV（14.3 ± 7.6）よりも有意に高かった（$p = 0.019$）。
結論　定量分析はMRONJの評価のために有益であると考える。

研究発表内容の紹介

ビスホスホネート製剤やデノスマブは骨粗鬆症や悪性腫瘍の骨転移などの治療に用いられる。しかし近年、これらの薬剤の副作用として顎骨壊死・骨髄炎が報告され、MRONJが問題となっている。MRONJの検査としては単純検査、CT検査、MRI検査、骨シンチグラフィーがあげられる。骨シンチグラフィーは病気の広がりや、CTでは判断が難しい部位で代謝の情報を得るために行う。放射性医薬品（^{99m}Tc HMDP、^{99m}Tc MDP）が骨表面に取り込まれ、骨代謝の亢進している部位に強く集積する。ソフトウェアを用いることでSPECT/CT診断を定量評価できる。現在、MRONJ患者には一刻も早い早期発見が求められている。この研究が少しでも多くの患者のために、そしてこれからの歯科医学発展につながれば幸いである。（ファカルティー・アドバイザー：小椋 一朗）

Volumetric analysis of medication-related osteonecrosis of the jaw with SPECT/CT

(Problem)

Bisphosphonates are inhibitors of osteoclastic bone resorption. They are effective in treating osteoporosis and prevent skeletal events associated with metastatic neoplasm. Although bisphosphonates are effective, they are also implicated in the development of medication-related osteonecrosis of the jaw (MRONJ).

(Hypothesis)

Recently, a standardized uptake value (SUV) has been applied for the evaluation of bone single -photon emission computed tomography/computed tomography (SPECT/CT). However, to the best of our knowledge, few reports have been published on SUV measurement in maxillofacial bone imaging using SPECT/CT scans with Tc-99m hydroxymethylene diphosphonate (^{99m}Tc HMDP). The aim of this study was to investigate quantitative SPECT/CT imaging for MRONJ.

(Methods)

Fifty-one patients with mandibular MRONJ (40 women and 11men; mean age, 77.5 years [range, 55-92 years]) underwent SPECT/CT after injection of ^{99m}Tc HMDP at our hospital from October 2018 to March 2021. Patients were considered to have MRONJ by the 2014 American Association of Oral and Maxillofacial Surgeons position paper.

SPECT/CT scans were obtained by a SPECT/CT scanner (Optima NM/CT 640, GE Healthcare, Tokyo, Japan), equipped with 4 slices CT scanner for attenuation correction (Fig. 1). The SPECT scan was acquired using low-energy high-resolution collimator, the 140 keV photoenergy peak for ^{99m}Tc, a 128×128 matrix of 4.2 mm pixel size, and a total of 60 projections (30 steps) over 360° with a dwell time of 10s/step. Subsequent to the SPECT acquisition, a low-dose CT transmission scan was performed with 120kV and 20mA using a 512×512 matrix size. The CT data were generated with a 2.5-mm slice thickness (Fig. 2).


Fig. 1

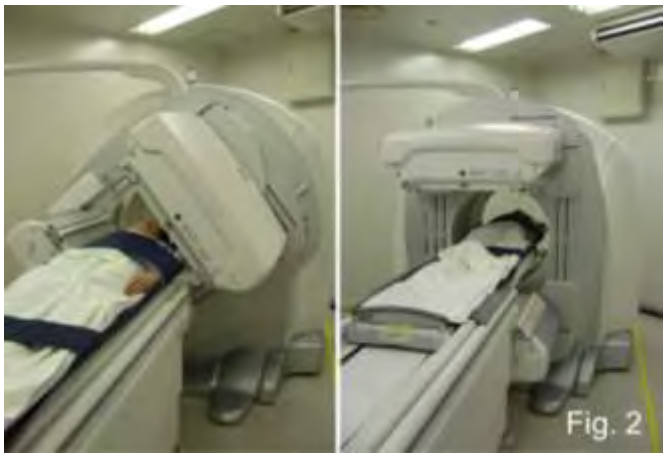


Fig. 2

The maximum SUV was obtained by using a software and workstation (Q. Volumetrix MI and GEniE-Xeleris 4DR, GE Healthcare, Tokyo Japan). By using the CT and SPECT transaxials, coronals and sagittals as the anatomical reference, the localization and size of volume of interest (VOI) was automatically drawn over the lesion (Fig. 3).

Then, the dosimetry software provided multiple quantitative data for a given VOI (Fig. 4). The maximum SUV in a given VOI was calculated as follows: maximum SUV = (maximum radioactivity/voxel volume)/(injected radioactivity/body weight).

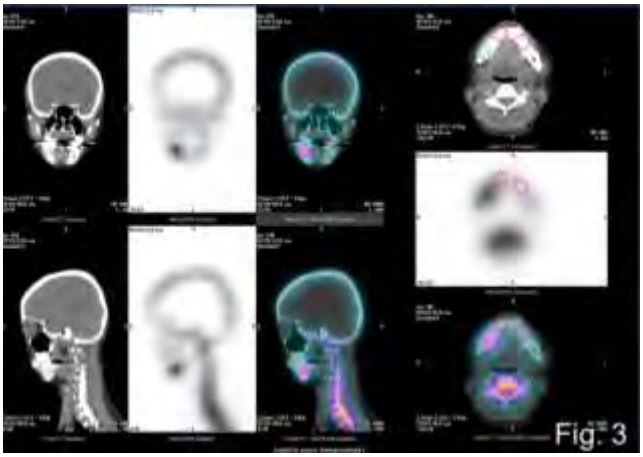


Fig. 3


Fig. 4

Parameters of MRONJ patients with osteoporosis and bone metastases were compared by Pearson chi-square test and Mann-Whitney U test. A p value lower than 0.05 was considered as statistically significant.

(Results)

The characteristics of patients enrolled in this study are summarized in Table 1. MRONJ patients with osteoporosis and bone metastases had significant relation to age (p = 0.001), gender (p = 0.002), medication (p = 0.001). Furthermore, the maximum SUV for osteoporosis (19.5 ± 9.0) was significantly higher than that for bone metastases (14.3 ± 7.6, p = 0.019).

Table 1

Parameters	Osteoporosis (n = 37)	Bone metastases (n = 14)	Total (n = 51)	P-value
Age				0.001
Mean ±SD	80.1 ± 7.0	70.6 ± 8.3	77.5 ± 8.5	
Range	64 - 92	55 - 84	55 - 92	
Gender				0.002
Men	4	7	11	
Women	33	7	40	
Medication				0.001
Denosumab	12	10	22	
Minodronate	13	0	13	
Alendronate	7	0	7	
Zoledronate	0	3	3	
Risedronate	3	0	3	
Ibandronate	2	0	2	
Bevacizumab	0	1	1	
Stage				0.338
Stage2	27	12	39	
Stage3	10	2	12	
SUV				0.019
Maximum ± SD	19.5 ± 9.0	14.3 ± 7.6	18.1 ± 8.9	
Range	7.7 - 52.5	6.8 - 36.1	6.8 - 52.7	

(Conclusion)

Volumetric analysis should be useful for the evaluation of MRONJ.

Additive alveolar bone mineralization in the dry socket may be induced by oral bacterial metabolites

日本大学歯学部 5年生 Nihon University School of Dentistry Class of 2022

髙田 紋花 Ayaka TAKADA

ファカルティー・アドバイザー：生化学講座 准教授 津田 啓方

A dry socket is an infectious condition that occurs on the surface of the alveolar bone because of displacement of blood clots after tooth extraction. Patients suffering from the condition often feel strong pain with delayed wound healing. Sometimes, the condition progress to osteomyelitis. Although periodontitis, which is also an infectious disease, exhibits bone resorption activity, an additive bone mineralization has often been observed on the surface of the alveolar bone in a dry socket. We evaluated the effects of short-chain fatty acids (SCFAs), a type of metabolite, produced by oral bacteria, on mineralization by osteoblasts and osteoclastogenesis. Several SCFAs induced mineralization by osteoblasts and repressed receptor activator of nuclear factor κ-B lingand (RANKL)-induced osteoclast formation. Furthermore, SCFA-mixtures mimicking culture supernatants of different oral bacteria were constructed and applied to the experiment. SCFA-mixtures mimicking culture supernatants of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* induced mineralization by osteoblasts and all mixtures reduced RANKL-induced osteoclast formation. These data suggest that the effects of SCFAs produced by oral bacteria might shift bone metabolism at the socket's surface to bone formation status.

口腔内細菌代謝産物がドライソケットにおける歯槽骨石灰化亢進に関与する可能性

ドライソケットは抜歯窩での血餅の脱落による細菌の抜歯窩骨壁への感染が原因と考えられており、抜歯窩の激痛に加え、骨髄炎へ移行するリスクを伴う。ドライソケットとなった部位ではX-線写真にて歯槽硬線の強調が観察されることがある。歯周炎では歯槽骨が吸収されるのに対し、同じく感染を伴うドライソケットでは石灰化促進が起こっている。血餅の脱落した抜歯窩では食物残渣の迷入もあることから、細菌が食物残渣を代謝し、その代謝産物が抜歯窩骨壁の石灰化亢進を行っている可能性を考えた。実験の結果、数種の短鎖脂肪酸が骨芽細胞による石灰化物形成を促進し、RANKL誘導の破骨細胞分化もしくは細胞の癒合を抑制した。次に、各口腔内細菌の培養上清と同濃度の短鎖脂肪酸混合物を作成し作用させたところ、*Porphyromonas gingivalis*および*Fusobacteirum nucleatum*の培養上清を模倣した短鎖脂肪酸混合物は石灰化を促進し、試した全ての短鎖脂肪酸混合物は全て破骨細胞形成を抑制した。これらの事より口腔内細菌の産生する短鎖脂肪酸がドライソケットにおける抜歯窩骨壁の石灰化度を亢進している可能性が示唆された。

研究発表内容の紹介

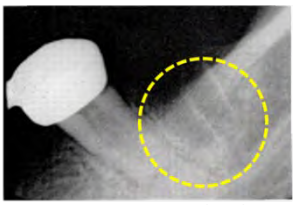
発表者は細菌感染によって起こるドライソケットでは骨石灰化が亢進する事を示唆する本の内容に出会いました。「同じ細菌感染症の歯周炎では歯槽骨吸収を伴うのにドライソケットでは何故逆の結果となるのか？」その時に生じた素朴な疑問から生まれたのが本研究内容です。本研究結果から、口腔内細菌が血餅を失った抜歯窩に入り込んだ食渣を代謝し、その産物が骨芽細胞による石灰化促進及び破骨細胞形成抑制を起こす可能性があることを明らかにしました。（ファカルティー・アドバイザー：津田 啓方）

Additive alveolar bone mineralization in the dry socket may be induced by oral bacterial metabolites

【Problem】

A dry socket is a type of infectious disease that develops on the surface of the alveolar bone socket after tooth extraction. Patients suffering from dry sockets often experience strong pain with delayed wound healing and have certain risks for progression from dry socket to osteomyelitis. Removing of blood clots from a socket after tooth extraction causes bacterial colonization on the socket's bone surface. Although both dry socket and periodontitis are infectious conditions, periodontitis accompanies bone resorption and by contrast dry sockets exhibit a highly mineralized alveolar hard line on the X-ray image (Fig. 1). This discrepancy remains a mystery.

Fig. 1, X-ray photo of dry socket.



Fukuda et al., Stomatology (1952)
doi.org/10.11277/stomatology1952.35.196

【Hypothesis】

The onset of dry sockets requires bacterial infection in socket cavities. Moreover, dietary fibers contained in food remnants are often supplied in this area. Bacteria adhering to the socket's surface ferment these dietary fibers in impacted food remnants in the socket and produce high concentrations (millimolar-order) of several types of short-chain fatty acids (SCFAs). Therefore, it is hypothesized that the SCFA production by bacteria could shift the bone metabolism to a bone-formation state. To consider bone metabolism, it is necessary to evaluate both mineralized tissue formation by osteoblasts and bone resorption by osteoclasts should be evaluated. Therefore, it is hypothesized that SCFAs induce mineralization by osteoblasts and reduce osteoclast formation from macrophages.

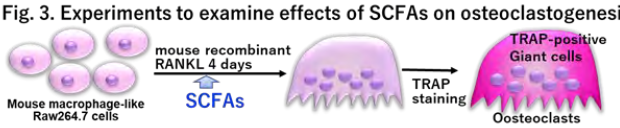
【Methods】

The mouse preosteoblastic MC3T3-E1 cell line was used to evaluate the bone formation activity of osteoblasts (Fig. 2). These cells were cultured for 3 weeks in a mineralization medium containing SCFAs. Alizarin red staining was performed to evaluate the amount of mineralization based on the staining intensity.

Fig. 2. Experiments to examine effects of SCFAs on osteoblastic mineralization.

The effects of SCFAs on osteoclast formation were evaluated using mouse macrophage-like Raw264.7 cells (Fig. 3). These cells were treated with SCFAs for 4 days in the presence of mouse recombinant RANKL, and TRAP staining was performed, in which TRAP-positive multinuclear giant cells were observed as osteoclasts under the microscope.

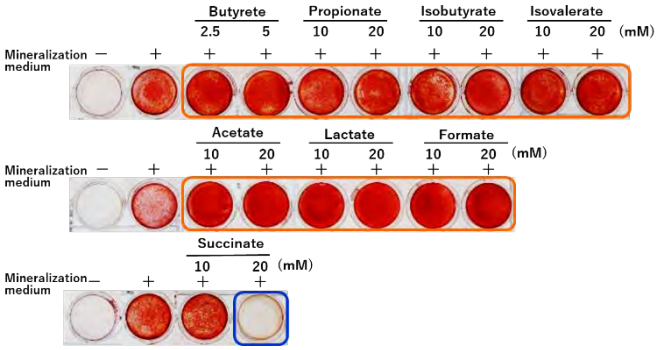
Fig. 3. Experiments to examine effects of SCFAs on osteoclastogenesis.



【Results】

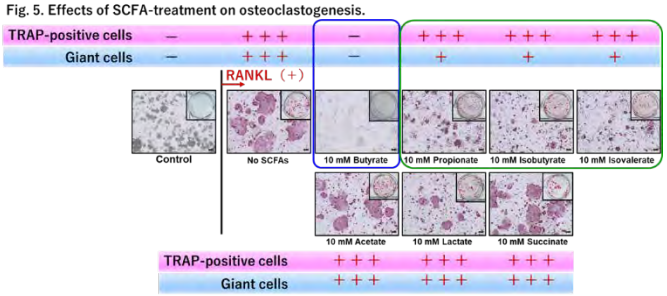
First, the effects of SCFA-treatments on MC3T3-E1 cells were examined (Fig.4). Treatment of MC3T3-E1 cells with butyrate (> 2.5 mM), propionate (>10 mM), isobutyrate (> 10 mM), isovalerate (> 10 mM), acetate (> 10 mM), lactate (> 10 mM), and formate (> 10 mM) induced mineralization as compared with that in the control (Fig.4). In contrast, treatment with 20 mM of succinate reduced mineralization (Fig.4).

Fig. 4. Effects of SCFA-treatment on osteoblastic mineralization.



The effects of SCFA treatments on RANKL-induced osteoclast formation were also investigated (Fig. 5). RANKL treatment induced osteoclast-like TRAP-positive, multinuclear giant cells. Butyrate-treatment strongly inhibited the generation of osteoclast-like cells (Fig. 5). Furthermore, treatments with propionate, isobutyrate, and isovalerate induced the generation of TRAP-positive cells, but only a small number of giant cells were observed (Fig. 5). However, treatments with acetate, lactate, and succinate had no effect on the RANKL-induced generation of osteoclast-like cells (Fig. 5).

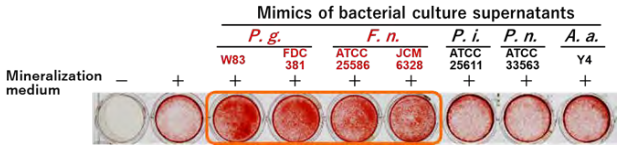
Fig. 5. Effects of SCFA-treatment on osteoclastogenesis.



The concentrations of each SCFA in the culture supernatants of oral bacteria is known. Based on this information, SCFA mixtures mimicking bacterial culture supernatants were constructed, and these were applied to the experimental systems to evaluate bone formation and osteoclastogenesis.

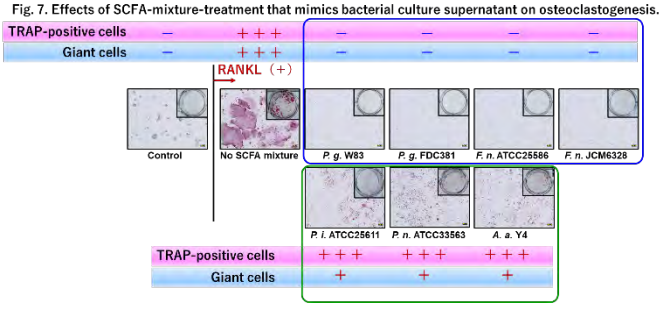
Treatment with mixtures mimicking *Porphyromonas gingivalis* (*P. g.*) and *Fusobacterium nucleatum* (*F. n.*), but not those mimicking *Prevotella intermedia* (*P. i.*), *Prevotella nigrescence* (*P. n.*), and *Aggregatibacter actinomycetemcomitans* (*A. a.*), induced mineralization (Fig.6).

Fig. 6. Effects of SCFA-mixtures that mimics bacterial culture supernatants on osteoblastic mineralization.



Moreover, treatment with SCFA mixtures mimicking *P. g.* or *F. n.* culture supernatants, which induced mineralization, completely inhibited the RANKL-induced generation of osteoclast-like cells (Fig. 7). In addition, treatment with SCFA mixtures mimicking *P. i.*, *P. n.*, and *A. a.* strongly inhibited giant cell formation, but the majority of these cells showed TRAP activities (Fig. 7).

Fig. 7. Effects of SCFA-mixture-treatment that mimics bacterial culture supernatant on osteoclastogenesis.



【Conclusion】

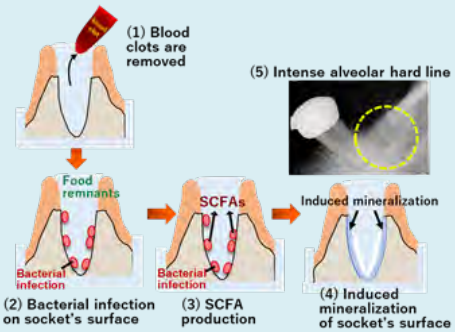
1. Treatment with SCFAs produced by oral bacteria can induce mineralization through upregulation of mineralization by osteoblasts and repression of osteoclastogenesis.
2. The mineralization-inducing effects of *P. gingivalis* and *F. nucleatum* are much stronger than those of other oral bacteria (Fig. 8).

Fig. 8. Effects of SCFA-mixtures that mimics bacterial culture supernatants on mineralization.

	Pi, Pn, Aa	*Pg, Fn*
Upregulation of mineralization	++	+++++
Inhibition of RANKL-induced osteoclastgenesis	++	+++++
Mineralization-inducing effect	++++	+++++ +++++

3. The presumed mechanism underlying the induction of cavity's bone surface mineralization is described as follows (Fig. 9): (1) When the blood clot in the socket cavities is not generated or removed by gargle, and so on, (2) The bacteria colonizing in the socket ferment dietary fibers in food remnants and (3) produce metabolites such as SCFAs. (4) These metabolites induce mineralization by osteoblasts and inhibit osteoclastogenesis. Therefore, bone mineralization becomes increasingly stronger at the cavity's surface. (5) This additive mineralization of the socket surface might appear as a highly mineralized hard line on the X-ray image.

Fig. 9. Presumed mechanisms for the induced mineralization on socket's surface.



Dry socket is one of the risk factors for the onset of osteomyelitis. When the socket's bone surface is highly mineralized, immune cells cannot migrate to the socket cavity. This induces bacterial growth in the socket cavity and the subsequent onset of osteomyelitis. Therefore, the patients must clean the oral cavity before undergoing the procedure of tooth extraction.

The induction of proinflammatory response in human umbilical vein endothelial cells by Hsp70-homolog DnaK of *Abiotrophia defectiva*

岩手医科大学歯学部　3年生　School of Dentistry Iwate Medical University　Class of 2024

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ファカルティー・アドバイザー：微生物学講座 教授　佐々木 実

Abiotrophia defectiva is a nutritionally variant streptococci inhabiting the oral cavity and one of the etiologic agents of infective endocarditis. We identified that one of the binding molecules of *A. defectiva* to fibronectin could be Hsp70-homolog DnaK, which is located on the cell surface. DnaK is a bacterial member of the highly conserved, family of 70-kDa heat-shock-induced chaperone proteins (Hsp70 proteins). However, several reports suggest that the bacterial Hsp70 protein, DnaK, is a cell surface protein that functions as a ligand for plasminogen and further stimulates the immune system to synthesize cytokines or chemokines. Therefore, bacterial Hsps could be a pathogenic agent. This study investigates the biological activities of *A. defectiva* DnaK to human umbilical vein endothelial cells (HUVECs) or macrophages to assess the pathogenicity in infective endocarditis. The expression of IL-8, CCL2, ICAM-1, and VCAM-1 were upregulated with the *A. defectiva* DnaK treatment in HUVECs depending on TLR4 signaling. Additionally, the expression of TNF-α in THP-1 macrophages was also upregulated by treatment with DnaK. The accumulation of leukocytes in infected areas and upregulation of adhesion molecules on host cells may cause serious inflammation to the blood vessel. Therefore, *A. defectiva* DnaK is a potent proinflammatory agent that causes infective endocarditis.

*Abiotrophia defectiva*のHsp70ホモローグDnaKはヒト臍帯静脈内皮細胞に炎症応答を誘発する

*Abiotrophia defectiva*は、口腔常在菌であるが条件さえ整えば感染性心内膜炎の起炎菌となる。これまでに我々は*A. defectiva*のフィブロネクチンへの結合分子の一つが菌体表層に存在するHsp70のホモローグ、DnaKである可能性を示唆してきた。近年、DnaKが菌体表層に局在し、宿主タンパク質へのリガンド として、さらにまた免疫系を刺激することが示唆されている。本研究では、感染性心内膜炎における*A. defectiva* DnaKの病原性を評価するために、ヒト臍帯静脈内皮細胞（HUVECs）またはマクロファージに対する*A. defectiva* DnaKの生物活性について検討した。*A. defectiva* DnaK処理によりHUVECsでのIL-8、CCL2、ICAM-1、VCAM-1の遺伝子ならびにタンパク質レベルでの発現は顕著に誘導された。さらに、THP-1マクロファージにおけるTNF-αの発現もDnaK処理により誘導された。これら誘導はTLR4のシグナル伝達阻害剤により抑制された。以上の成績より感染局所へのケモカインによる白血球動員と宿主細胞上の白血球接着分子の発現誘導は、局所血管に深刻な炎症を引き起こす可能性がある。従って*A. defectiva* DnaKは、感染性心内膜炎における重要な病原因子となる可能性が示唆された。

研究発表内容の紹介

*Abiotrophia defectiva*のDnaKは熱ショックタンパク質Hsp70のホモローグであり、菌体表層に存在することが明らかにされている。本研究では、*A. defectiva* DnaKの感染性心内膜炎における病原因子としての可能性をその生物活性から評価した。DnaKはヒト臍帯静脈内皮細胞またはマクロファージに対してケモカインや炎症性サイトカイン、白血球接着分子発現を誘導し、血管局所に深刻な炎症を引き起こす可能性が示唆された。したがって、*A.defectiva* DnaKは、感染性心内膜炎の病原因子として機能する可能性があり、将来的に感染性心内膜炎予防ワクチンの開発におけるターゲットの一つになると考える。（ファカルティー・アドバイザー：佐々木 実）

The induction of proinflammatory response in human umbilical vein endothelial cells by Hsp70-homolog DnaK of *Abiotrophia defectiva*

PROBLEM

Although *Abiotrophia defectiva*, commensal bacteria in the oral cavity, is one of the etiologic agents of infective endocarditis, the pathological factors are still unclear. We previously discovered that the binding activity of *A. defectiva* to fibronectin and human umbilical vein endothelial cells (HUVECs). Furthermore, the binding molecule was identified as heat shock protein 70 (Hsp70) homolog, DnaK, located on cell surfaces (Figure 1). Some reports suggest that the DnaK is a cell surface protein in a growing number of bacteria that functions as a ligand to host protein receptor. This was followed by the report that cell surface DnaK in *Mycobacterium tuberculosis* binds to plasminogen, DNA, and CCR5. *M. tuberculosis* Cpn60 proteins revealed cytokine-inducing ability, suggesting involvement in granuloma generation. Additionally, Kol et al. reported the involvement of *Chlamydia pneumoniae* Hsp60 in atherosclerotic plaques and the ability of this protein to stimulate monocyte proinflammatory cytokine and metalloproteinase synthesis suggesting these activities could be because of the pathogenic factors of these bacteria. However, there are still few reports the biological or immunological activities of Hsp. This study investigates the biological activities of *A. defectiva* DnaK to HUVECs and macrophage cell lines to prove the pathogenicity of *A. defectiva* as regards infective endocarditis.

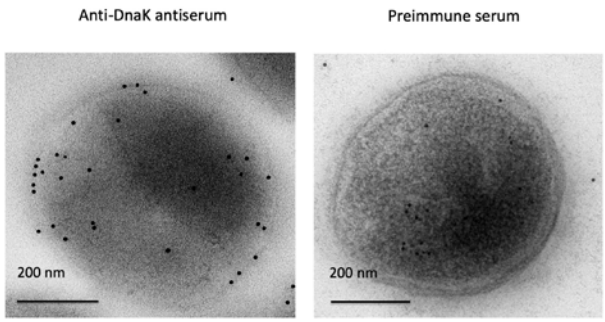

Figure 1 Localization of *A. defective* DnaK on bacterial cell surface

HYPOTHESIS

Our hypothesis is that DnaK of *A. defectiva* upregulates the inflammatory cytokines, chemokines, and adhesion molecules in HUVECs or macrophages. That could make *A. defectiva* DnaK a potent proinflammatory agent that causes infective endocarditis.

MATERIALS AND METHODS

1. Construction of the expression vector for *A. defectiva* DnaK protein and the expression and purification of *A. defectiva* rDnaK

PCR was used to generate a DNA fragment containing the dnak gene with *A. defectiva* chromosomal DNA as the template, the fragment was cloned into pQE60, and transfected into an LPS-eliminated *Escherichia coli*, ClearColi™ BL21 (DE3). The recombinant protein expression was induced by IPTG and purified from cells using TALON affinity chromatography.

2. Induction of cytokines and adhesion molecules in HUVECs or THP-1 cells stimulated with *A. defectiva* DnaK

A. defectiva recombinant DnaK (rDnaK) was added to the wells of a 24-well micro-plate with a semiconfluent culture of

HUVECs or THP-1cells. After incubation at 37ºC for two or four hours, total RNA was extracted from cells using the RNeasy mini kit according to the manufacturer's instructions. Complementary DNA was synthesized from total RNAs using PrimeScript RT Master Mix. Quantitative real time reverse-transcription polymerase chain reaction (RT-PCR) analysis was conducted using the Thermal Cycler Dice Real Time System according to the manufacturer's instructions. The primers for TNF-α, CCL2, IL-8, ICAM-1, VCAM-1, and GAPDH were used. The contents of cytokine culture supernatants were measured using ELISA kits (TNF-α, IL-8, and CCL2). In some experiments, HUVECs or THP-1cells was pretreated with TAK242, an inhibitor of TLR4 signaling. The expression of adhesion molecules on HUVECs stimulated with *A. defectiva* rDnaK was conducted via immune-staining assay. The cells were treated with monoclonal anti-ICAM-1 antibody or anti-VCAM-1 antibodies, followed by Alexa Fluor 594-conjugated goat anti-rabbit IgG.

RESULTS

1. Cytokine induction in HUVECs or THP-1 stimulated with *A. defectiva* DnaK

The upregulation of CCL2, IL-8 mRNA, and those of protein were revealed by treating HUVECs with *A. defectiva* rDnaK (Fig. 2a). Cytokine induction was inhibited using TAK242, an inhibitor of TLR4 signaling pathway (Fig. 2a). Additionally, TNF-α mRNA expression and protein production were enhanced in THP-1 cells treated with rDnaK, and inhibited also by TAK242 (Fig. 2b).

2. Adhesion molecules induction in HUVECs stimulated with *A. defectiva* DnaK

mRNA expression of adhesion molecules ICAM-1 and VCAM-1 in HUVECs were upregulated with the rDnaK (Fig 3a). Furthermore, as exhibited in Fig. 3b, HUVECs, the expression of these molecules treated with rDnaK were enhanced by analyzing immunostaining analysis using anti-VCAM-1 and anti-ICAM-1 antibodies.

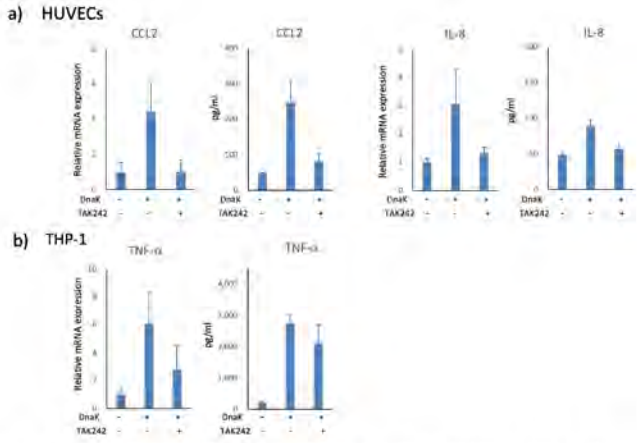

Figure 2 Chemokine and inflammatory cytokines induction in HUVECs or THP-1 cells stimulated with *A. defectiva* DnaK

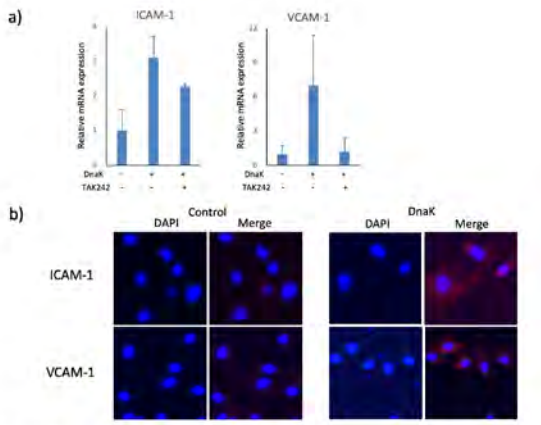

Figure 3 Induction of adhesion molecule expressions in HUVECs stimulated with *A. defectiva* DnaK

CONCLUSION

The study investigated the biological activities of *A. defectiva* DnaK to HUVECs and THP-1 cells with the tendency to cause infective endocarditis. First, we studied the inductions of chemokine and adhesion molecule expressions. The IL-8 and CCL2 mRNA expressions and the proteins of HUVECs or THP-1 cells were induced via treatment with DnaK depending on TLR4 signaling. Additionally, ICAM-1, VCAM-1 mRNA, and protein expression on HUVECs surfaces were also upregulated with the DnaK. This indicates that DnaK activates HUVECs and THP-1 cells through a receptor on these cells, which is a TLR4, and as a result, induces the expression of IL-8, CCL2, and adhesion molecules. The activities of *A. defectiva* DnaK can be used to explain the pathogenicity in infective endocarditis of the bacteria. This antigen may be a target in the development of a vaccine for infective endocarditis in the future.

Relation between oral hypofunction and nutrient intake condition in the elderly

九州大学歯学部 5年生 Kyushu University School of Dentistry Class of 2022

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Problem/hypothesis: Japan has a become super-aging society. Roles of dentists has come to a wide variety from the treatment to the improvement of function and the prevention of flail, Dementia, nursing. Analyzing oral function and nutrient intake in the elderly will help to following to improve general health following avoidance of oral hypofunction.
Methods: Twenty-seven subjects with more than 65 years-old were enrolled and received dental treatment and oral function test. Experimental protocols were approved by University Institutional Review Board for Clinical Research .The oral function and nutrient intake condition were evaluated.
Results: 1) Seventeen subjects (63%) were diagnosed as oral hypofunction. 2) Hypofunction with tongue and lip motor function, tongue pressure, occlusal force were observed in 50% of subjects. 3) Although 38 % subjects were categorized to loss occlusal support in Eichner classification under only remaining tooth, 92% subjects were categorized to keep full support under including denture support. 4) We evaluated the relationship between oral hypofunction and nutrient intake condition by questionnaire and record.
Conclusion: It was suggested that dental treatment affect to prevention oral hypofunction and malnutrition.

高齢者の口腔機能低下症と栄養摂取状態の関連

問題点・仮説：超高齢社会を迎えた日本では歯科医師の役割も治療だけでなく、機能改善やフレイル、認知症、介護の予防などへと変化してきた。高齢者の口腔機能、栄養摂取を分析することで、口腔機能低下の進行を抑制し健康増進へ繋げることができる。
方法：歯科治療のため受診した65歳以上の外来患者のうち、口腔機能検査を希望し、研究への同意を得た患者27名を対象とした。臨床研究倫理審査委員会の承認を得た。被験者に対し、口腔機能検査を行った。また、質問票ならびに摂取食品記録をもとに栄養状態の評価を行った。この結果から口腔機能の状態と栄養状態の分析を行った。
結果：1)口腔機能検査結果で63%の17名が口腔機能低下症と診断された。2) 50%以上に低下が認められた項目は「舌口唇運動機能」「舌圧」「咬合力」であった。3)残存歯のみのEichner分類では咬合支持を持たない対象者が38%であったが、義歯を含めたことで92%が4支持域有となった。4)質問票と摂取記録から栄養摂取と口腔機能の関連を分析することができた。
結論：歯科治療により口腔機能の回復と栄養状態の改善に影響することが示唆された。

研究発表内容の紹介

超高齢社会を迎えた日本において、我々歯科医師の責務も大きく変化してきた。「8020運動」では歯を保つことの重要性を啓蒙してきたが、今では広く国民に認知され、多くの高齢者により実現されている。本研究で得られた結果より、次のステージの「健康長寿」に向けて、口腔機能低下の予防や早期発見について高齢者に認識を促し、同時に栄養状態に関する患者教育を行うことで、「心身ともに健康な長寿」を実現するための一助となると考える。（ファカルティー・アドバイザー：王丸 寛美）

Relation between oral hypofunction and nutrient intake condition in the elderly.

【Problem】

Japan has become a super-aging society. Roles of dentists have come to a wide variety from the treatments with dental caries, periodontal disease or missing teeth to the improvement of oral function and the prevention of flail, dementia or eldercare. However, there is a serious concern that oral hypofunction or alteration of nutrient intake is often overlooked, as many of the elderly populations live alone or as elderly couples.

【Hypothesis】

We hypothesized that avoidance of oral hypofunction would help to improve general health. We analyzed whether trouble of oral condition would influence oral function and nutrient intake in the elderly and viewed countermove.

【Methods】

1) Types of Research designs: Observation study

2) Subjects: Twenty-seven subjects with more than 65 years-old were enrolled. They were the patients who visited to receive dental treatments including regular maintenance in university hospital and agreed to be included in the study.

3) Measurement items: ① Examination of oral function (oral hygiene, dry mouth, occlusal force, tongue and lip motor function, tongue pressure, masticatory function, and swallowing) ② Questionnaire (Mini Nutritional Assessment: MNA®) ③Record of food intake (Balance guide made by Ministry of Agriculture)

4) Statistical analysis: The relationship between oral function and nutrient intake condition was evaluated. Statistical analyses were performed using a Spearman's correlation coefficient by rank （SPSS statistic, IBM）.

5) Experimental protocols were approved by University Institutional Review Board for Clinical Research.

【Results】

1) Seventeen subjects (63%) were diagnosed as oral hypofunction.

2) Hypofunctions with tongue and lip motor function (81%), tongue pressure (63%) and, occlusal force (52%) were observed in more than 50% of the whole subjects. On the other hand, hypofunction of oral hygiene (26%), dry mouth (26%), masticatory function (22%) were relatively low prevalence.

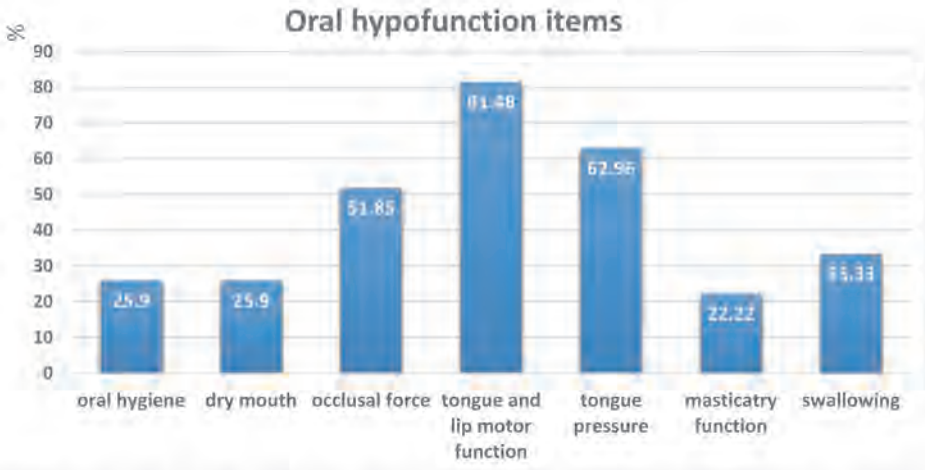

Fig.1

3) Although 38 % of the subjects were categorized to lose occlusal support in Eichner classification under their own teeth, 92% of the subjects were categorized to keep full support in case of including denture support. It was indicated that this might influence the low percentage of masticatory hypofunction and that it was important to recover masticatory area by introducing denture. (fig.2 and 3)

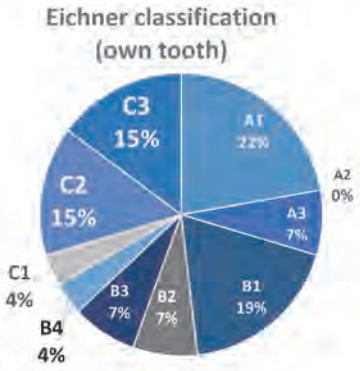

Fig.2

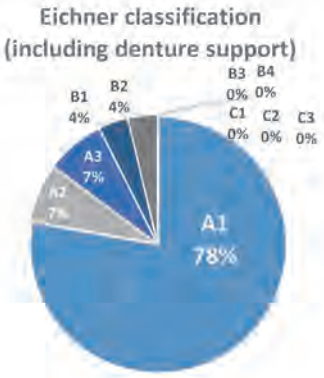

Fig.3

4) In analysis of the relation between oral hypofunction and nutrient intake condition with five subjects who fully completed data, the amount of foods other than staple food was relatively small, compared with standard value of similar age. Three of them were diagnosed as a "risk of malnutrition" or "malnutrition" in MNA examination. Actually, their BMI were relatively low, but there was only one subject who was diagnosed as "oral hypofunction". (table.1)

	carbohydrate	vitamin and mineral and dietary fiber	protein and lipid	dairy product	fruit	MNA	BMI	oral hypo-function
subject 1	2.7	4.1	3.6	0.5	0.8	w-n	≧23	-
subject 2	3.1	1.4	3.1	0.8	0.9	w-n	≧23	-
subject 3	2.8	4.7	5.8	1.9	0.8	r.m.	16	-
subject 4	4.1	3.5	4.8	0.5	0.5	r.m.	17.5	+
subject 5	3.1	2.9	2.3	2.3	1.6	m	17.9	-
average	**3.16**	**3.32**	**3.92**	**1.2**	**0.92**			
standard value	4～5	5～6	3～4	2	2			

＊Showing in red indicated the value less than 50% of standard value

＊＊ w-n : well-nourished, r. m. : at risk of malnutrition, m : malnourished

Table.1 Evaluation of nutrient intake condition and oral hypofunction

MNA is a useful screening tool for short time evaluation. On the other hand, "intake records" could reveal the detailed nutritional balance, though it takes a long time for recording meal contents and analyzing process. However, with the records, it is not enough to grasp the exact amount of intake yet. It may be required that both or either tool is properly selected by opportunity for appropriate nutrition counseling by dentists.

There were a positive correlation between occlusal force and MNA score and a negative correlation between occlusal force and oral hygiene (table.2). From these results, it was suggested that maintenance of occlusal support could improve the nutritional status. Based on the findings so far, it was considered that recovery of occlusal force following improvements of occlusal support or masticatory area by dental treatment could improve nutritional status. In addition, the decline of occlusal force might be related to muscle weakness such as flail. Also, the decline of activity by flail might affect the oral hygiene. It was suggested that dentists could notice a sign of flail by evaluating oral status.

Because of the study with small number of subjects, further research has been required for detailed consideration.

Correlation	occlusal force vs MNA	occlusal force vs oral hygiene
n	19	27
Spearman r	0.583	-0.615
P value (two-tailed)	0.001	0.009

Table.2 Factors correlating with occlusal force

【Conclusion】 Dentists could notice a sign of flail by evaluation of oral function and nutrient intake. It was suggested that dental treatment affect prevention of oral hypofunction and malnutrition.

Induction of macrophages into the small intestine by oral inoculation with *Fusobacterium nucleatum*

日本大学松戸歯学部　4年生　Nihon University School of Dentistry at Matsudo
Class of 2023

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ファカルティー・アドバイザー：感染免疫学講座 助教　小林 良喜

Periodontopathogenic bacteria and their pathogenic factors that enter the lower gastrointestinal tract and bloodstream due to chronic periodontitis have been contribute to pathogenesis in various organs.
Recently, *Fusobacterium nucleatum* (*Fn*), one of the periodontopathogenic bacteria, has been detected in the mucosal sites of colorectal cancer, and suggest that *Fn* is closely related to carcinogenesis and progression of colorectal cancer. However, the mechanism by how *Fn* influences the intestinal environment remains unclear.
The intestinal tract has a tolerate and suppressive immune system. Disruption of the intestinal macrophages response can induce an excessive immune response in the intestinal tract, leading to inflammatory bowel disease.
In this study, we attempted to investigate the effect of Fn bacteria on the dynamics of intestinal macrophages. BALB/c mice were orally inoculated with Fn (1×10^9 CFU/100μl /mouse) for 15 days. The control group was given only 5% CMC. The small intestine was isolated from euthanized mice, and sections were prepared by embedding in paraffin.
One day after the last inoculation in the *Fn* group, macrophages were detected in the lamina propria of the small intestine (SiLP) of the jejunum, and a further increase was observed after 30 days but not the ileum.
Although the immune response in the intestine is thought to be centered in the small intestine, the fact that the migration of intestinal macrophages differs by the site may contribute to the elucidation of the pathogenesis of inflammatory bowel disease and other diseases.

Fusobacterium nucleatum の口腔内接種によるマクロファージの小腸への誘導作用の検討

慢性化した歯周炎により下部消化器や血流を介して流入した歯周病原性細菌やそれらを由来とする病原因子は全身の様々な臓器における病態形成に寄与することが注目されている。

近年、大腸癌の病原粘膜部位から歯周病原性細菌の一つである*Fusobacterium nucleatum (Fn)*が検出され、*Fn*が大腸癌の発癌や進行に深く関連しているとの報告が多数みられる。しかしながら, *Fn*が腸内環境に影響を及ぼす機序は不明な点が多い。

腸管は他の臓器と異なり寛容系・抑制系が優位な免疫システムであり、中でも腸管マクロファージの応答破綻は腸管における過剰な免疫応答を惹起させ、炎症性腸疾患などを引き起こすことが考えられるが不明な点は多い。そこで本研究は、*Fn*菌が腸管マクロファージの動態に及ぼす影響を試みた。BALB/c マウスに*Fn*菌 (1×10^9 CFU/100μl /mouse) を15日間の口腔内接種を行った。対照群には5%CMCのみを投与させた。安楽死させたマウスから小腸を単離しパラフィン切片を作成した。

*Fn*群の口腔内接種1日後,腸管マクロファージを空腸の粘膜固有層 (SiLP) に認め、30日後にはさらに増加していることが認められたが、回腸では認められなかった。腸管における免疫応答は小腸が中心と考えられるが、腸管マクロファージの遊走が部位別に異なることは炎症性腸疾患などの発症の解明に寄与すると考えられる。

研究発表内容の紹介

慢性化した歯周炎による全身疾患の増悪化・重篤化が指摘されている。高齢者の残存歯数の増加は歯周炎の発症のさらなるリスクであるが, 加齢による免疫応答の減弱に伴い口腔内だけでなく全身に炎症リスクが波及すること考えられる。近年注目されている*Fusobacterium nucleatum*と炎症性腸疾患の発症機序を解明することで、口腔ケアから全身のケアに繋がることを発信することは、我が国のみならず諸外国の歯科臨床・歯科医学の発展に寄与すると考えられる。（ファカルティー・アドバイザー：小林 良喜）

Induction of macrophages into the small intestine by oral inoculation with *Fusobacterium nucleatum*

(Problem)

It has been suggested that periodontopathogenic bacteria and pathogenic factors that enter the digestive tract and bloodstream due to chronic periodontitis contribute to pathogenesis in various body organs. Since the oral cavity is anatomically contiguous with the intestinal tract, oral bacteria may enter the intestinal tract and affect the composition ratio of the intestinal microbiota and immune cells. In addition, 60% of the immune cells in the body are located in the intestinal tract, and they are activated by cooperation with the intestinal microflora. They are involved in maintaining and improving biological homeostasis in the intestinal tract and the whole body. Therefore, changes in the oral environment are thought to affect the intestinal environment and the entire body. Recently, *Fusobacterium nucleatum* (Fn), one of the embryonic pathogenic bacteria, has been detected in the pathogenic mucosal sites of colorectal cancer. Many reports that Fn is deeply related to the carcinogenesis and progression of colorectal cancer. However, the mechanism by which Fn affects the intestinal microbiota and intestinal mucosal tissues remain unclear.

(Hypothesis)

The fact that the causes of death and morbidity of colorectal cancer are rapidly increasing in both men and women in their 60s, and the fact that the number of remaining teeth in the elderly increases year by year and the incidence of periodontal disease increases suggests that periodontitis in the elderly may induce inflammatory bowel disease, which may become severe and cancerous due to chronicity. In this study, we attempted to examine the effects of Fn on the intestinal environment from the oral cavity by examining the dynamics of macrophages, which are immune cells.

(Method)

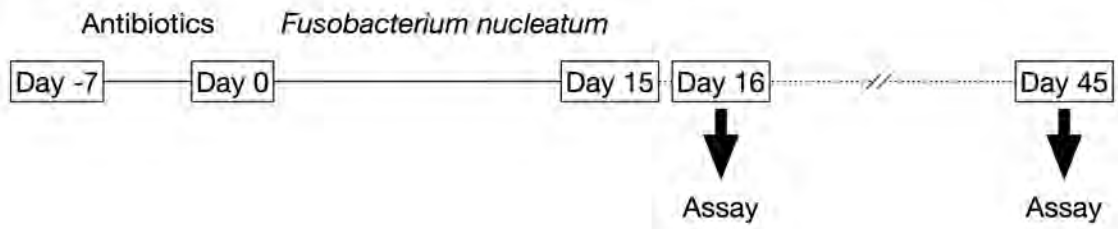
1) Preparation of *Fusobacterium nucleatum* (Fn)

Fusobacterium nucleatum (ATCC:23726) was cultured on sheep blood agar medium for CDC anaerobes (Becton Dickinson) in an anaerobic box containing 10 % H2, 80 % N2, and 10 % CO2 for 3 to 5 days. The cultured cells were centrifuged at 4 °C, 8000 rpm, for 15 min and resuspended in 5 % carboxymethylcellulose (CMC) to obtain an inoculum of 1×10^9 CFU/100 µl /mouse. After that, Fn was collected from the blood agar medium and seeded into a Brain-heart infusion (BHI, Becton Dickinson) medium. The cells were transferred to a new BHI medium by taking 1 ml from the BHI medium daily until the absorbance reached OD540nm = 0.8, corresponding to 10^9 CFU/ml.

2) Animal experiments and breeding conditions

Seven-week-old female BALB/c Cr Slc (BALB/c) mice (Sankyo Lab Service Co., Ltd.) were obtained and housed in a temperature-controlled, pathogen-free clean rack with a 12-h light/dark cycle. Autoclaved food and water were freely available. The Animal Experiment Committee approved this experiment.

Figure 1



3) Oral inoculation schedule

Mice were randomly divided into two groups: one group inoculated with Fn (Fn group) and the other group inoculated only with 5 % CMC solution (Sham group). The Fn solution and CMC solution were prepared each time and inoculated 15 times.

4) Immunohistochemical study of small intestinal mucosal tissue

One day after the last oral inoculation and 30 days after the last oral inoculation, mice were intraperitoneally injected with a mixture of three anesthetics (medetomidine hydrochloride, midazolam, and butorphanol tartrate), washed with heparinized solution, perfused, and fixed with 10 % neutral buffered formalin solution, and small intestine was collected. The small intestine was embedded in paraffin, and sections were prepared at a thickness of 4 μl. The small intestine was stained with PE-conjugated anti-mouse F4/80 as primary antibody and DAPI as contrast stain.

(Results)

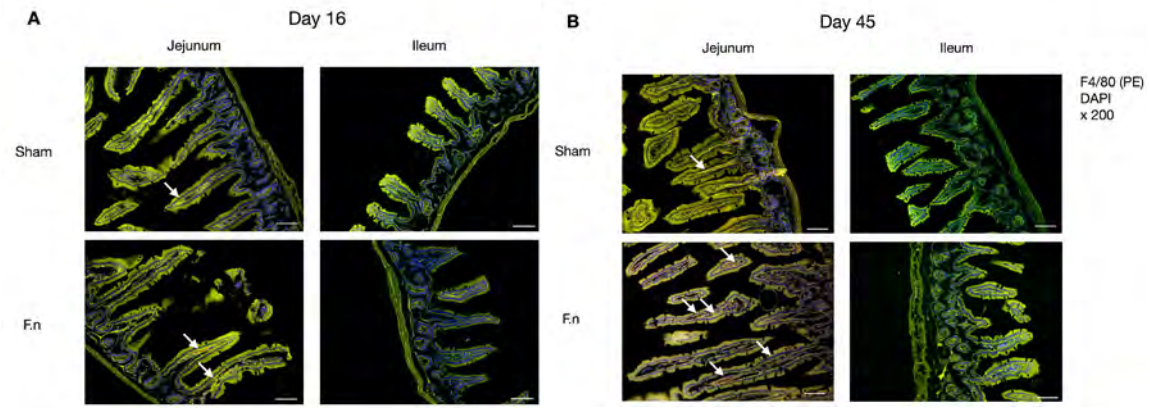
We examined the localization of macrophages in the small intestine. We found that they were localized in the intrinsic layer of mucosa (SiLP) of the jejunum one day after oral inoculation of Fn bacteria (Day 16). To examine temporal changes, an increase in SiLP in the jejunum was observed 30 days after the last inoculation (Day 45). This was not observed in the control group inoculated with 5% CMC. On the other hand, no F4/80+ macrophages were found in the ileum (Fig 2A,2B).

(Conclusion)

The gastrointestinal tract is a unique organ with a symbiotic relationship with intestinal bacteria, and unlike other organs, it has a predominantly permissive and inhibitory immune response. Usually, the intestinal mucosal tissues, which are constantly exposed to food-derived antigens and intestinal bacteria, maintain homeostasis by suppressing excessive immune responses to these antigens. In particular, intestinal macrophages are representative immune cells of the innate immune response. It has become clear that they play an essential role in infection defense and play an inhibitory role in the intestinal immune system. In this study, we investigated the effect of periodontal pathogens on the immune responses of periodontal macrophages.

In this experiment, we investigated whether inoculation of periodontopathogenic Fn bacteria into the oral cavity would induce oral inflammation and intestinal inflammation and disrupt the immune response and found that F4/80^{+} macrophages migrated into the intrinsic layer of the small intestinal mucosa. In addition, we found that F4/80^{+} macrophages migrated into the intrinsic layer of the small intestine mucosa, and the increase was sustained. Interestingly, the localization of macrophages was found in the jejunum and limited in the ileum. Thus, although the immune response in the intestine is thought to be centered in the small intestine, the fact that macrophage migration differs by the site may contribute to the understanding of the pathogenesis of inflammatory bowel disease and other diseases.

Figure 2



Analysis of relationship between ectopic pain and somatotopy of rat trigeminal ganglion neurons

鹿児島大学歯学部 4年生 Kagoshima University Faculty of Dentistry Class of 2023

福島 慎 Makoto FUKUSHIMA

ファカルティー・アドバイザー：歯科機能形態学分野 教授 後藤 哲哉
研究指導協力者：歯科機能形態学分野助教 倉本 恵梨子

Ectopic pain and non-odontogenic toothache are common in clinical dentistry, but their causes are not clear. The primary trigeminal neurons that transmit sensation in the maxillofacial region form somatotopy within the trigeminal ganglion according to their three branches. If there is overlap in their somatotopy, it may result in satellite cell-mediated crosstalk between neurons. In this study, retrograde tracers were injected into the maxillofacial region of rats to clarify whether there is overlap of somatotopy within the trigeminal ganglion, and the localization of the neurons to be labeled was more accurately reconstructed in three dimensions in combination with the tissue transparency method. The results showed that the cell bodies of each trigeminal neuron, from the first to the third branch, were distributed in different regions within the ganglion, however, considerable overlap was observed at the boundary area. One area of high percentage of overlap was found in the combination of mandibular molars and tongue or masseter muscle, which are areas where ectopic pain is frequently observed in clinical practice. In fact, it was confirmed that nerve cell bodies in the areas where ectopic pain occurs with high clinical frequency were in close proximity to each other, suggesting that crosstalk between nerve cell bodies via satellite cells, etc., may be a factor in ectopic pain.

異所性疼痛とラット三叉神経節体部位局在との関連についての研究

異所性疼痛や非歯原性歯痛は歯科臨床ではよく見られるが、その原因は明らかではない。顎顔面領域の知覚を伝える三叉神経1次ニューロンはその3枝に応じて三叉神経節内で体部位局在を形成するが、もしそれらの局在部位に重複があれば、神経細胞同士で衛星細胞を介したクロストークを生じる可能性がある。本研究では、三叉神経節内で体部位局在のオーバーラップが存在するかどうかを明らかにするために逆行性トレーサーをラットの顎顔面部位に注入し、標識される神経細胞の局在を組織透明化法と組み合わせてより正確に三次元再構築を行った。その結果、第1枝から3枝までの、それぞれの三叉神経の細胞体は、神経節内で、異なる領域に分布していたが、移行部ではかなりのオーバーラップが観察された。高い割合のオーバーラップを示した部位として、下顎臼歯と舌または咬筋の組み合わせが見られたが、これらは臨床でも異所性疼痛が高頻度で認められる領域である。実際に、臨床的に高い頻度で異所性疼痛を生じる領域の神経細胞体が近接して存在することが確認できたことから、衛星細胞等を介した神経細胞体間のクロストークが異所性疼痛の一因となる可能性が示唆された。

研究発表内容の紹介

異所性疼痛は歯科診療でしばしば見られるが、その原因や発生メカニズムが明確ではないため、その治療も困難である。本研究では逆行性蛍光トレーサーと組織透明化法を使い、正確に三叉神経節内での神経細胞体・体部位局在の重複が確認できた。臨床でも異所性疼痛が高頻度で認められる領域に、体部位局在の重複が見られたことから、三叉神経節における神経細胞体間のクロストークが顎顔面領域の異所性疼痛の一因となる可能性が示唆された。（ファカルティー・アドバイザー：後藤 哲哉）

Analysis of relationship between ectopic pain and somatotopy of rat trigeminal ganglion neurons

【Problem】

In the clinical practice of dentistry," non-odontogenic toothache" that cause pain in the teeth even though there is no direct cause in the teeth has become a problem. For example, as shown in Figure 1, some patients complain of toothache even though the cause of the pain is in the masseter muscle. This can lead to problems such as invasion of healthy teeth and pain that does not go away even after treatment. This mechanism has not been fully elucidated. One possible cause of ectopic pain is the somatotopy in the trigeminal ganglion (TG), which is responsible for somatosensory perception in the orofacial region.

Fig 1. Ectopic pain

【Hypothesis】

The trigeminal nerve is divided into three major branches, each innervating a different region (Fig. 2). Individual ganglion cells of the three branches are distributed in different parts in the trigeminal ganglion (TG; Fig. 2), it is called somatotopy. It has been reported that when a nerve in one region is damaged, the neurons and surrounding satellite cells release inflammatory mediators such as cytokines, causing inflammation to spread to nearby neurons that have not been damaged (Fig. 3). If this cross-talk between ganglion neurons via satellite cells is one of the causes of ectopic pain, then the neurons innervating the regions with high frequency of ectopic pain would be expected to closely appose to each other within the trigeminal ganglion. To examine this logic, in the present study, we revealed the details of the somatotopy of rat trigeminal ganglion neurons in three dimensions.

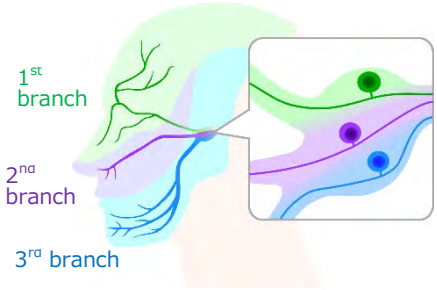


Fig 2. Somatotopy of the TG

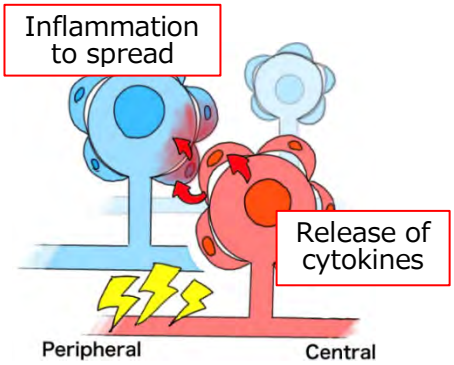


Fig 3. Crosstalk of TG cells via satellite cells

【Methods】

The fluorescent dye Fast Blue was used as a retrograde tracer and injected into the rat orofacial regions (maxillary first and second molars, mandibular first and second molars, masseter muscle, tongue, upper eyelid, cornea, infraorbital nerve, and palate; n=5 each). After cell bodies of trigeminal ganglion neurons were labeled, the rat trigeminal ganglia were harvested. The trigeminal ganglia were cut into three slices (500-μm-thick), cleared using a modified 3DISCO method, and z-stack images were captured using a confocal laser microscope (Zeiss). Cell bodies of tracer-positive neurons in the trigeminal ganglion were three-dimensionally (3D) plotted using Neurolucida (MBF) (Fig. 4). In double labeling, DiI and Fast blue were injected into different orofacial regions, and observed by confocal laser microscopy.

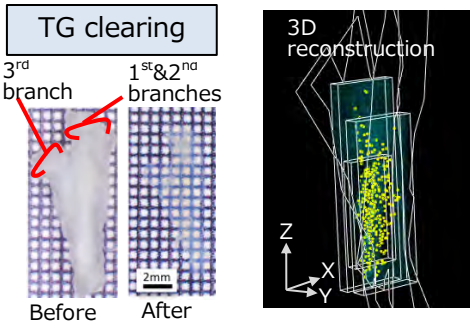


Fig 4. Tissue clearing and 3D reconstruction

【Results】

As shown in Figure. 5, neuronal cell bodies of the trigeminal ganglion were distributed in the area indicated by light blue, which was divided into two regions: the main trunk and lateral branch, in the present study. Neuronal cell bodies of the first, second and third branch of the trigeminal nerve were distributed in different regions of the ganglion. However, considerable overlap was observed in the transition zone.

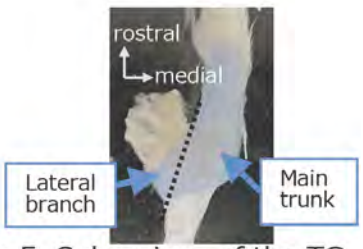


Fig 5. Subregions of the TG

Neuronal cell bodies innervating the upper eyelid and cornea of the first branch field were concentrated in the dorsomedial part of the main trunk of the trigeminal ganglion (Fig. 6A, B). Neuronal cell bodies innervating the maxillary first and second molars of the second branch region were scattered to the lateral and main trunk (Fig. 6C). Neurons innervating the palate were abundantly distributed on the ventral part of the main trunk (Fig. 6D). Neuronal cell bodies of the infraorbital nerve were widely distributed throughout the main trunk, with the largest number of tracer-positive cell bodies (Fig. 6E). All the neurons innervating the third branch region (Fig. 6F, mandibular first and second molars; G, tongue; H, masseter muscle) were mainly distributed in the lateral branch of the trigeminal ganglia. However, neurons were also distributed extensively in the main trunk.

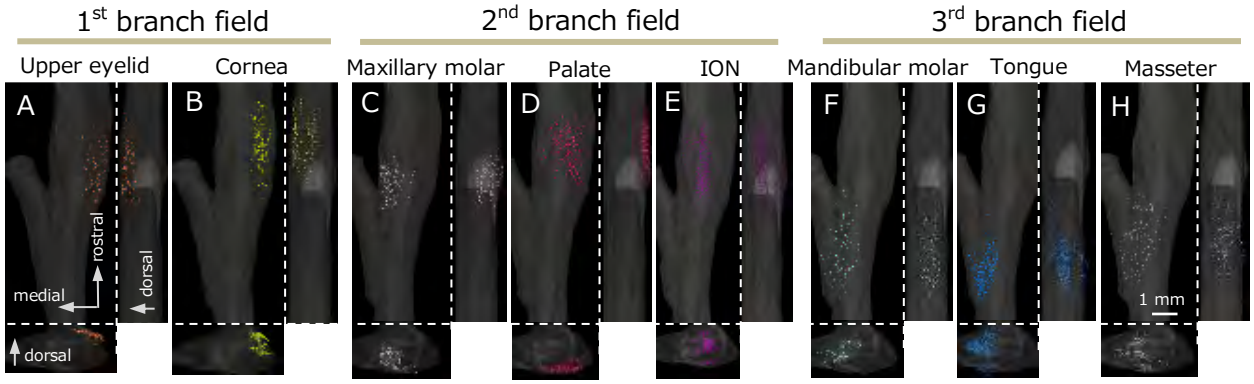


Fig. 6 Distribution of retrogradely labeled TG cells

The three-dimensional somatotopy of the trigeminal ganglion was summarized in Figure 7A. Next, we quantified the overlapping ratio of each cell-body-distribution area. The pairs of areas which showed high percentage of overlap were listed in Figure 7B. Among these pairs, the pair of mandibular molars and tongue or masseter muscle is a region where ectopic pain is frequently observed in clinical practice.

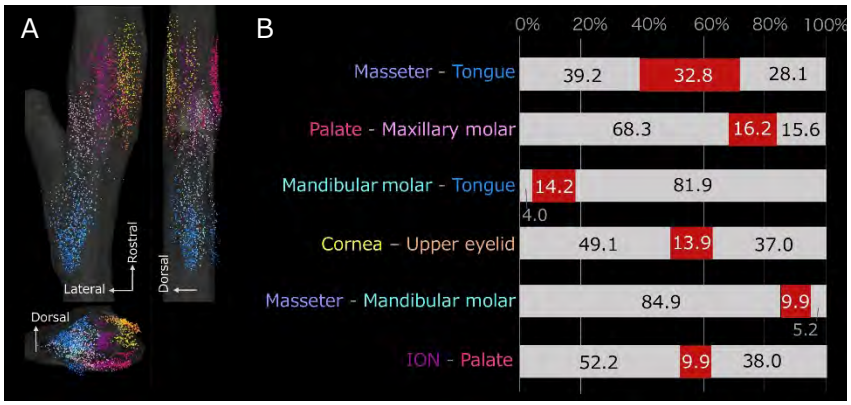


Fig. 7 Percentage of overlap of cell-body-distribution-area

Finally, to confirm whether neuronal cell bodies are closely apposed to each other enough to allow crosstalk in these regions, we used the fluorescent double labeling method. As shown in Figure 8, cell bodies in apposition were observed, suggesting the possibility of crosstalk between ganglion neurons as a mechanism of ectopic pain.

【Conclusion】

By combining retrograde tracers and tissue clearing technique, we succeeded to reconstruct the somatotopy of the rat trigeminal ganglion three dimensionally. Neurons innervating the first, second, and third branch fields were mainly distributed in different areas of the trigeminal ganglion, while considerable overlap were observed in transitional zones. In fact, the neuronal cell bodies in the regions of clinically frequent ectopic pain were found to be close proximity to each other, suggesting that crosstalk between neuronal cell bodies via satellite cells is a contributing factor to ectopic pain.

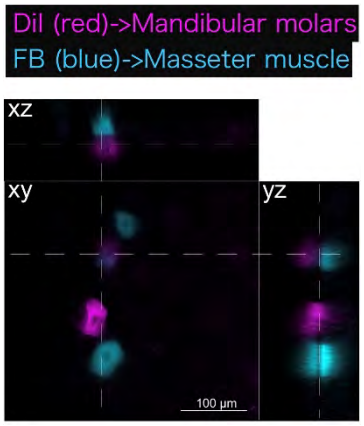


Fig. 8 TG cell bodies in close proximity

Metagenomic analysis of oral and intestinal microflora in a rat model of chronic restraint stress

北海道医療大学歯学部 5年生 School of Dentistry Health Sciences University of Hokkaido Class of 2022

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Alteration of intestinal microflora has been often observed in the stress-related systemic diseases. It is, however, not known how chronic stress affect oral microflora. It is not known whether changes in oral microflora affect alteration of intestinal microflora caused by chronic stress. In this study, we performed metagenomic analysis of oral and intestinal microflora in a rat model of chronic restraint stress. These experiments were approved by the animal experiment center in our institution. The metagenomic analysis of oral bacteria showed significant differences in relative abundance of bacteria such as *Facklamia*, *Aerococcaceae*, *Prevotella*, *Corynebacterium*, *Clostridium* and others. between stress group rats and control. The metagenomic analysis of intestinal microbiome showed significant differences in bacteria such as *Bifidobacterium*, *Lactobacillus*, *Butyricicoccus*, *Dehalobacterium*, *Oscillospira* and others between stress group and control. We found significant alterations in oral flora as well as intestinal microflora under chronic stressed conditions. However, no relationship between oral and intestinal microflora could be found in this study.

慢性拘束ストレスラットモデルにおける口腔および腸内細菌叢のメタゲノム解析

腸内細菌叢の変化は、ストレス関連の全身性疾患で観察されている。しかしながら、慢性ストレスが口腔細菌叢にどのように影響するかは不明である。また、口腔細菌叢の変化が慢性ストレスによって引き起こされる腸内細菌叢の変化に影響を与えるかも不明である。本研究では、慢性拘束ストレスラットモデルにおける口腔および腸内細菌叢のメタゲノム解析を行なった。実験に際し動物実験センターの承認を得た。口腔細菌のメタゲノム解析では、ラットストレス群とコントロール群間で、*Facklamia*、*Aerococcaceae*、*Prevotella*、*Corynebacterium*、*Clostridium*などの細菌の相対的な存在量に有意差が認められた。腸内細菌叢のメタゲノム解析では、ストレス群とコントロール群間で、*Bifidobacterium*、*Lactobacillus*、*Butyricicoccus*、*Dehalobacterium*、*Oscillospira*などの細菌属に有意差が認められた。結果から慢性的なストレス条件下で、口腔細菌叢と腸内細菌叢に有意な変化が認められた。一方、口腔微生物叢と腸内細菌叢の関連性は認められなかった。

研究発表内容の紹介

ストレスによる全身への悪影響に、腸内細菌叢の変化も一因とされている。近年、歯周病原菌により腸内細菌叢が変化することが示され、脳―腸相関に加え、口腔―腸連関が注目されてきている。本研究はラットを用いた実験から、ストレスによる口腔環境の変化を、口腔細菌叢の変化及び腸内細菌叢との連関から明らかにしたものである。現代のストレス社会における口腔細菌叢観察の重要性を提唱しており、歯科医学への寄与が大きい。（ファカルティー・アドバイザー：安彦 善裕）

Metagenomic analysis of oral and intestinal microflora in a rat model of chronic restraint stress

[Problem]

Many people are suffering from psychological stresses probably linked to many systemic diseases in the society. Alteration of intestinal microflora has been often observed in the stress-related systemic diseases. It is, however, not known how chronic stress affect oral microflora. Oral microbes can be ingested and will naturally translocate to the digestive tract, where they can potentially form ectopic colonies in the upper and lower digestive tracts. It is not known whether changes in oral microflora affect alteration of intestinal microflora caused by chronic stress.

[Hypothesis]

Both oral and intestinal microflora may be changed in a rat model with chronic stress. Changes in oral microbe may affect alteration of intestinal microflora under stressed condition.

[Methods]

These experiments were approved by the animal experiment center in our institution. Six-weeks old Sprague Dawley rats were given restraint stress by enclosing them in a plastic tube 4 hours daily for 1 month. Behavior analyses (elevated plus maze test), adrenal gland and serum corticosterone were evaluated as stress markers. After a month of stress protocol, the rats were sacrificed to collect oral swab, intestinal stool and serum from blood. The bacterial DNA was extracted from oral swab and intestinal stool. Metagenomic analyses was done using 16S rRNA sequencing. The obtained data was analyzed using various software. The significant differences in bacterial taxonomic abundance, alpha diversity, beta diversity and predicted functional pathway were analyzed between stress group and control.

[Results]

The low body weight (Fig.1), high adrenal gland weight (Fig.2), high serum corticosterone (Fig.3) and lower number of entries (Fig.4) and time spent in the open arm of elevated plus maze confirmed high stress level in stress group rats as compared to control.

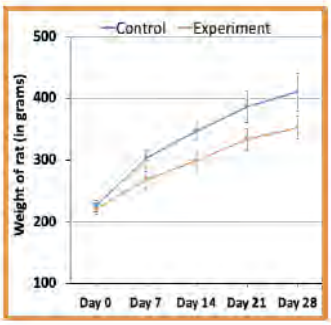

Fig.1

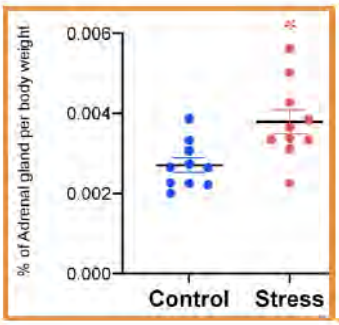

Fig.2

Fig.3

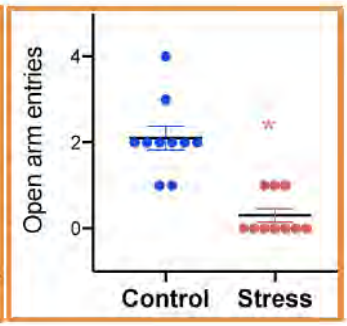

Fig.4

The metagenomic analysis of oral bacteria showed significant differences in relative abundance of bacteria such as *Facklamia, Aerococcaceae, Prevotella, Corynebacterium, Clostridium* and others (Fig.5) between stress group rats and control. Also, reduction of alpha diversity of oral microbiome in stress group was observed (Kruskal-Wallis test, $p<0.05$) (Fig.6).

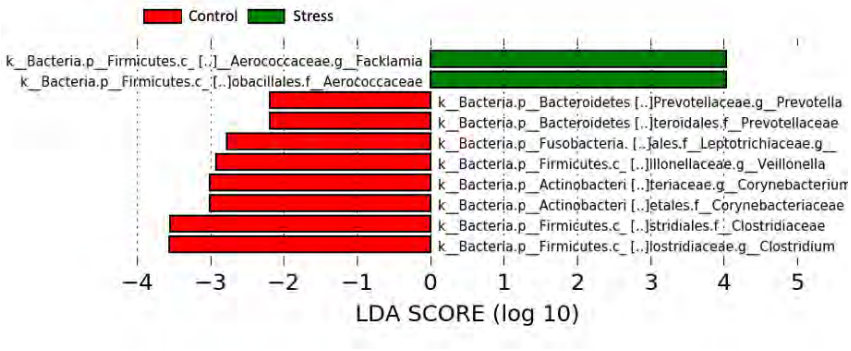

Fig.5

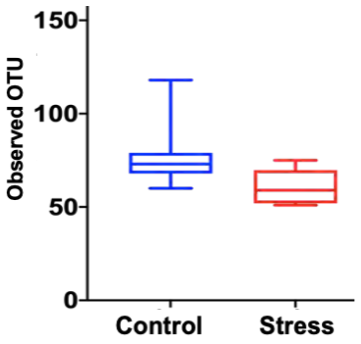

Fig.6

The metagenomic analysis of intestinal microbiome showed significant differences in bacteria such as *Bifidobacterium, Lactobacillus, Butyricicoccus, Dehalobacterium*, *Oscillospira* and others between stress group and control (Fig.7). Also, significant difference in beta diversity was observed (PERMANOVA, $p<0.01$) (Fig.8). The prediction of microbial function showed 37 significantly altered functional pathways in oral cavity and 150 significantly altered functional pathways in intestine between stress group and control (Welch's t tests, $p<0.05$). No significant correlation between altered oral and intestinal microbiome could be observed.

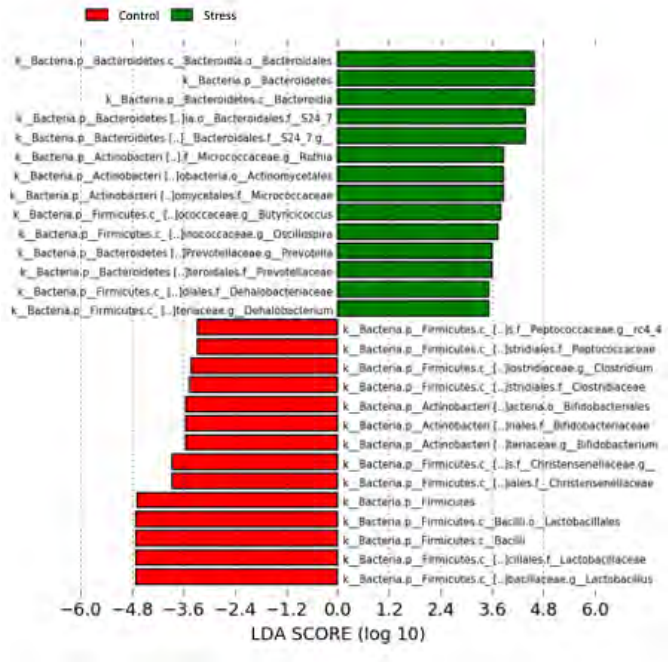

Fig.7

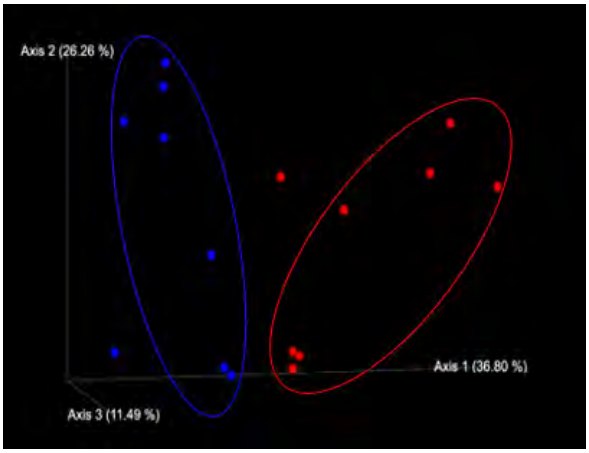

Fig.8

[Conclusion]

We found significant alterations in oral flora as well as intestinal microflora under chronic stressed conditions. However, no relationship between oral and intestinal microflora could be found in this study.

Impact of fear of contracting COVID-19 on refraining from the dental visits: A nationwide internet survey

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Objectives: During the COVID-19 pandemic, people tended to refrain from seeing a doctor or dentist, but the reason remained unclear. The aim of present study was to investigate the association between the fear of contracting COVID-19 and refraining from dental visits.
Methods: This was a cross-sectional study using the Japan Society and New Tabaco Internet Survey (JASTIS) in 2021. This web survey was based on the self-reported questionnaires. People aged 20 to 80 years were included. We used the refraining from dental visits within last two months as dependent variable and the degree of fear of contracting COVID-19 as independent variable. Possible confounders were used as covariates. We estimated adjusted prevalence ratio (aPR) and 95% confidence intervals (CIs) using Poisson regression model.
Result: Among 7,687 participants, the mean age was 54.3 years (1SD=16.4) and 48.1% were man. Among them, 2,019 (26.3%) answered that they refrained from dental visits. The participants of 67.7% have higher fear of COVID-19, and 1.38 times more people with higher fear refrained from dental visits (95%CI=1.25-1,53: p <0.001).
Conclusion: The fear of contracting COVID-19 is associated with refraining from dental visits.

新型コロナウイルス感染症に対する恐怖心の歯科受診控えに対する影響：インターネット全国調査による検討

新型コロナウイルス（以下COVID-19）の蔓延は人々の行動に大きな影響をもたらした。歯科の現場では、COVID-19流行下において歯科の受診を控える人が増加しているが、要因が明らかでない点が多い。本研究の目的はCOVID-19に対する恐怖心とCOVID-19の流行下における歯科の受診控えとの関連を分析することであった。2021年2月～3月に実施された自記式調査票を用いたウェブ調査データ（JASTIS）を用いた。20～80歳代の男女で調査回答時の最近2か月以内に歯科医院への通院を予定していた者を対象とした。ポアソン回帰モデルを用いた多変量回帰分析を行い、存在率比および95%信頼区間を算出した。分析対象者は7,687人（男性48.1%、平均年齢54.3歳（1標準偏差=16.4））であった。歯科の受診を控えた人は2,019人（26.3%）であり、COVID-19に対する恐怖の度合いが高い人は5,202人（67.7%）であった。ポアソン回帰モデルを用いて交絡因子を調整した結果、恐怖心が高かった人では歯科受診を控えた人が1.38倍多かった。（95%信頼区間=1.25 - 1,53: p<0.001）本研究結果よりCOVID-19への恐怖心が強いことと歯科の受診控えとの間に有意な関連が見られた。歯科医師はCOVID-19の感染予防を適切に行い、患者が安心して受診できるような環境を整備し、口腔の健康維持に貢献していく必要がある。

研究発表内容の紹介

新型コロナウイルス（以下COVID-19）の流行下において、歯科の受診控えが問題となっている。本研究ではCOVID-19に対する恐怖心が歯科の受診行動とどのように関連しているかについて大規模ウェブ調査を用いて明らかにした。新型コロナウイルス（以下COVID-19）の流行下において、歯科の受診控えが問題となっており、本研究ではCOVID-19に対する恐怖心が歯科の受診行動とどのように関連しているかを分析した。その結果から、COVID-19に対する恐怖が大きいほど、歯科の受診を控えていたことが分かった。歯科の受診控えは、口腔内環境の悪化につながることが予想されるため、歯科医師はCOVID-19の感染予防を適切に行い、歯科の受診控えを抑制し、口腔の健康維持に貢献していく必要がある。（ファカルティー・アドバイザー：小坂 健）

Impact of fear of contracting COVID-19 on refraining from dental visits: A nationwide internet survey

(Problem)

The spread of COVID-19 had a huge impact on people's behavior. Many people were forced to refrain from going out for unnecessarily and urgently purpose by the government. The multiple channels of mass media also broadcasted the news related to COVID-19 every day, and many people were afraid of the emerging virus. In addition, a number of people refrained from seeing doctors because of the unknown risk of infection with COVID-19. Even in dental settings, the number of people also avoided dental visits. There would have occurred oral health problems such as worsening periodontal disease or dental caries due to interruption of consultation, and there were possibilities of worsening oral health due to the inability to get oral management. However, it remained unclear what factors were associated with refraining from dental visits under the COVID-19 pandemic.

(Hypothesis)

We hypothesized that a strong fear of contracting COVID-19 has led people to refrain from dental visits. (fig. 1). The aim of this study was to investigate the association between the fear of COVID-19 and refraining from dental visits during the COVID-19 pandemic.

Fig.1 The hypothesized mechanism between the fear of COVID-19 and refraining from dental visits.

(Method)

This was a cross-sectional study using the data of the Japan Society and New Tabaco Internet Survey (JASTIS) in 2021.

JASTIS is a huge web survey based on self-reported questionnaires conducted from February to March 2021 from the candidates, who were selected from the panelists at a Japanese Internet research company (Rakuten Insight, Inc.) Among the total sample of 26,000 participants, we targeted those who planned to go to dental clinics in the last two months. We used “refraining from dental visits” for the independent variable. We asked the participants, "Have you ever been unable, postponed, or had any medical or illness events in the last two months?", and have them answered “yes” or “no” for dental visits. We used the fear of COVID-19 infections as the dependent variable. The degree of the fear of COVID-19 was used as independent variable. We recategorized the five-point Likert-scale answer for the feeling of the fear of COVID-19 into “low”, “medium”, and “high”. We used the following covariates including gender, age, equivalent income, education, household size, and employment.

Statistical analysis: Multivariate regression analysis using the Poisson regression model was performed to calculate adjusted prevalence ratios (aPRs) and 95% confidence interval (CIs). We also considered the possibility that the relationship may differ by gender, and we also conducted the analysis stratified by gender.

(Result)

The participants of 7,687 (male 48.1%, average age 54.3 years (1 standard deviation = 16.4)) were included into the analysis, of which 2,019 (26.3%) answered that they refrained from dental visits. The distribution of the degree of fear of contracting COVID-19 was "low": 837 (10.9%), "medium": 1,648 (21.4%), and "high": 5,202 (67.7%). The proportions of those who refrained from dental visits were higher in the group with higher degree of the fear of contracting COVID-19 (low: 11.7%, medium: 22.9%, high: 65.4%) (Fig. 2).

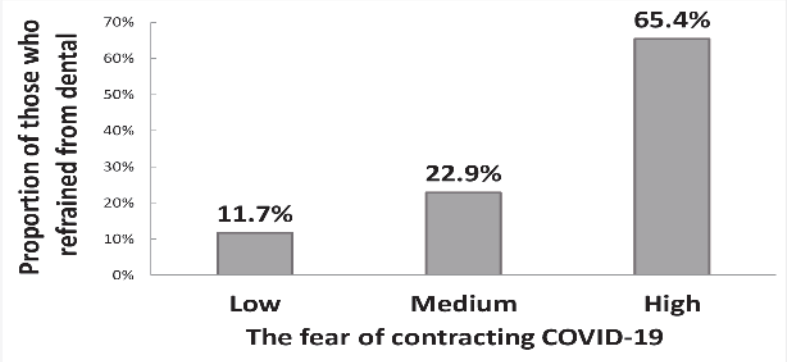

Fig.2 The proportion of those who refrained from dental visits by the fear of contracting COVID-19 (n=7,687)

Table 1 shows the results of multivariate regression analysis after adjusting the possible confounders. There was no statistically significant difference in the prevalence of refrained from dental visits between those with low degree of the fear compared and those with moderate (aPR=0.95, 95%CI=0.80-1.12); however, 1.38 times higher in people with higher degree of fear (95% confidence interval = 1.25-1,53: p <0.001). Although the interaction with gender was not significant (p = 0.90), the association between higher degree of fear and refraining from dental visits was stronger among women.

Table 1. Relationship between gender-specific fear of contracting COVID-19 and refraining from dental visits

	All (n=7,687) aPR (95% CI)	Men (n=3,698) aPR (95% CI)	Women (n=3,989) aPR (95% CI)
The fear of contracting COVID-19			
Low	0.95 (0.80 - 1.12)	0.90 (0.72 - 1.12)	0.96 (0.74 - 1.24)
Medium	Ref.	Ref.	Ref.
High	1.38 (1.25 - 1.53)***	1.28 (1.10 - 1.50)**	1.48 (1.29 - 1.70)***

(**p < 0.01, ***p < 0.001 aPR=adjusted prevalence ratio, 95%CI=95% confidence interval, Ref=Reference)

(Discussion)

We found that the higher degree of fear of contracting COVID-19 was associated with refraining from dental visits even in the period of more than a half year after COVID-19 pandemic in Japan. The result suggested that the influence of fear of contracting COVID-19 on refraining from dental visits may prolong for a certain period. As a possible mechanism, it has not been reported in Japan that the infection occurred due to dental treatment, but there was uncertain information that infection is likely to occur at dental clinics on some media. It may have led people with fear of contracting COVID-19 to refrain from dental visits.

(Conclusion)

From this study, we found that the fear of contracting COVID-19 is associated with refraining from dental visits. Dentists should undertake measures for preventing the transmission of COVID-19 and must provide a safe opportunity of maintaining optimal oral health to patients.

Effects of Propolis and a PPARγ inhibitor, GW9662, upon the biological activities of stimulated spleen cells and undifferentiated mesenchymal cells

朝日大学歯学部　2年生　Asahi University School of Dentistry Class of 2025

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In this study,we examined the effects of Brazilian propolis (BP) and GW9662 on cytokine production of activated immune cells and osteoblastgenesis of premature stromal cells, and evaluate the potential usage as a pulp capping material.

Our results demonstrated that;

1. Production of pro-inflammatory cytokines, IFN-γ, IL-6 and IL-17,in the stimulated spleen cells was significantly reduced; regulatory cytokines, IL-4 and IL-10, were almost unchanged; IL-2 was significantly enhanced by BP at concentrations sustaining high viability(80%). These effects on the modification of the cytokine production were similarly triggered by artepillin C, a major component of BP and an agonist of PPARγ.
2. GW9662 and artepillin C synergistically enhanced the production of IL-2, IL-4 and IL-10, while suppressing that of IFN-γ, IL-6 and IL-17 in the stimulated spleen cells.
3. The expression of Osterix mRNA in 10T1/2 cells was significantly elevated in the presence of BP and GW9662.

We concluded as follows;

1. Artepillin C, representing immune-modulatory effects of BP, and GW9662, an agonist of artepillin C, could curatively regulate host immune cells.
2. Artepillin C and GW9662 might also induce hard tissue deposition in premature stromal cells.
3. These properties of BP and GW9662 suggest that their combined use could be an effective alternative for dental pulp treatment by reducing inflammation and enforcing dentin formation.

プロポリスおよびPPAR-γ阻害因子（GW9662）が活性化脾細胞や未分化間葉系細胞の生物学的活性におよぼす影響

ブラジル産プロポリス（BP）とGW9662が活性化免疫細胞のサイトカイン産生および未分化間葉系細胞の硬組織形成能に及ぼす影響を調べ両者併用による歯髄治療薬としての可能性を検討した。

その結果；

1. 高い生存率（80%）を維持する濃度のBPにより、刺激脾細胞脾臓細胞における炎症誘発性サイトカイン、IFN-g、IL-6およびIL-17の産生は有意に低下し制御性サイトカインIL-4とIL10はほとんど変化せず、IL-2は有意に増加した。BPによるサイトカイン産生の修飾効果はBPの主成分でありPPARgのアゴニストであるアルテピリンCによってほぼ同様に誘発された。
2. GW9662とアルテピリンCは協調的に刺激脾臓細胞のIL-2、IL-4、IL-10の産生を増強し、IFN-g、IL-6、IL-17の産生を抑制した。
3. 10T1/2細胞における*Osterix* mRNAの発現は、BPおよびGW9662の存在下で有意に上昇した。これに対して*Runx2*mRNA発現は変化しなかった。

結　論；

1. BPの主成分であるアルテピリンCとGW9662は炎症性および抑制性サイトカインを治癒的に調節する可能性が示された。
2. BPおよびGW9662は未分化間葉系細胞から骨芽細胞形成を誘発する可能性が示された。
3. BPとGW9662の特性からこれらを組み合わせれば炎症を軽減し二次象牙質形成を促進する歯髄治療薬として使用できる可能性が示された。

研究発表内容の紹介

BPは、GW9662と相乗的に炎症反応を抑制する作用を発揮することが示された。一方でこれらは協調的に、未分化間葉系細胞の骨分化誘導因子を活性化することが示唆された。この二つの相乗作用により、これらが炎症を抑えながら二次象牙質形成を促進するという、理想的な覆髄材の素材としての効果を発揮する可能性が示された。（ファカルティー・アドバイザー：近藤 信夫）

Research title: Effects of Propolis and a PPAR-γ inhibitor, GW9662, upon biological activities of stimulated spleen cells and undifferentiated mesenchymal cells

(Problem)

Propolis is a natural extract from honeybee glue and has been proven to have several beneficial biological effects. It exerts anti-inflammatory effects through the inhibition of inflammatory cytokines and also participates in tissue regeneration through the stimulation of regulatory cytokines (Machado JI, 2012).

Dental pulp often suffers from caries, which results in inflammation and damage to dentin. The application of calcium hydroxide is a conventional standard for pulp capping, however it also induces pulp tissue necrosis caused by fibroblast cell death, resulting in delayed generation of secondary dentin. It has been reported that propolis combined with pulp capping material can stimulate the process of pulp tissue repair (Rahayu RP, 2020). However, green propolis extract inhibits osteogenic differentiation, while also promoting adipogenesis and chondrogenesis of bone marrow stromal cells (Elkhenany H, 2019). Adipocyte differentiation is activated by a major component of Brazilian propolis (BP), artepillin C, via the activation of PPARγ (Choi SS, 2011). This inhibitory effect of propolis could spoil hard tissue regeneration, such as secondary dentin deposition.

(Hypothesis)

It has been reported that the PPARγ-dependent adipogenesis could be blocked and facilitate osteoblastogenesis by GW9662, a potential antagonist of PPARγ (David V2007). Therefore, we assume that the combined use of propolis and GW9662 could exert preferable effects from both substrates, i.e., inhibition of inflammation together with enforcement of hard tissue generation. Dental pulp is a complex tissue consisting of several cell types including premature mesenchymal origin and immune cells. Therefore, In this study, we examined the effects of Brazilian propolis and GW9662 on cytokine production in activated immune cells and osteoblastogenesis of pre-mature fibroblasts, and evaluated their integration as a curative reconstruction of dental pulp.

(Methods)

1. Preparation of spleen cells

   Spleens were removed from male C3H/HeN mice older than 24 weeks, then mashed with a cell strainer in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% FBS, 50 μM 2-mercaptoethanol. Cells were collected and the red blood cells were removed using a red blood cell lysis buffer. The spleen cells were washed and filtered using a cell strainer to remove residue.

2. Cytokine detection using enzyme-linked immune sorbent assay (ELISA)

   The spleen cell suspension (4×10^5/well) was added to a 96‐well plate, on which 1 μg/mL of anti‐CD3 monoclonal antibody was immobilized (0.1 mL/well) at 4°C overnight. The propolis extracts or artepillin C, and GW9662 were added to the wells and co-cultured with the spleen cells in RPMI RPMI‐1640 basal medium for 48 hours in 5% CO_2 at 37°C. The concentration of cytokines in the supernatant of the cell culture was assayed by ELISA.

3. RNA extraction and RT-PCR analysis

   C3H 10T1/2 mouse embryonic fibroblast were seeded in a 10 cm dish and diluted in RPMI supplemented with 10% FBS, and cultured in 5% CO_2 at 37°C. The propolis extracts and/or GW9662 were added to the medium, then incubated for 72h.

Total RNA was extracted using ISOGEN. RT-PCR was performed using primer pairs for mouse *Runx2*, forward 5'- CTT CAT TCG CCT CAC AAA CAA CCA C-3' and reverse 5'- TGC TTG CAG CCT TAA ATG ACT CGG T-3'; mouse *Osterix* , forward 5'- AGC CCA CCT AAC AGG AGG ATT TTG-3' and reverse 5'- CTT TCT CGT GGC TTC TAG GCA CC-3'; ribosomal protein S5 (*RPS5*), forward 5'- GAG CGC CTC ACT AAC TCC ATG ATG A-3' and reverse 5'- CAC TGT TGA TGA TGG CGT TCA CCA-3', respectively. mRNA expression levels were normalized to those of *RPS5*.

(Results)

1. Production of pro-inflammatory cytokines, IFN-g, IL-6 and IL-17, in the stimulated spleen cells was significantly reduced; regulatory cytokines, IL-4 and IL10, was almost unchanged; IL-2 was significantly enhanced, by BP at concentrations sustaining high viability (80%). These effects on the modification of the cytokine production were similarly triggered by artepillin C, a major component of BP and also an agonist of PPARg (Figure 1)
2. GW9662 and artepillin C synergistically enhanced the production of IL-2, IL-4 and IL-10, while, suppressed that of IFN-g, IL-6 and IL-17 in the stimulated spleen cells (Figure 2).
3. The expression of *Osterix* mRNA in 10T1/2 cells was significantly elevated, while that of *Runx2* was unchanged in the presence of BP and GW9662 (Figure 3).

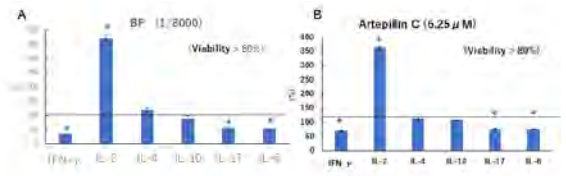

Figure 1. Comparison for varied effects of BP and artepillin C on the cytokine productions in anti CD3 antibody-stimulated mouse spleen cells.

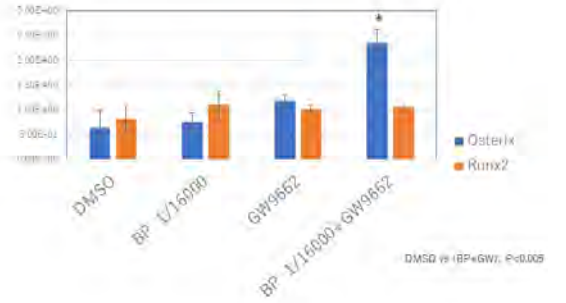

Figure 3 Expression of *Osterix* and *Runx2* mRNAs in 10T1/2 cells treated with BP and GW9662. DMSO denotes control.

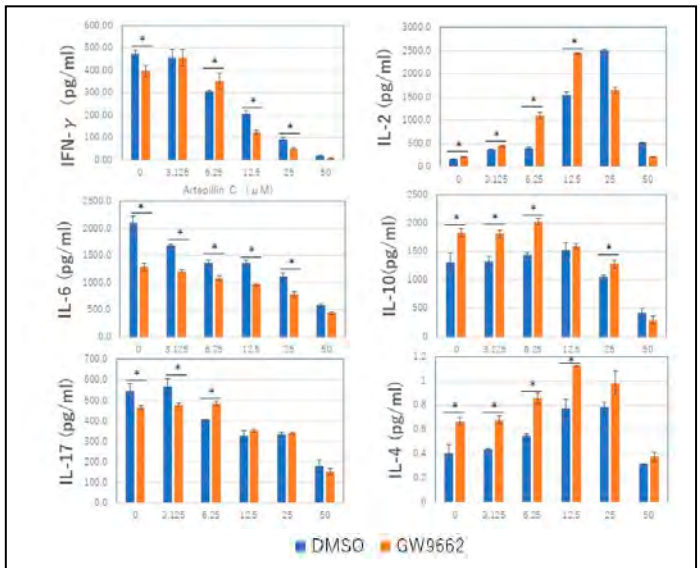

Figure 2 Effects of GW9662 on the cytokine production in anti CD3-antibody-stimulated spleen cells treated with varied amounts of artepillin. As a control, only solvent (DMSO) was added.

(Conclusions)

1. Artepillin C, a major component of BP, and GW9662 could curatively regulate pro-inflammatory and regulatory cytokines.
2. BP and GW9662 might induce osteoblastgenesis in premature stromal cells.
3. These properties of BP and GW9662 suggest that the combination usage could be a remedy for dental pulp treatment by reducing inflammation and enforcing dentin formation.

Immunolocalization of podoplanin-reactive/PHOSPHO1-positive osteoblasts in murine femora with intermittent administration of parathyroid hormone

北海道大学歯学部 5年生 Hokkaido University School of Dental Medicine Class of 2022

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Osteoblasts on the bone surface are known to synthesize the bone matrix, in which they gradually become embedded to differentiate into osteocytes. In this study, the speaker has attempted to clarify the issues; 1) whether osteoblasts' differentiation into osteocytes takes place simultaneously with osteoblastic bone mineralization in a normal state, and 2) whether the distribution pattern of PHOSPHO1-positive bone-mineralizing osteoblasts and podoplanin-positive osteoblasts ready to differentiate into osteocytes are changed after the intermittent administration of parathyroid hormone (PTH). In order to verify the issues, the speaker has examined the murine femoral metaphyses received vehicle or human PTH by immunohistochemical and statistical analysis. As a result, the distribution of podoplanin-positive osteoblasts and PHOSPHO1-reactive osteoblasts is not co-localized in the control mice. In contrast, many podoplanin-positive osteoblasts and PHOSPHO1-reactive osteoblasts were observed in the PTH administered bone, implicating accelerated osteoblast differentiation into osteocytes and osteoblastic bone mineralization. Furthermore, double staining of PHOSPHO1 and podoplanin clearly demonstrated the co-localization of PHOSPHO1-positive and podoplanin-reactive osteoblasts in the PTH-administered secondary trabeculae but not the primary trabeculae. Thus, intermittent PTH administration appears to stimulate osteoblasts' bone mineralization and their differentiation into osteocytes, inducing synchronous bone mineralization and osteocytic differentiation in the secondary trabeculae.

副甲状腺ホルモン間歇投与マウス大腿骨におけるpodoplanin/PHOSPHO1陽性骨芽細胞の免疫局在

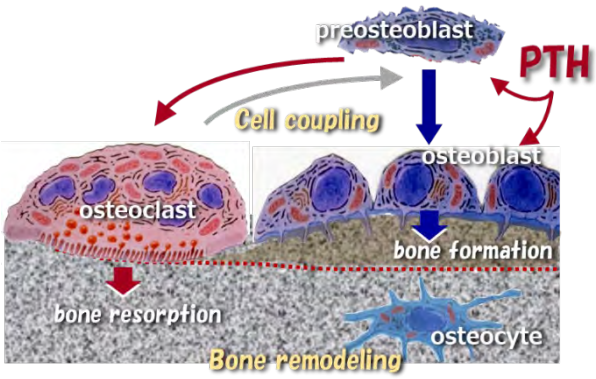
骨芽細胞は、骨基質形成・石灰化を営む一方、骨細胞へ分化する。本研究は、正常状態で骨芽細胞による骨基質石灰化と骨細胞分化が同時に起こるのか、また、骨代謝回転の上昇時に、骨芽細胞の石灰化基質合成と骨細胞分化のタイミングが正常状態と同様に生じるのかを明らかにする目的で、PTH間歇投与による高骨代謝回転状態のマウス大腿骨を解析した。その結果、コントロールマウスでは、骨基質石灰化を行うPHOSPHO1陽性骨芽細胞と、骨細胞に分化しつつあるpodoplanin陽性骨芽細胞の局在が一致せず、骨芽細胞による骨基質石灰化と骨細胞分化は異なるタイミングで生じていることが示唆された。一方、PTH間歇投与マウスでは、多くの骨芽細胞でPHOSPHO1とpodoplaninの陽性反応を認めた。また、一次骨梁では骨芽細胞の基質石灰化と骨細胞分化は一致しなかったが、二次骨梁のモデリング領域では骨芽細胞の基質石灰化と骨細胞分化は一致していた。以上から、PTH間歇投与は、骨芽細胞による骨基質石灰化と骨細胞分化を促進させるとともに、二次骨梁の骨芽細胞における骨基質石灰化と骨細胞分化を同時に誘導してゆく可能性が推測された。

研究発表内容の紹介

本研究は、骨芽細胞の細胞機能である骨基質形成・石灰化と骨細胞への分化という異なる現象がどのようなタイミングで生じているのか、また、これらは骨代謝回転に影響を受けるのか、という点に着目し解析を行いました。本研究のような骨代謝に関する基礎研究は、歯周組織再生治療など様々な歯科臨床における治療法・予防法開発の基盤として、歯科医学の発展に大きく寄与するものと考えています。（ファカルティー・アドバイザー：長谷川 智香）

Immunolocalization of podoplanin-reactive/PHOSPHO1-positive osteoblasts in murine femora with intermittent administration of parathyroid hormone

【Problem】

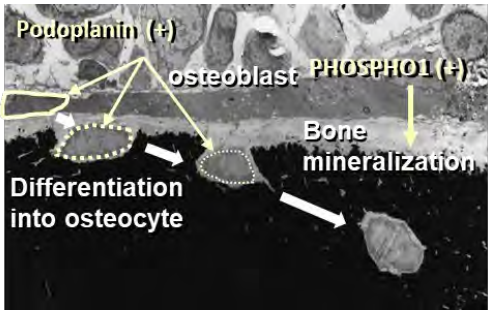


Osteoblasts are known to synthesize bone matrix, in which they are gradually embedded to differentiate into osteocytes. The research questions to be resolved are: 1) whether osteoblasts' differentiation into osteocytes takes place simultaneously with osteoblastic bone mineralization in a normal state, and 2) whether the distribution of bone-mineralizing osteoblasts and osteoblasts that are ready to differentiate into osteocytes is changed after administration of parathyroid hormone, or PTH which accelerates bone remodeling. PHOSPHO1 can be a hallmark of bone-mineralizing osteoblasts, while podoplanin is a cell surface marker of the osteoblasts that are ready to differentiate into osteocytes.

【Hypothesis】

Regarding the abovementioned issues, the presenter hypothesizes the following:

1) The distribution of PHOSPHO1-reactive osteoblasts and podoplanin-positive osteoblasts is variable in a normal state, indicating that bone mineralization by osteoblasts and their differentiation into osteocytes occur in different stages in a normal state.
2) After intermittent PTH administration, osteoblasts show both PHOSPHO1 reactivity and podoplanin positivity, given that the high bone turnover driven by PTH shortens the periods of bone mineralization and subsequent osteoblastic differentiation into osteocytes.



【Methods】

Six-week-old C57BL/6J mice received vehicle (control group, N = 6) or 20 μg/kg/day of human PTH [1-34] (hPTH; PTH group, N = 6) two or four times per day for two weeks. The primary trabeculae and secondary trabeculae of femoral metaphyses were employed for histochemical examination and statistical analyses as follows.

1) Immunohistochemical analyses of alkaline phosphatase (ALP), PHOSPHO1, and podoplanin
2) Calcein labeling for bone mineralization
3) RT-PCR for PHOSPHO1, ALP and so forth
4) Statistical analysis on the number of podoplanin-reactive osteoblasts, and the index of PHOSPHO1-positive osteoblasts/bone volume (one-way ANOVA followed by Tukey–Kramer multiple comparisons test)

【Result】

1) Osteoblasts' differentiation into osteocytes does not take place simultaneously with osteoblastic bone mineralization in a normal state

In a normal state, the different distribution of the brown colored PHOSPHO1 and podoplanin were seen in the femora metaphysis (Fig. 1). The distribution of podoplanin-positive osteoblasts and PHOSPHO1-reactive osteoblasts is not co-localized both in the primary and secondary trabeculae in a normal state, as hypothesized.

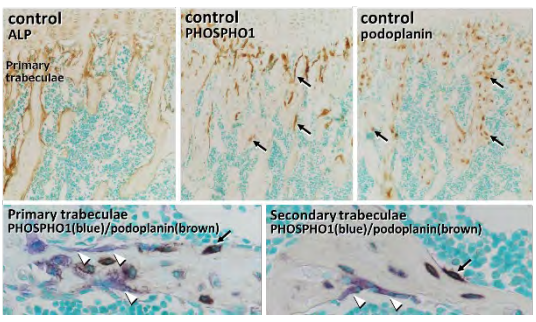


Fig. 1

2) PTH appears to promote bone mineralization and osteoblastic differentiation into osteocytes; however, synchronous bone mineralization and osteocytic differentiation by osteoblasts take place in the secondary trabeculae but not the primary trabeculae

After PTH administration, many podoplanin-reactive osteoblasts (brown) and PHOSPHO1-positive osteoblasts (blue) can be seen in the primary and secondary trabeculae (Fig. 2). The statistical analysis revealed a significant increase in the number of podoplanin-positive osteoblasts in PTH administered bones compared with the control bone (Fig. 3). RT-PCR results showed increased mRNA encoding PHOSPHO1 (Fig. 3). Interestingly, PTH administered bone exhibited not only short ranges but also long span of osteoblastic layer (arrows, Fig. 4). The long span of calcein-positive, alkaline phosphate-reactive and PHOSPHO1-positive osteoblasts were seen in the secondary trabeculae, but not the primary trabeculae in PTH administrated bone. This means that the bone formation phase is expanded in the secondary trabeculae following PTH administration, despite PTH-administered bone showing frequent bone remodeling. In addition, double staining of PHOSPHO1 and podoplanin clearly demonstrated the co-localization of PHOSPHO1-positive and podoplanin-reactive osteoblasts in the PTH-administered secondary trabeculae but not the primary trabeculae (Fig. 2). Thus, PTH appears to stimulate bone mineralization and osteoblastic differentiation into osteocytes. However, synchronous bone mineralization and osteocytic differentiation by osteoblasts take place in the secondary trabeculae but not the primary trabeculae.

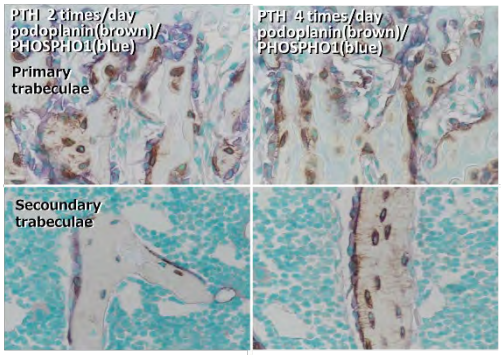


Fig. 2

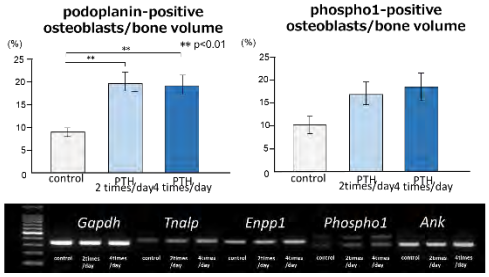


Fig. 3

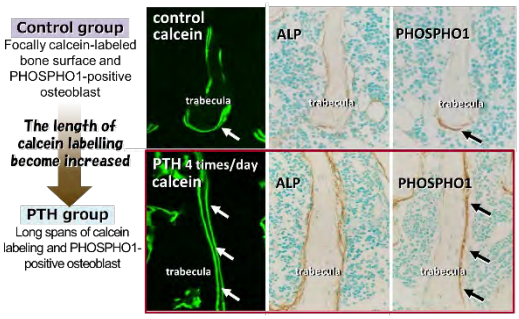
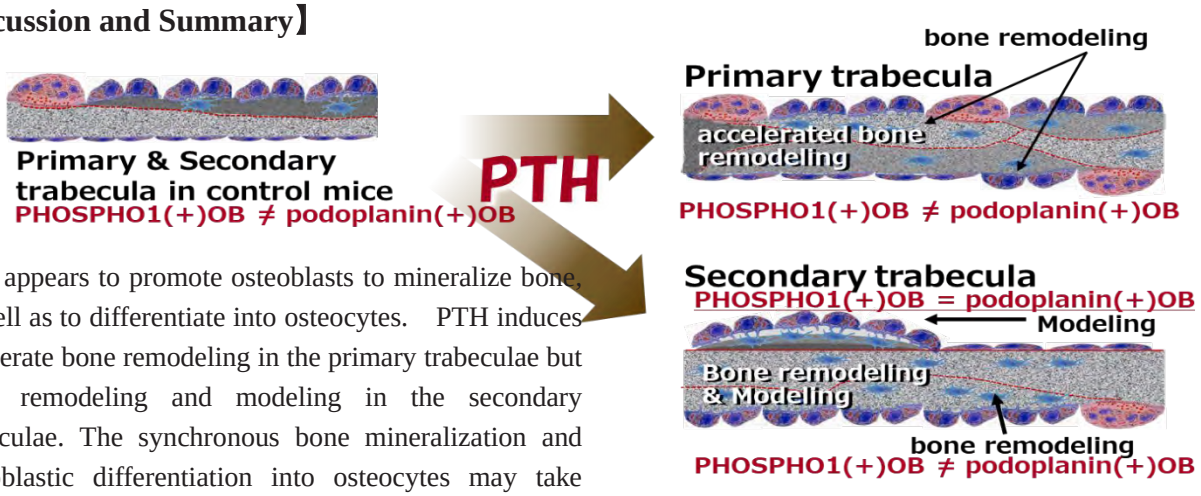


Fig. 4

【Discussion and Summary】



PTH appears to promote osteoblasts to mineralize bone, as well as to differentiate into osteocytes. PTH induces accelerate bone remodeling in the primary trabeculae but bone remodeling and modeling in the secondary trabeculae. The synchronous bone mineralization and osteoblastic differentiation into osteocytes may take place in the modeling sites of the secondary trabeculae.

【Conclusion】

Intermittent PTH administration appears to stimulate osteoblasts' bone mineralization and their differentiation into osteocytes, inducing synchronous their bone mineralization and osteocytic differentiation in the secondary trabeculae.

Development of a new dental disinfectant containing peracetic acid for oral care of the elderly

大阪歯科大学　3年生　Osaka Dental University　Class of 2024

栗山 実久　Miku KURIYAMA

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欠損歯列補綴咬合学講座 助教　張 泓灝
中央歯学研究所 助教 神田 龍平

Introduction: With the rapid increase in the number of people requiring nursing care worldwide, development of novel oral care materials that circumvent aspiration pneumonia because of high viscosity, bactericidal activity, and safety could alter nursing care fields. In this study, we prepared a prototype of peracetic acid (PA)-cellulose nanofiber (CNF) slurry, and conducted material, bactericidal, and cytotoxicity evaluations to preliminarily examine the optimal mixing conditions for the development of novel oral care materials.
Results: The results of Fourier transform infrared spectroscopy, X-ray diffraction measurements, and scanning electron microscopy confirmed that the material used was CNF, with peaks specific to CNF and fibrous material with a width of 50 nm. All PA-CNF slurries showed a white color, evading any esthetic problems. The viscosity of slurries increased in a dose-dependent manner of CNF. The PA solution is generally acidic, whereas the PA-CNF with 20 ppm PA and CNF dissolved in phosphate-buffered saline showed a neutral pH that was above the critical pH. In addition, the PA-CNF slurry containing 20 ppm PA and 500 mg/mL CNF showed high bactericidal activity against Staphylococcus aureus and high cytocompatibility against oral gingival epithelium-derived cell line Ca9-22.
Conclusion: PA-CNF slurry containing an optimal mixture of PA and CNF, may be a candidate for the development of a novel oral care material with high viscosity, bactericidal activity, safety, and esthetics.

高齢者の口腔ケアのための過酢酸を含む新しい歯科用消毒剤の開発

【目的・方法】要介護者の世界的な急増の中、誤嚥性肺炎を誘発しない高粘性・高殺菌性・高安全性の口腔ケア材料は、介護現場に変革をもたす材料となりうる。本研究では、将来的な新規口腔ケア材料の創製に向け、過酢酸（PA）とセルロースナノファイバー（CNF）の混合物を試作し、材料評価、殺菌実験、細胞毒性評価を行い、最適混合条件を予備的に検討した。
【結果】フーリエ変換分光測定、X線回折測定、走査型電子顕微鏡観察を行った結果、CNF特有のピークと、50nm幅の繊維物質を確認し、使用した材料がCNFである事を確認した。マクロ観察において全てのPA-CNFが白色を示し、審美的問題点は観察されなかった。更に、粘性試験を行った結果、CNFの増加に応じ粘性が増加した。更に、PA溶液は酸性を示すが、20ppmPAおよびCNFをリン酸緩衝食塩水（PBS）に溶解させたPA-CNFでは、臨界pHを超え中性pHを示した。また、20ppm PAと500mg/mL CNFを含むPA-CNFにおいて、*Staphylococcus aureus*に対して高い殺菌性を、口腔歯肉上皮由来細胞株Ca9-22に対し高い細胞親和性を示す結果を得た。
【結論】PAとCNFを最適混合させたPA-CNFスラリーは、高粘性を具備しつつ、高殺菌性、安全性、審美性を担保した新規口腔ケア材料候補となる可能性が示唆された。

研究発表内容の紹介

超高齢化社会の今、要介護者や高齢者の口腔内環境が悪化し、誤嚥性肺炎等の全身疾患に陥るケースが散見される。本研究では、歯科領域では馴染みの薄い消毒剤の過酢酸と、SDGsに繋がるセルロースナノファイバーを用いて、誤嚥性肺炎を回避しうる高粘性の新規口腔ケア材料の開発に取り組んでいる。将来的に製品が完成した暁には、日常のケアを担う家族や介護職員などの負担軽減に大きく繋がり、口腔ケア領域に貢献しうる。（ファカルティー・アドバイザー：本田 義知）

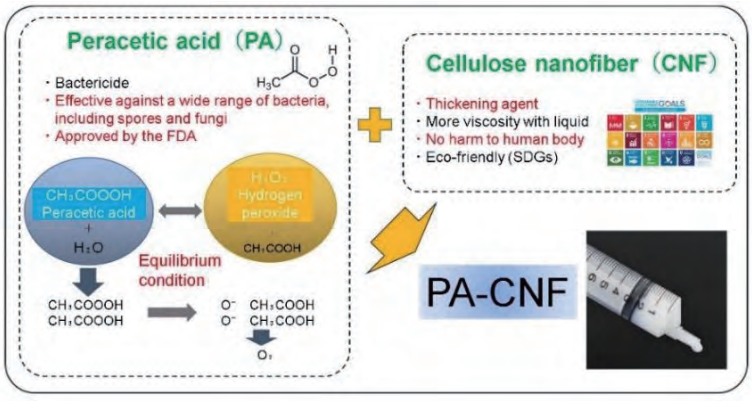
Development of a new dental disinfectant containing peracetic acid for oral care of the elderly

【Problem】

In a super-aging society, it is challenging to maintain a clean oral environment for the rapidly increasing number of people who are bedridden and patients under nursing care. Nevertheless, caregivers and family members mainly focus on systemic management and excretion control. Oral care is limited to simple treatment, resulting in deteriorated oral environments that leads to a decline in oral function, inducing systemic frailty in patients. In addition, aspiration of plaque or bacteria occasionally leads to aspiration pneumonia, which directly elevates mortality risk. Elderly people are at higher risk of aspiration because of their reduced swallowing and spitting functions. Therefore, developing novel oral care methods to circumvent aspiration pneumonia is an urgent and essential issue.

Thus far, materials containing disinfecting ingredients such as chlorhexidine, benzethonium chloride, and isopropyl methyl phenol have been commercially available for use as oral care materials. However, many of these materials are in a low-viscosity state, and aspiration has to be induced. Therefore, highly functional oral care materials with high viscosity are urgently required. Peracetic acid (PA) is a disinfectant that disrupts bacterial cell membrane through its oxidizing effect. The molecule, having a broad spectrum against various pathogenic microorganisms, has also been proven safe when swallowed because it has already been approved for sterilization of food surfaces at concentrations below a certain level. However, PA alone has a low viscosity, which is why it may lack the ability to be retained in the affected area. Meanwhile, cellulose nanofiber (CNF) is a biomass material prepared by refining wood to nano-order. The combinatory use of this fiber and other materials can change material properties. In particular, highly concentrated cellulose nanofibers can work as a thickening agent. In addition, cellulose nanofibers have attracted wide attention as a material contributing to the SDGs, which is a global goal, owing to their low environmental impact on productivity and disposal.

【Hypothesis】



We hypothesized that the combinatory use of CNF and PA solution would significantly improve the viscosity of PA solution and can be applied as an oral care material with high maneuverability, retention in the affected area, anti-bactericidal effect, and safety and would prevent the risk of aspiration pneumonia. This study was designed to preliminarily investigate the optimal mixing conditions of PA and CNF to develop a new oral care material in the future.

【Methods】

In general, to develop a new oral care material, achieving the following goals is necessary. 1) material preparation and evaluation, 2) bactericidal and biocompatibility experiments, 3) biosafety tests, and 4) clinical testing. In this study, we have performed stages 1) and 2).

1: Preparation and material evaluation of PA-CNF slurry

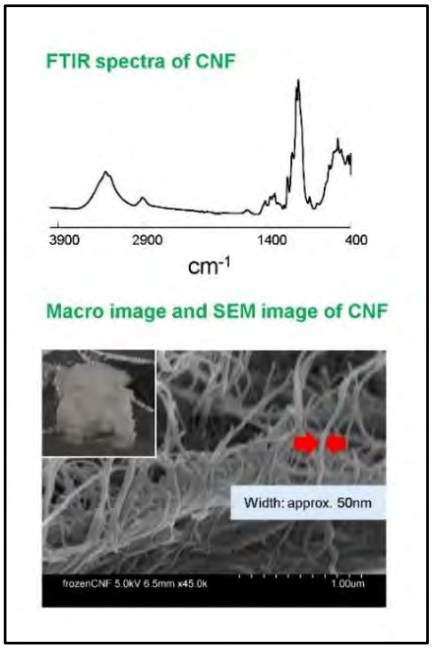
PA (Kanto Kagaku Co.) and CNF (BinFis-S, Moly Machinery Co.) were mixed in phosphate buffer saline (PBS) at various ratios to prepare PA-CNF slurries. The properties of each material were evaluated using a Fourier transform infrared spectrophotometer (IRAffinity-1s, Shimadzu), an X-ray diffractometer (XRD-6000, Shimadzu), a scanning electron microscope (S-4800, Hitachi), a pH meter (Metteler Toledo), and a thickening measuring plate (Saraya).

2: Bactericidal experiment

Staphylococcus aureus (ATCC 12600) was used to evaluate the bactericidal effect of PA-CNF slurry. Its bactericidal effect was evaluated using the bacterial community calculation method (CFU calculation) and other methods.

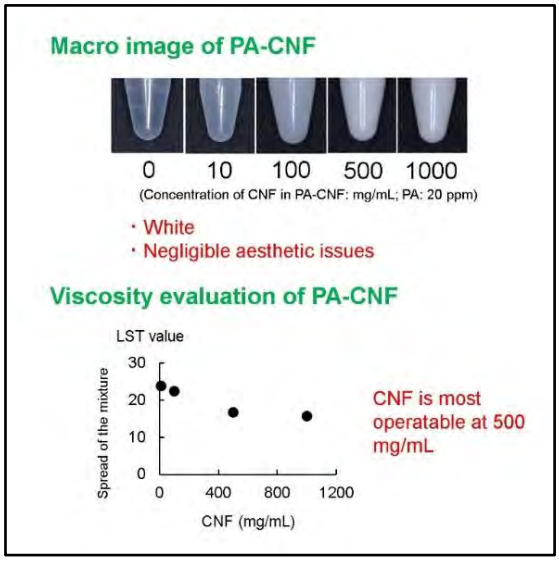
3: Cytotoxicity test for cells

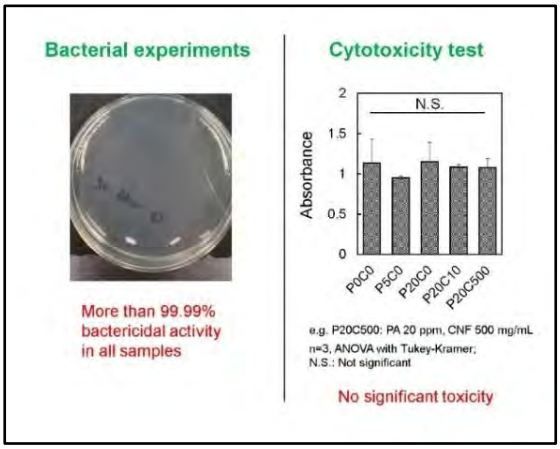
Ca9-22, an oral-derived epithelial cell line, was used to evaluate the toxicity of the PA-CNF slurry. After the cell seeding, PA solution or PA-CNF slurry was administrated at various doses. Cytotoxicity was estimated using the WST-8 kit.



【Results and discussion】

FTIR and XRD measurements revealed the specific peaks of CNF, confirming that the used material was cellulose. SEM observation showed a thin fibrous material with a width of 50 nm, confirming that the material was a nano-sized cellulose fiber (Fig at the top). Macroscopic observation revealed that the mixture of 20 ppm PA and different concentrations of CNF showed a white color, indicating only minor esthetic problems in case residue remained (Fig at the middle). In the simple viscosity test using a thickening measuring plate, PA solutions containing low concentrations of CNF showed high line spread test (LST) values (high value means low viscosity), while PA-CNF slurry exceeding 500 mg/mL showed a decrease in LST value, indicating that an increase in CNF concentration attenuates the spread of PA. Intact PA solution is acidic, potentially causing acid demineralization of the tooth surface when diluted with ultrapure water. Consequently, we used PBS as a solvent. The pH of PA-CNF slurry containing 20 ppm PA was higher than the critical pH and was maintained at a neutral value. The presence of CNF had no effect on the pH. Although PA is a substance that exhibits an acetic acid odor similar to that of vinegar, the odor was hardly perceptible in the PA-CNF slurry containing PA at a low concentration of about 20 ppm PA. The results of bacterial experiments showed that the PA-CNF slurry containing 20 ppm PA or higher had an antibacterial rate of more than 99.99% within 180 seconds (Fig at the bottom). As for the cytotoxicity test, there was no apparent cytotoxicity to the oral-derived epithelial cell line Ca9-22 at the range of conditions of PA-CNF slurry used in the study.





【Conclusion】

We demonstrated that PA-CNF slurries containing PA and CNF could be a promising candidate for use as an oral care material that shows high viscosity, high bactericidal activity, and safety. We are planning to conduct additional experiments in the future to elucidate further the properties of the slurries, such as antibacterial spectrum, safety for different cells, stability test, and the bactericidal effect on the tooth surface, to prove its usefulness as an oral care material.

Single-cell genomics approach for identification of antimicrobial resistance genes and virulence factors in oral bacteria

大阪大学歯学部 4年生 Osaka University School of Dentistry Class of 2023

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Objective/Methods: Recent studies have presented findings showing that the oral condition of an individual is related to systemic lesions, such as those associated with diabetes, arteriosclerosis, and rheumatoid arthritis, which has resulted in acceleration of oral microbiome studies. For the present investigation, single-cell genome sequencing of saliva collected from a healthy donor was performed. The genome sequences thus obtained enabled identification of the distribution of antimicrobial genes as well as genes encoding virulence factors.
Results: Bacterial cells were isolated and cultured in 96 wells, of which 88 showed genomic amplification, resulting in production of genome sequences. Most of the genera were *Streptococcus*, followed by *Prevotella* and *TM7x*. Furthermore, there was evidence of antimicrobial resistance genes containing *cfxA*, *ermF*, and *tet*, and virulence factors containing *cps4, lbpA, nanB, pavA*, and *psaA*.
Conclusion: The present results indicated the high reliability of single-cell analysis of oral bacteria. A single bacterium was found to contain multiple resistance genes, while five streptococcal species possessed multiple virulence factors. In addition, genetic diversity in each of the bacterial cells was noted, even in a case of bacteria colonized from the same host and belonging to the same genus. The present collaborative approach for bacterial single-cell analysis and bioinformatics may be a useful model to clarify the distribution of antimicrobial resistance genes and virulence factors in individual bacterial cells.

シングルセル解析によって得られた口腔細菌のゲノム情報を用いた耐性遺伝子と病原因子の探索

【目的・方法】 近年、微生物叢が全身疾患に関連するとともに重要な役割を果たすことが報告され、口腔微生物叢の解析が活発に行われている。本研究では、健常人の唾液を用いた細菌シングルセル解析を行い、一菌体ごとのゲノム配列を解読するとともに、耐性遺伝子と病原因子の分布を検索した。

【結果】 96 wellに菌体をシングルセル分離し、88 wellでゲノムの増幅と配列の解読がなされた。得られた菌の分類を行ったところ、*Streptococcus*属や*Prevotella*属、*TM7x*属が多く検出された。また、耐性遺伝子として*cfxA*、*ermF*、*tet*、病原因子として*cps4*、*lbpA*、*nanB*、*pavA*、*psaA*を保有している菌体が存在した。

【結論】 本結果から、口腔細菌叢のシングルセル単離が良好に行われることが示唆された。複数の耐性遺伝子を保有する菌体は1菌体のみであるのに対し、複数の病原因子を保有する菌体は5菌体でその全てが*Streptococcus*属であった。
本研究から同じ属の菌体でも異なる遺伝子分布を示すこと、シングルセル解析技術と情報解析手法の組み合わせによって菌体ごとの耐性遺伝子と病原因子の分布を解析できることが示された。

研究発表内容の紹介

現在、細菌叢解析で主に用いられている解析では、個別の菌がどのような遺伝子を持っているか解明することができない。
本研究では、唾液を用いた細菌シングルセル解析によって、口腔細菌の個別のドラフトゲノム配列を決定するとともに、耐性遺伝子や病原因子の分布を同定した。これらの情報は、口腔細菌叢の情報から全身疾患に及ぼす影響や、関連する全身疾患の新たな予防策や治療法を探索する基盤となると考えられる。（ファカルティー・アドバイザー：山口 雅也）

Single-cell genomics approach for identification of antimicrobial resistance genes and virulence factors in oral bacteria

(Problem)

Oral health literacy has become recognized as an important determinant of health. As for dental caries, prevalence is limited in Japan, with over 50% of individuals aged 80 years possessing 20 or more teeth, as most seniors receive appropriate dental consultation and/or care. Recent studies have highlighted that oral condition is related to systemic lesions such as those associated with diabetes, arteriosclerosis, and rheumatoid arthritis, as well as local lesions, findings that have led to acceleration of oral microbiome studies. Human saliva is estimated to contain 1×10^9 CFU/mL bacteria, including over 700 species. However, most oral bacteria cannot be cultured, making them difficult to study. Major metagenomics approaches, such as 16S rRNA amplicon and metagenome shotgun sequencing, can provide a snapshot of microbial composition or the complete genomic content of a microbial community, though the genetic distribution in individual bacterial strains still remains unknown.

(Hypothesis)

In this study, single-cell genome sequencing of saliva collected from a healthy donor was performed. Genome sequences enable identification of microbial composition details, as well as the distribution of antimicrobial genes and genes encoding virulence factors. Such information provides a base to explore the relationship between the oral microbiome and systemic lesions, leading to establishment of novel preventive measures and drugs for oral microbiome-related diseases.

(Methods)

After fasting for more than 30 minutes in the morning, 5 mL of saliva was collected from a healthy donor. One mL was added to OMNIgene ORAL solution, which inactivates but stabilizes bacterial cells, and then stored at room temperature until single-cell analysis. To investigate the presence of live bacteria, another 3 mL of saliva was centrifuged at 8000 rpm for 10 minutes, suspended in 800 µL of 50% glycerol/RPMI1640 solution, and stored at -30˚C until single-cell analysis. Using 48 wells, single cell isolation, genome amplification, and paired-end genome sequencing of both saliva samples were performed by bitBiome. Quality control and preprocessing of FASTQ files obtained from next-generation sequencing were performed using fastp, v.0.20.0. Antimicrobial resistance gene and virulence factor profiling were determined with the cleaned sequencing data using ARIBA, 2.14.4. Bacterial species were identified by use of the Genome Taxonomy Database (GTDB) and GTDB-Tk.

(Results)

Bacterial cells in the two samples were isolated in a total of 96 wells, of which 88 showed genomic amplification, resulting in production of genome sequences. Findings showed that 36 were the genus *Streptococcus*, 8 were *Prevotella*, 7 were *TM7x* (a Candidatus Saccharibacteria bacterium), 6 were *Gemella*, 5 were *Fusobacterium*, 5 were *Leptotrichia*, 3 were *CAG-793* (of the *Clostridiales* order), 3 were *Rothia*, 2 were *Eubacterium*, 2 were *Lachnoanaerobaculum*, 2 were *Latonella*, 2 were *Neisseria*, 2 were *F0040* (of the *Bacteroidaceae* family), 1 was *Mogibacterium*, 1 was *Metamycoplasma*, 1 was *Granulicatella*, and 2 were unknown. The *Streptococcus* genus was the most abundant (41.0%), followed by *Prevotella* (9.1%) and *TM7x* (8.0%).

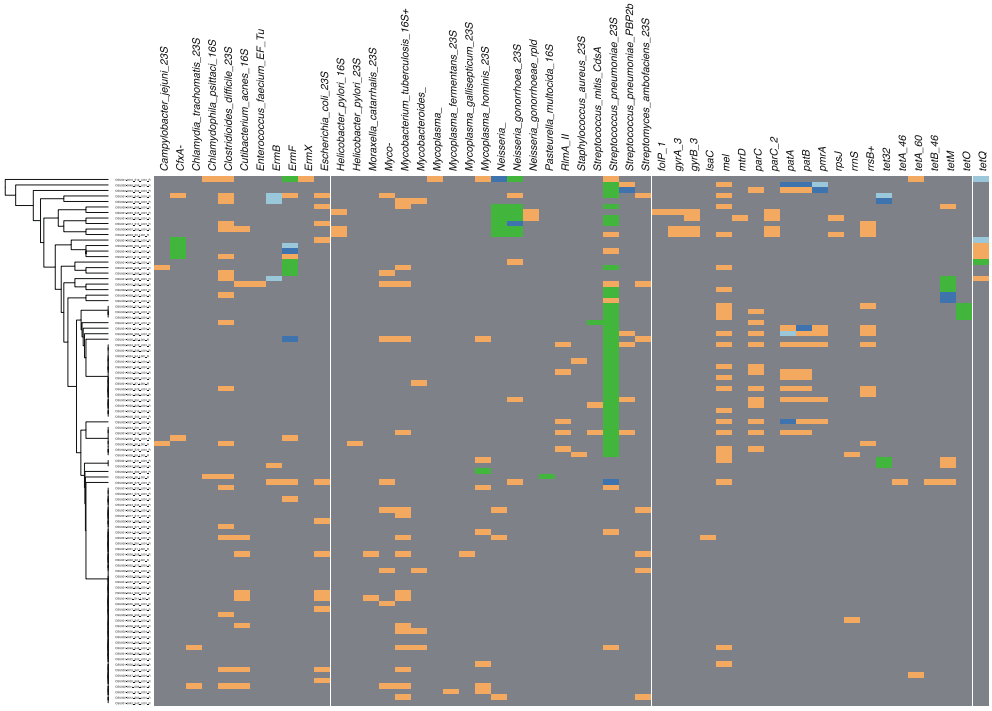

Figure 1. Burden of antimicrobial resistance genes in single-cell isolated bacteria.

Green, light blue, blue, orange, and gray colors indicate matches to reference, interrupted, fragmented, partial, and lacking genes, respectively. The clustering tree was generated by ARIBA based on gene distribution. Graphical data was obtained using Phandango.

In addition, obtained sequence data of 41 (46.6%) of the bacteria genera covered >80% of the reference genome, while 16 showed >90% completeness.

As for antimicrobial resistance genes (Fig. 1), four bacteria possessed *cfxA* encoding β-lactamase, while another four possessed *ermF*, which confers erythromycin-resistance. Moreover, regarding tetracycline resistance, two bacteria possessed *tet32*, three had *tetM*, three had *tetO*, and one had *tetQ*. In total, nine possessed tetracycline resistance genes.

Next, virulence factors were explored. Three bacteria possessed pneumococcal polysaccharide capsular genes; two with *cps4J*, *cps4K*, and *cps4L*, and the other with *cps4J* and *cps4L*. In addition, one was found to have *nanB* encoding pneumococcal sialidase, one *pavA* encoding pneumococcal fibronectin-binding protein, and eight *psaA* encoding the pneumococcal ABC transporter functioning as an adhesin. The *Neisseria* genus possessed two types of resistance genes, *ermF* and *tetQ*, and five *Streptococcus* genera showed pneumococcal virulence factors.

(Conclusion)

The present results indicated that bacterial single-cell genome sequencing can identify the burden of antimicrobial resistance genes and virulence factors in individual bacteria present in oral flora. Since the present samples in 88 of 96 wells showed genomic amplification and produced genome sequences, it was concluded that single-cell analysis of oral bacteria has high reliability. The obtained oral bacteria were classified into 16 genera, with the most abundant found to be *Streptococcus*, followed by *Prevotella* and *TM7X*. Genome profiling of the oral flora of the healthy donor also revealed several bacteria possessing antimicrobial resistance genes, whereas only one of the bacteria found in the 88 wells contained multiple resistance genes. On the other hand, five streptococcal species showed multiple virulence factors, which suggested that oral streptococci share virulence factors with *S. pneumoniae*. Interestingly, other genus bacteria examined in this study did not possess known virulence factors. Single-cell analysis indicated genetic diversity in bacterial cells, even when bacteria colonized the same host and belonged to the same genus.

A collaborative approach using bacterial single-cell analysis and bioinformatics is suggested be a useful model for clarification of the distribution of antimicrobial resistance genes and virulence factors among bacterial cells present in saliva of a healthy donor.

Clinicopathological study on odontogenic keratocysts

広島大学歯学部　5年生　Hiroshima University Faculty of Dentistry Class of 2022

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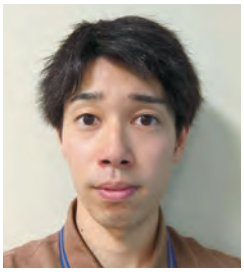


Odontogenic keratocyst (OKC), which has local aggressiveness and high recurrent rate, had been classified into benign odontogenic tumor in 2005 WHO classification. And it has moved back to odontogenic cyst again in 2017 WHO classification. However, it has not yet been concluded whether OKC includes lesions with neoplastic nature or not. In the present study, I examined clinicopathologic features of OKC to clarify the important recurrent-related factors and the presence of OKC with neoplastic nature.
Syndromic OKCs are 5.1%, frequently occur in young generation, and show high recurrent rate.
The recurrence rate of non-syndromic OKC cases was about 37%, and the recurrence rate was relatively high. Factors, which are likely to induce recurrence include multilocular lesion, atypia in the basal cell layer, budding suggesting high proliferative activity, and the presence of daughter cysts. Although careful removing is required, most cysts are considered to be non-neoplastic cystic disease. In addition, continuous follow-up observation is required for 3 years after surgery.
Two possibilities occur 1) some OKCs recur as they have a primarily inherent neoplastic potential and some OKCs recur due to some secondary changes in lining epithelium leading to tumor-like solid growth.

歯原性角化嚢胞の臨床病理学的検討

歯原性角化嚢胞（OKC）は、他の嚢胞では見られない侵襲性性格や高い再発率を示す事から、2005年のWHO分類では角化嚢胞性歯原性腫瘍として良性歯原性腫瘍に分類されたが、2017年のWHO新分類では、再び嚢胞として扱われるようになった。しかしながら腫瘍性性格を持つOKCの存在については、未だ結論が出ていない。そこで、私はOKCの再発に関係する臨床病理学的な所見を検討し、再発に関わる所見や腫瘍性性格を持つ症例の存在を明らかにすることを目的とした。1973年から2018年までの間に当大学病院を受診、加療したOKC及び角化嚢胞性歯原性腫瘍のうち、加療後3年の経過観察が可能であった334症例を対象とした。症候性OKCは 5.1%で、若年者に多く、高い再発率を示した。非症候性OKCの再発率は37%程度で、比較的高いが、多房性のレントゲン像、基底細胞の異型性や増殖活性、嬢嚢胞の存在が再発と関わる傾向を示した。ほとんどの嚢胞が非腫瘍性であるが、注意深く切除し、術後3年間は定期的な経過観察を行う必要がある。例外として、初めから裏層上皮の核分裂像や異型性を示す症例や再発時に扁平上皮胞巣の充実性増殖が見られた症例が含まれており、腫瘍性性格を有するOKCが存在する可能性が示唆された。

研究発表内容の紹介

良性腫瘍として分類されていたOKCが歯原性嚢胞に再分類され、腫瘍性性格を持つOKCの存在の可能性は未だ明らかでありません。本研究は広島大学症例344例の臨床病理的な所見を解析し、摘出あるいは開窓後摘出の術式で丁寧に対応すれば再発率に大きな違いはないこと、処置後、3年間経過観察を行い、再発に留意する必要があることが示された。また、まれに腫瘍性の性格を示す症例が含まれることも呈示できた。歯科臨床に有益と考える。（ファカルティー・アドバイザー：宮内 睦美）

Clinicopathological study on odontogenic keratocysts

(Problem)

Odontogenic keratocysrt (OKC) is jaw cyst originated from odontogenic epithelium. It has characteristic lining epithelium, which consist of thin parakerainized squamous epithelium with flat basement membrane and palisading basal cell layer. It is well accepted that OKCC has relatively high rate of recurrence, potentially destructive growth, and relatively high potential of proliferation, supporting for neoplastic nature unlike other jaw cysts. So, OK was classified into benign odontogenic tumors under the name of ketatinizing cystic odontogenic tumor. In the new WHO classification in 2017, it reclassified into cyst category, because most of OKCs show an indolent behavior like non-neoplastic lesions. However, it has not yet been concluded whether all cases of OKC can be classified as cysts or it includes lesions with neoplastic nature.

(Hypothesis)

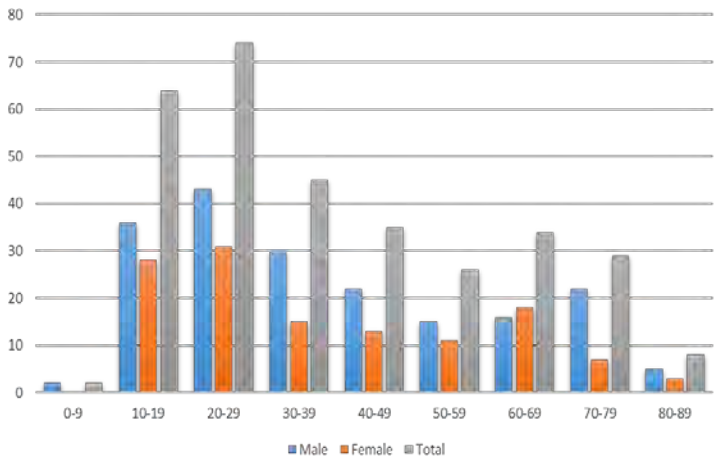
In my department, histopathological examination specimens of university hospital for more than 40 years are accumulated, and many histopathological specimens of OKCs are available. In addition, dental radiology and oral surgery have a collection of x-ray findings and clinical data of OKC cases. Therefore, by surveying the clinical and pathological data of OKCs, I will clarify the recurrent-related clinicopathological factors and will consider whether all OKCs are just cystic diseases without neoplastic nature, describing in the current WHO classification or not.

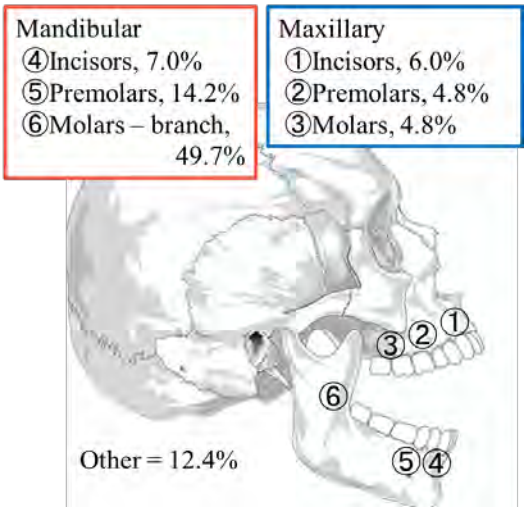
(Methods)

The study protocol is approved by ethic committee of my university. We enrolled 334 cases of OKCs and keratinizing cystic odontogenic tumors with more than 3-years follow-up period of my university hospital records. Firstly, I investigated relationships between OKCs with and without basal cell nevus syndrome and recurrence. Moreover, in nonsyndromic OKCs, relationships between recurrence, and clinicopathologic features, X-ray finding, treatment and pathological findings are observed.

(Results and discussion)

OKC cases include syndromic OKCs of cases and nonsyndromic OKCs of cases. Recurrent rate are % and %, respectively. Nonsyndromic OKCs include 191 male and 126 female, the ratio of male/female is 1.5:1. Average age is 37 years old (range from 9 to 87. There is no relationship among sex, age and recurrence.





A total of 317 patients(191 male and 126 female) with OKCs were evaluated in this study. The male: female ratio was 1.5:1. Range was 9-87 years. The mean age was 37 years. Mandibular molar to ramus area is most prevalent site. Recurrent duration range is from 3 months to 24 years and 80% recurrences occurred within 3 years. So, continuously follow-up is needed during 3years after operation.

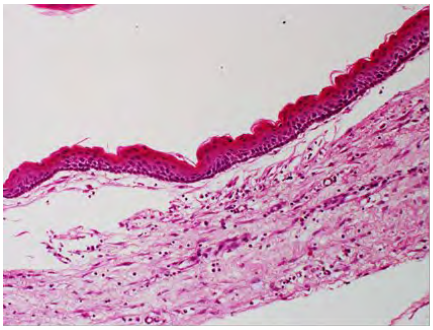
We investigated the relevance between X-ray findings (191 cases) and recurrence of OKCs. The recurrence rate of multilocular cysts (31.3%) was significantly higher than that of unilocular cysts (13.2%), indicating that removal of multilocular cyst, which is potentially local aggressiveness may be more difficult than the unilocular one.

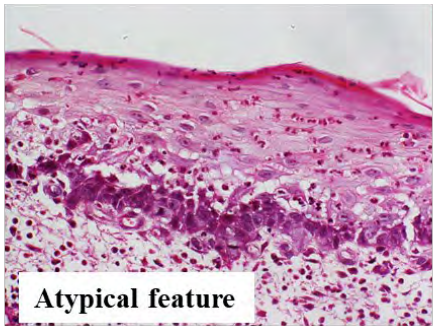
Regarding the treatment method, recurrence was observed in 14 cases (22.68%) with fenestration, and in 34 cases (16.58%) with excision, respectively, but there was no significant difference between the two surgical methods.

At our university hospital, all patients with fenestration used to get excision after reduction in size. that was why there was no significant difference in recurrence rates between two procedures.

Relationship between pathological features and recurrence

Atypism including mitosis in basal cell layer, present of daughter cyst tend to be higher in cases with recurrence, but significant differences were not observed.





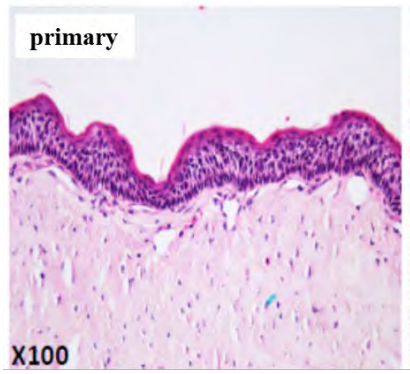
Pathological "Epithelium thickness" and recurrence (p = 0.0606)

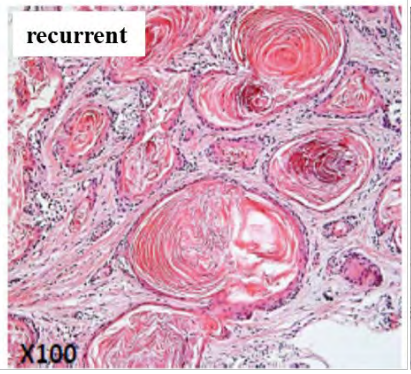
	Recurrence	Nonrecurrence	Recurrence rate
Thin	28	78	25.00%
Thick	7	9	47.06%
Total	34	87	

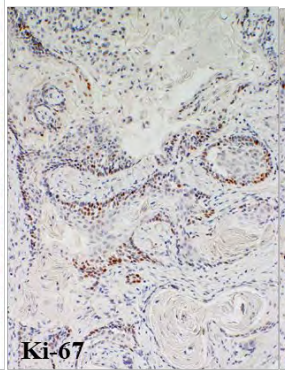
Pathological "Presence or absence of Daughter cyst" and recurrence (p =0.1123)

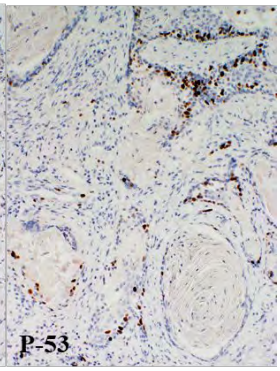
	Recurrence	Nonrecurrence	Recurrence rate
Presence	5	5	50.00%
Absence	29	81	26.36%
Total	34	86	

In the recurrence cases with the primary lesion showing a typical findings of OKC, one case of OKC, consisting of solid proliferation of keratinized stratified squamous epithelial nests was included. Since this case showed a relatively high Ki-67 positive rate and p-53 positivity It is possible to be neoplastic nature.









(Conclusion)

Syndromic OKCs are 5.1%, frequently occur in young generation, and show high recurrent rate.

The recurrence rate of non-syndromic OKC cases was about 37%, and the recurrence rate was relatively high. Factors, which are likely to induce recurrence include multilocular lesion, atypia in the basal cell layer, budding suggesting high proliferative activity, and the presence of daughter cysts. Although careful removing is required, most cysts are considered to be non-neoplastic cystic disease. In addition, continuous follow-up observation is required for 3 years after surgery.

Two possibilities occur 1) some OKCs recur as they have a primarily inherent neoplastic potential and some OKCs recur due to some secondary changes in lining epithelium leading to tumor-like solid growth.

上位入賞結果

● 優勝

岡山大学歯学部 4年生　棚井 あいり　Airi TANAI

歯周病と胎児の成長障害：*Porphyromonas gingivalis*はマクロファージの細胞外小胞を介して胎盤の血管形成を阻害する

Periodontitis & abnormal pregnancies: *Porphyromonas gingivalis* utilizes macrophage extracellular vesicles and inhibits placental angiogenesis

● 準優勝

大阪歯科大学 3年生　栗山 実久　Miku KURIYAMA

高齢者の口腔ケアのための過酢酸を含む新しい歯科用消毒剤の開発

Development of a new dental disinfectant containing peracetic acid for oral care of the elderly

● 第3位

日本大学歯学部 5年生　髙田 紋花　Ayaka TAKADA

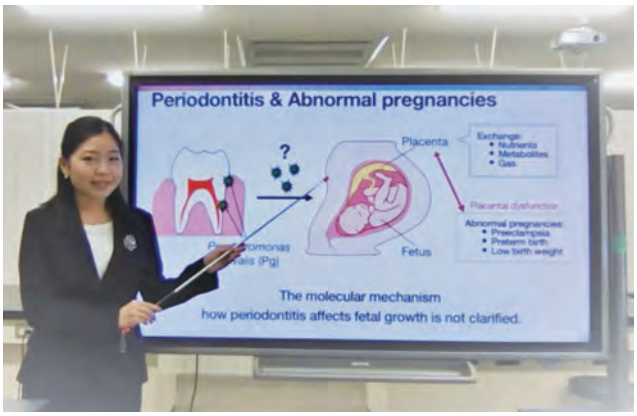
口腔内細菌代謝産物がドライソケットにおける歯槽骨石灰化亢進に関与する可能性

Additive alveolar bone mineralization in the dry socket may be induced by oral bacterial metabolites

鹿児島大学歯学部 4年生　福島 慎　Makoto FUKUSHIMA

異所性疼痛とラット三叉神経節体部位局在との関連についての研究

Analysis of relationship between ectopic pain and somatotopy of rat trigeminal ganglion neurons



優勝　岡山大学歯学部　棚井 あいり

二次審査風景　2021年8月20日　於歯科医師会館

審査講評

審査講評をさせて頂きます。本年度のSCRP日本代表選抜大会は、新型コロナウイルス感染症が猛威を振るい、昨年度に続きオンライン開催となりました。このような状況にもかかわらず、21校からエントリーをいただきましたこと、誠に感謝申し上げます。

一次審査では、全員に研究発表抄録・発表ビデオ・発表スライドをご提出いただきました。そして、審査員6名ならびに正副審査員長の合計8名により、厳正に審査採点させて頂きました。審査は審査表に採点をつける形で、上位4名を二次審査に選出いたしました。

二次審査では、4名のスチューデント・クリニシャンがオンラインにより実際の発表を行い、次いで審査員による質疑応答がなされました。英語での発表および研究内容の理解度を中心に審査し、審査員6名の最高点と最低点を削除し、残りの審査員4名の平均点を算出し、順位を決定させていただきました。なお、審査員にスチューデント・クリニシャンの大学出身者がいる場合には、該当審査員の点数を削除いたしました。

すべてのスチューデント・クリニシャンの発表はすばらしく、甲乙つけがたいものでありました。来年度はコロナも落ち着き、歯科医師会館にて対面形式で審査できることを望んでおります。

最後になりますが、スチューデント・クリニシャンを指導された各大学の先生方には敬意を表します。また、本年度の大会を経験されたスチューデント・クリニシャンの皆様は、将来の日本の歯科医療に貢献できる、立派な歯科臨床医または歯科研究者になられ、国際的に活躍されることを期待いたします。

副審査員長　井上　孝

審査員一覧

審査員長

平野　裕之* Hiroyuki HIRANO
京都府開業

副審査員長

井上　孝* Takashi INOUE
東京歯科大学　名誉教授

審査員

影山　幾男 Ikuo KAGEYAMA
日本歯科大学新潟生命歯学部
解剖学第1講座　教授

審査員

細川　隆司 Ryuji HOSOKAWA
九州歯科大学
口腔再建リハビリテーション学分野　教授

審査員

上條竜太郎 Ryutaro KAMIJO
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歯科保存学教室　教授

審査員

小川　祐司* Hiroshi OGAWA
新潟大学大学院医歯学総合研究科
予防歯科学分野　教授

審査員

澁川　義幸 Yoshiyuki SHIBUKAWA
東京歯科大学
生理学講座　教授

*公益社団法人 日本歯科医師会　国際渉外委員会 委員

スチューデント・クリニシャン・リサーチ・プログラム（SCRP）の歴史

スチューデント・クリニシャン・リサーチ・プログラム（SCRP）は、1959年にスチューデント・クリニシャン・プログラム（SCP）として始まりました。その歴史は、アメリカ歯科医師会（ADA）が創立100周年を迎えるにあたり、当時の専務理事Dr.ハロルド ヒレンブランドがデンツプライ インターナショナル インク（現：デンツプライシロナ インク）の会長ヘンリー M.ソーントンに対し、歯科学生による研究の実践発表という斬新で意義ある記念企画の後援を依頼したことに由来します。研究発表形式は、当時から2006年頃までテーブルクリニックで行なわれましたが、翌年以降はポスター発表に変わり、同時期に'SCP'から'SCRP'へ名称変更いたしました。2017年には国際歯科研究学会米国部会（AADR、現：AADOCR）学術大会における発表に移行し、Student Competition for Advancing Dental Research and its Application（SCADA）と更に名称変更となりました。

SCRPは約60年間に世界5大陸に拡大し、オーストラリア、オーストリア、ブラジル、カナダ、中国、デンマーク、英国、エストニア、フィジー、フィンランド、フランス、ドイツ、香港、アイスランド、インド、インドネシア、アイルランド、日本、ラトビア、リトアニア、マレーシア、メキシコ、ミャンマー、オランダ、ニュージーランド、ノルウェー、フィリピン、シンガポール、南アフリカ、韓国、スウェーデン、スイス、台湾、タイ、トルコ、ベトナムで開催されました。各国代表は米国大会においてコンペティションは行なわれず、招待発表ならびに学術交流機会を得ることができます。日本においては1995年に日本歯科医師会主催、デンツプライジャパン株式会社（現：デンツプライシロナ社）後援により米国と同様SCPの名称で第1回日本代表選抜大会が開催され、4校の参加でスタートしました。日本代表選抜大会は長年に亘る同社からの後援を経て、2020年（第26回）から本会による単独開催となりました。本年度も新型コロナウイルス感染症による影響を受けながらも21校からの参加があり、来場を伴わない二次審査方式により開催されました。

1959年 ADA/SCP創設当時
（左）ADA専務理事Dr.ハロルド ヒレンブランド
（右）デンツプライインターナショナル インク 会長 ヘンリー M.ソーントン

先輩SCからのメッセージ　日本大学松戸歯学部感染免疫学講座 助教　小林 良喜

私は1999年、第5回大会に日本大学松戸歯学部の代表として発表の機会を得ました。当時の発表形式はパネルによるTable clinicでした。20年以上を経過した今、当時を思い起こすと緊張感や達成感が蘇ります。本大会を知るきっかけは、第3回大会に出場する先輩がいることを同期から聞きつけたことでした。在学当時に研究に魅力を覚えた自分としては、チャンス！と、前田隆秀名誉教授（当時ファカルティーアドバイザー）に願い出たことを記憶しています。卒業後は米国にて粘膜免疫学の研究に関わり、帰国後も母校で研究活動を継続して、ファカルティーアドバイザーとして本大会の活動に関わることができたのは、リサーチマインドを持ち研究に情熱を注ぐ同世代と出会えたことでした。Student Clinician・Co-Clinicianとして出場後は、SCADA会員として研究から臨床と幅広く、交流を広げていけることもSCRPの魅力です。次回は、会場で多くの皆様とお会いできることを楽しみにしています！
Show the spirits of the scientist !

1999年（平成11年度） 第5回大会 歯科医師会館
日本大学松戸歯学部（当時）5年生 小林 良喜

日本歯科医師会雑誌2020年3月号「国際交流だより」より転載

スチューデント・クリニシャン・リサーチ・プログラム（SCRP）とのご縁

米国・ニューイングランド大学歯学部教授　駒林　卓

■はじめに

2019年9月に米国サンフランシスコで米国歯科医師会・FDI世界歯科大会（ADA/FDI World Dental Congress）が共同開催され，筆者は直接覆髄（Current Status of Direct Pulp-Capping Materials for Permanent Teeth）とシーラー（Assessing and Selecting Endodontic Sealers in Clinic）の2つの講演演者として参加しました。そこで開催されたソーシャルイベント（ジャパンナイト）の中で，日本歯科医師会（日歯）の国際渉外関連をはじめとする派遣団の先生方にお目にかかりました（**図1**）。

筆者は，広島大学歯学部，東京医科歯科大学大学院（博士），カリフォルニア大学歯学部，コネチカット大学［エンド（歯内療法）専門医・修士］の4大学を卒業後，ベイラー大学助教，ウエストバージニア大学准教授を経て，2015年からニューイングランド大学歯学部で教授・エンド専門指導医として教育・研究・臨床に従事しています。ポートランド市に学舎を構えるニューイングランド大学は100年以上の歴史を持つ伝統校ですが，2013年に地元の強いニーズを受けて歯学部を新設しました。ポートランド市は，米国東海岸メイン州南西部の港湾都市で，ボストンの北北東約170kmに位置します。大学での業務に加えて，2010年からは米国陸軍歯科医師（将校）として人材育成・国際歯科医療貢献などに携わっております。

広島大学歯学部6年在学中の1997年に，各大学代表の歯科学生が研究の実践発表を英語によるテーブルクリニックという形式（現在はポスター発表）で行う，スチューデント・クリニシャン・プログラム（SCP）日本代表選抜大会で第2位になり（**図2**），渡米後は2005年にHenry M. Thornton賞，2006年にAlan J. Davis賞をいただき，2008年から現在に至るまで，SCRP米国大会の審査委員を務めております。

こういった経緯から，このたびSCRP日本大会経

図1　FDIサンフランシスコ大会でのジャパンナイトにて（2019年9月）
左から，平野裕之日歯国際渉外委員会委員長（FDI教育委員会委員），筆者，尾松素樹日歯常務理事。日歯主催のジャパンナイトでは多くの国の参加者と交流を持てました。

図2　1997年SCP大会にて第2位の表彰を受ける筆者
授与者は梅田昭夫日歯副会長（当時），右後方がデンツプライ・インターナショナル社ジョージ・R・ローズ副社長（当時）

験者として日歯会員の先生方へメッセージを送る大変名誉な機会を頂戴した次第です。

■筆者と SCRP 日本大会

幼少のころから在日米軍日本人高官だった祖父から極めて厳しく英語の指導を受け，筑波大学附属駒場高校在学中，東京都友好親善使節団生徒代表としてニューヨーク市に派遣されました。広島大学歯学部在学中には，アジア競技大会（広島県）やユニバーシアード大会（福岡県）などで英語通訳者・通訳指導者の機会を得ました。これらを通じて異文化理解・国際性・多様性の実践的な経験を積むことができた一方，こういった実地経験と歯科での研鑽との関係が薄いことに筆者自身疑問を感じ始めていました。そんな時，日本で1995年から SCP（現在の SCRP）大会が始まり，幸運にも1997年に参加の機会をいただけたことは，現在に至る筆者の進路・人生に大きな影響を与えました。

SCRP の特色は第一に，研究的態度を備えた臨床家を育成する機会を与える点です。歯科学生だった筆者が，大学教官や日歯会員の先生方から直接親身のご指導を受けられたおかげで，普段大学の授業や実習だけではなかなか学ぶことができない，より踏み込んだ数多くのことを学べました。第二に，SCRP の参加学生以外にも大きな刺激や積極的な良い影響を与えることです。それは参加学生の友人知人にとどまらず，先輩後輩，参加大学の学生全体が歯科における国際性・多様性に目覚め，一人ひとりがそれぞれの夢と希望を持ち，その実現に邁進できる絶好の機会となることです。筆者自身は，競争は熾烈な一方で努力した結果が自分自身に反映され，真の意味での実力で挑戦できる米国での人生を選ぶきっかけになりました。第三に，SCRP は年に一回のイベントではあるものの，開催当日のみならず年単位で，大学や国・地域を越えた大きな交流が続きそれが発展していくことです。このたびのサンフランシスコ大会での日歯の先生方との出会いがその具体例です。したがって，SCRP 日本大会は日歯の事業として核心的で極めて重要であると感じています。

■米国の歯科医療と SCRP 米国大会

米国で歯科医師になるには，高校卒業後の大学４年間で自然科学系科目を選択した成績優秀な卒業生が，高倍率の選抜試験を経て，さらに４年間の労力・高額な授業料を投資し歯学部へ進学する必要があります。専門医（病理，放射線，口腔外科，公衆衛生，矯正，小児，補綴，歯周，歯内）になるには，歯科医師免許取得後さらに最低２年間の専門医プログラムを修了しなくてはなりません。

米国の歯科医師は一般歯科医（約80％）と専門歯科医（約20％）に分かれます。筆者はエンド専門医なので，患者はまず一般歯科医を受診します。症例の難易度を一般歯科医の先生が判断され，難症例の場合，我々専門医に紹介されます。エンド専門医はエンドのみに専念し，患者は紹介元の一般歯科医で歯冠修復などの続きの治療を行います。一般歯科医の先生は紹介した患者が戻ってくることから，安心して紹介ができます。

米国でのこのシステムの長所は，専門医がより高度な治療を患者に提供できることや，一般歯科医の先生が無理をした結果の医療過誤が減少することです。短所は，歯科医療費の高騰が続き，歯科治療を受けられない多くの患者が存在することや，米国歯科医師会年次総会への専門医の参加が疎くなることです。

SCRP 米国大会は日本同様，歯科学生を対象としています。たとえ専門医であっても全員歯学部は卒業するので，SCRP はすべての米国歯科医師の次なるステップへの登竜門なのです。その意味では SCRP 経験者は現役学生のみならず経験豊富な指導者まで幅広い人材を輩出する出発点と言えるでしょう。

■ふるさと日本の先生方への感謝と想い

SCRP は日本と世界の歯科医療の発展の礎として，地域医療，学術研究，大学教育，生涯研修，国際交流，指導者育成などあらゆる可能性を無限に秘めている日歯の宝物です。SCRP 日本大会が今後も日歯の事業として継続発展していくことを願います。

今後も筆者は，米国歯科大学と陸軍で，教育・研究・臨床に国際的な貢献をしていきたいと考えています。日本はふるさとであり，プロフェッショナルの始まりであり，深い感謝の念を忘れたことはありません。一方，近年歯科に限らず，日本から米国を目指す，留学生・研究者・客員教授・駐在員・移民が激減していることを大変心配しています。米国はまるでオリンピックの選手村のように，世界各国から人々が集まり切磋琢磨し，その後，それぞれの母国に帰国し活躍されたり，筆者のように移民として米国で暮らしたりする，とても特色のある国です。一人でも多くの先生方が米国にお越しになられることを切望します。今後も初心を忘れずに真摯な気持ちで地道に努力を続けていく所存です。

日本歯科医師会雑誌2021年3月号「国際交流だより」より転載

新たなスタートを切った日本歯科医師会 令和2年度 SCRP 日本代表選抜大会

日本歯科医師会常務理事　尾松素樹

■ SCRP 日本代表選抜大会とは

SCRP（スチューデント・クリニシャン・リサーチ・プログラム）日本代表選抜大会は，各歯科大学／歯学部の代表歯科学生が研究発表を行い，その中から国際歯科研究学会米国部会（AADR）主催による学術大会に日本代表として発表する歯科学生を選抜する大会である（図１）。SCRP は，1959年米国歯科医師会（ADA）が設立100周年を迎えにあたり，デンツプライ・インターナショナル・インク（現：デンツプライシロナ・インク）に，歯科学生による研究の実践発表という記念企画の後援を依頼したことに始まる[1]。現在では世界各国に広がり，2018年から AADR 学術大会における発表に移行され，歯科界の発展を担う研究者・教育者・開業医等を多く輩出している[2]。

日本では，将来の歯科界を担う歯科学生の研究意欲を啓発・高揚させ，国際的な視野に立脚した歯科医師像の育成を目的に，日本歯科医師会主催による日本代表選抜大会を，1995年に全国歯科大学／歯学部29校のうち４校からスタートした。日本代表選抜大会は歯科医師会館を会場とし，ポスターによるプレゼンテーションを審査員が評価して代表者を選抜してきた。参加校も年々増加し，2019年は24校からの参加があった。

図１　2018年　AADR/SCADA*フォートローダーデール大会

*Student Competition for Advancing Dental Research and its Application

■日歯単独事業として新たなスタートを切った SCRP

第26回となる令和２（2020）年度大会からは，これまで後援をしていたデンツプライシロナ株式会社が退き，日歯単独の主催で国際渉外委員会の事業として新たにスタートした。例年通り募集を開始したが，新型コロナウイルス感染症の拡大により，学生が大学での勉学や研究活動ができない状況となり，大会が開催できるだけの参加があるかどうか。また，会館での開催ができるかどうかも懸念された。

そこで，まず，予定していた審査書類の提出締切日や審査日を遅らせ，審査形式も会館でのポスター発表から，研究発表抄録・発表ビデオ，発表スライドによる一次審査および一次審査通過者によるオンラインでのプレゼンテーションの二次審査方式に変更した（図２）。その結果，参加校が例年よりも少なくなったが，18校からの参加応募があった。

■優勝は北海道大学　６年生の吉野弘菜さん

９月に行われた一次審査において基礎部門の上位２名，臨床部門の上位１名が二次審査に進出した。二次審査は10月４日（日），オンラインでのプレゼンテーション・質疑応答で行われ，基礎部門第１位の北海道大学歯学部６年生・吉野弘菜さんが研究テーマ「アレンドロネート投与による骨特異的血管の組織学的変

図２　令和２年度日本代表選抜大会　二次審査の模様
（於：日本歯科医師会会議室）

図３　令和２年度優勝（日本代表）北海道大学歯学部６年生
吉野弘菜さん

化」で優勝した（図３）。なお，吉野さんは2021年７月に米国・マサチューセッツ州ボストン市で開催されるAADR学術大会に招待され，日本代表として発表するとともに，世界各国のSCRP代表学生，歯学研究者等との交流を図る予定である。

準優勝は臨床部門第１位の九州大学歯学部５年生・高本侑立子さんで，研究テーマは「欠損補綴治療による起立運動機能への影響」であった。基礎部門第２位は大阪歯科大学４年生・鈴田真裕さんで，研究テーマは「力学的閾値同定を主軸とした破骨細胞分化（促進／抑制）制御法の確立」であった。

今年度の審査結果については，本会ホームページに堀憲郎会長の主催者挨拶，審査結果発表，審査講評を動画にてアップしている。また，参加者全員の研究発表は「SCRP研究発表抄録集」として参加した歯科学生，関係者に配布し，本会ホームページにも掲載した。このように研究発表という学生時代の貴重な経験を抄録集に記録として残し，大学での研究を志すこれからのスチューデント・クリニシャンの参考となるようにした。

新型コロナウイルス感染症の拡大の状況下で，本年度大会に参加した18名のスチューデント・クリニシャン，共同研究者，また，スチューデント・クリニシャンが安心して実験等発表準備に専念できるよう環境を整え研究指導に当たられたファカルティー・アドバイザーならびに研究指導協力者の方々に敬意を表したい。

次年度も多くの参加を期待したい。

■ SCRP日本代表選抜大会参加校・代表者

北海道大学歯学部・吉野弘菜（６年），岩手医科大学歯学部・羽金雅登（２年），東北大学歯学部・古内聖弓（６年），奥羽大学歯学部・今井千穂子（４年）日本大学松戸歯学部・植草信乃介（４年），東京医科歯科大学歯学部・渡部準也（６年），日本大学歯学部・黒澤佑介（５年），昭和大学歯学部・根岸宗一郎（５年），松本歯科大学・小野亜美（６年），大阪大学歯学部・都原志穂（４年），大阪歯科大学・鈴田真裕（４年），岡山大学歯学部・村田志穂（４年），徳島大学歯学部・喜田悠太（５年），九州大学歯学部・高本侑立子（５年），九州歯科大学・高田知佳（３年），福岡歯科大学・池本梨央南（５年），長崎大学歯学部・園木美結（５年），鹿児島大学歯学部・佐藤大幹（５年）
（計18校）

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口腔粘膜診断支援プログラムの作成 舌編
Shuntaro KURODA - Tokyo Dental College
“A visualized assist system for clinical diagnosis of the tongue”

第 2 回（H.8/1996 年）■日本大学歯学部　松山 智子
塩素濃度の異なる 2 種類の酸化水の殺菌効果および保存条件による経時的変化
Tomoko MATSUYAMA - Nihon University School of Dentistry
"Bactericidal effects and temporal changes in preservation conditions of two kinds of oxidized water with different concentrations of chlorine"

第 3 回（H.9/1997 年）■東京医科歯科大学歯学部　五十川 伸崇
新しいチューインガムを用いた咀嚼機能の評価
Nobutaka ISOGAWA - Tokyo Medical and Dental University Faculty of Dentistry
"Evaluation of masticatory performance by using new chewing-gum"

第 4 回（H.10/1998 年）■東京歯科大学　阿部 修
要介護高齢者口腔内には肺炎起因菌が高頻度に検出される
Shu ABE - Tokyo Dental College
"High incidence of pneumonia pathogens in oral cavity of elderly patients requiring daily nursing care"

第 5 回（H.11/1999 年）■日本歯科大学歯学部　横山 享子
簡易血糖測定機器による不正咬合者の咀嚼能率の評価
Yukiko YOKOYAMA - The Nippon Dental University School of Life Dentistry at Tokyo
“Evaluation of masticatory efficiency in persons with malocclusion using a simplified blood glucose measuring device”

第 6 回（H.12/2000 年）■大阪大学歯学部　中島 正裕
支台形成実習用デンタルミラーの改良
Masahiro NAKAJIMA - School of Dentistry Osaka University
"Advancement in dental mirror to assist tooth preparation for students"

第 7 回（H.13/2001 年）■日本大学松戸歯学部　金親 あや乃
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Ayano KANEOYA - Nihon University School of Dentistry at Matsudo
“Development of novel disclosing agents”

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ブラックスチェッカーを用いた睡眠ブラキシズム時のグラインディング運動パターンの分析
Toshimi KAWAGOE - Kanagawa Dental University
“Study of grinding pattern during sleep bruxism with a simple device: Bruxchecker”

第 9 回（H.15/2003 年）■鶴見大学歯学部　角田 衣理加
精油の歯周病原性細菌に対する抗菌効果および口臭抑制効果の検討
Erika KAKUTA - Tsurumi University School of Dental Medicine
"The Anti-microbial activity and anti-halitosis of essential oils against oral bacteria causing periodontitis"

第 10 回（H.16/2004 年）■東京医科歯科大学歯学部　佐藤 智子
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Tomoko SATO - Tokyo Medical and Dental University Faculty of Dentistry
“Evaluation of articulation of children with open-bite using acoustic analysis of speech”

第 11 回（H.17/2005 年）■日本歯科大学新潟生命歯学部　宇波 雅人
デジタルカメラにおけるマクロ撮影の可能性（携帯カメラを含めて）
Masato UNAMI - The Nippon Dental University School of Life Dentistry at Niigata
“Development of micro mode digital camera and cell phone”

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チェアサイドで使用可能な簡易型偏性嫌気性菌培養キットの開発
Toshimitsu OHSAKO - School of Dentistry Health Sciences University of Hokkaido
“Development of the simple chair side obligate anaerobic culture kit practical at the general dental clinic”

第 13 回（H.19/2007 年）■日本大学歯学部　秋山 祐子
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Yuko AKIYAMA - Nihon University School of Dentistry
“Shade determination using visibly optimal custom shade guide”

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Etsuko AIDA - Nihon University School of Dentistry at Matsudo
“Examination on the effects of brushing applied mobile telephone attached camera”

第 15 回（H.21/2009 年）■日本大学歯学部　梶 佳織
撤去容易な熱膨張性矯正用ブラケット接着材の開発
Kaori KAJI - Nihon University School of Dentistry
“Development of easy debondable orthodontic bracket adhesive by heating”

第 16 回（H.22/2010 年）■大阪歯科大学　岸田 瑠加
う蝕予防を目的としたまんじゅうの製作と研究
Luka KISHIDA - Osaka Dental University
“Trial production of manju, Japanese style cake stuffed with adzuki bean paste, with alternative sweeteners for prevention of dental caries”

第 17 回（H.23/2011 年）■広島大学歯学部　髙才 東
歯周病予防と治療を目的としたラクトフェリンの応用
Azuma KOSAI - Hiroshima University Faculty of Dentistry
“Application of lactoferrin for periodontitis prevention and treatment”

第 18 回（H.24/2012 年）■北海道大学歯学部　大畑 八重
線維芽細胞は腫瘍微小環境で PTHrP により CAF へ誘導される
Yae OHATA - Hokkaido University School of Dental Medicine
“Fibroblasts in tumor microenvironment are induced to Cancer-Associated Fibroblast (CAF) by PTHrP”

第 19 回（H.25/2013 年）■岡山大学歯学部　王 碩
なぜ煙草をやめると太るのか？
WANG Shuo - Okayama University Dental School
"Why do people get fat if they stop smoking ?"

第 20 回（H.26/2014 年）■昭和大学歯学部　道家 碧
歯周病原細菌の産生するヌクレアーゼの解析
Midori DOKE - Showa University School of Dentistry
"Molecular characterization of nuclease enzymes from periodontal Bacteria"

第 21 回（H.27/2015 年）■東京医科歯科大学歯学部　田中 大貴
閉経後骨粗鬆症モデルにおける FactorX 発現制御機構
Daiki TANAKA – Tokyo Medical and Dental University Faculty of Dentistry
"The FactorX expression mechanisms in a model of postmenopausal osteoporosis"

第 22 回（H.28/2016 年）■鹿児島大学歯学部　神園 藍
Syk 活性阻害は間葉系幹細胞の骨分化を促進し脂肪分化を抑制する
Ai KAMISONO – Kagoshima University Faculty of Dentistry
"Syk inactivation induces to promote osteogenic differentiation and suppress adipogenic differentiation of mesenchymal stem cells"

第 23 回（H.29/2017 年）■広島大学歯学部　吉野 舞
単一細胞トランスクリプトミクスによる骨芽細胞の多様性の解析
Mai YOSHINO – Hiroshima University School of Dentistry
"Single-cell transcriptomics uncovers the diversity of osteoblasts"

第 24 回（H.30/2018 年）■北海道大学歯学部　阿部 未来
骨リモデリングとモデリングの骨芽細胞活性化における細胞学的相互作用
Miki ABE – Hokkaido University School of Dental Medicine
"Cellular interaction activating osteoblastic bone formation during bone modeling and remodeling"

第 25 回（R.1/2019 年）■広島大学歯学部　前川原 思惟子
***Porphyromonas gingivalis*（*P.g.*）-fimA type2 と type4 血清抗体価の上昇は歯周炎の関連する早産のマーカーとなる**
Shiiko MAEKAWARA – Hiroshima University School of Dentistry
"*Porphyromonas gingivalis (P.g.)*-fimA (Type2 and Type4) serum antibody titer is a possible marker for preterm birth associated with periodontitis"

第 26 回（R.2/2020 年）■北海道大学歯学部　吉野 弘菜
アレンドロネート投与による骨特異的血管の組織学的変化
Hirona YOSHINO – Hokkaido University School of Dental Medicine
"Histological alteration of bone-specific blood vessels by alendronate administration"

参加大学関係者一覧

大学	学長/学部長	ファカルティー・アドバイザー	研究指導協力者	スチューデント・クリニシャン	共同研究者	SC No.
北海道大学歯学部	八若 保孝	長谷川 智香 硬組織発生生物学教室 助教	網塚 憲生 本郷 裕美	中嶋 悠斐	—	18
北海道医療大学歯学部	古市 保志	安彦 善裕 臨床口腔病理学分野 教授	植原 治 パウデル ドウルガ 森川 哲郎	呂 令凱	野口 裕季子 宮本 康佑	15
岩手医科大学歯学部	三浦 廣行	佐々木 実 微生物学講座 教授	—	増田 彩	—	11
東北大学歯学部	髙橋 信博	小坂 健 国際歯科保健学分野 教授	草間 太郎	岡田 嘉奈子	—	16
日本大学松戸歯学部	小方 賴昌	小林 良喜 感染免疫学講座 助教	—	熨斗 優樹	—	13
東京医科歯科大学歯学部	依田 哲也	宇尾 基弘 先端材料評価学分野 教授	和田 敬広	髙村 彩	—	4
日本大学歯学部	本田 和也	津田 啓方 生化学講座 准教授	—	髙田 紋花	—	10
昭和大学歯学部	槇 宏太郎	山田 篤 口腔生化学講座 講師	—	四宮 寛大	—	1
鶴見大学歯学部	大久保 力廣	大島 朋子 口腔微生物学講座 学内教授	溝辺 智美 向井 陽子	田﨑 智也	—	6
新潟大学歯学部	前田 健康	井上 誠 摂食嚥下リハビリテーション学分野 教授	真柄 仁 ステガロユ・ロクサーナ	安藤 まな	—	3
日本歯科大学新潟生命歯学部	中原 賢	小椋 一朗 歯科放射線学講座 教授	—	田邊 由佳	—	9
朝日大学歯学部	田村 康夫	近藤 信夫 口腔生化学分野 教授	神谷 真子	西口 真矢	—	17
大阪大学歯学部	今里 聡	山口 雅也 口腔細菌学教室 講師	川端 重忠	小山 愛結	—	20
大阪歯科大学	川添 堯彬	本田 義知 中央歯学研究所 准教授	橋本 正則 張 泓灝 神田 龍平	栗山 実久	—	19
岡山大学歯学部	長塚 仁	岡村 裕彦 口腔形態学分野 教授	池亀 美華 福原 瑶子 江口 傑徳	棚井 あいり	—	7
広島大学歯学部	谷本 幸太郎	宮内 睦美 口腔顎顔面病理病態学分野 教授	柿本 直也 虎谷 茂昭 武知 正晃	安田 雅空斗	小林 明弘 片岡 奈菜子	21
徳島大学歯学部	馬場 麻人	市川 哲雄 口腔顎顔面補綴学分野 教授	渡邉 恵	深田 有希	—	5
九州大学歯学部	中村 誠司	王丸 寛美 口腔総合診療科 助教	和田 尚久	平田 薫子	松本 和大	12
九州歯科大学	西原 達次	向坊 太郎 口腔再建リハビリテーション学分野 助教	細川 隆司	赤司 妃咲	晝間 歩未	8
長崎大学歯学部	村田 比呂司	住田 吉慶 硬組織疾患基盤研究センター 准教授	岩竹 真弓	長野 敏樹	—	2
鹿児島大学歯学部	西村 正宏	後藤 哲哉 歯科機能形態学分野 教授	倉本 恵梨子	福島 慎	—	14

SCADA-Japanへようこそ

SCADA Associates in Japan
代表　井田 有亮　Yusuke IDA

本年もSCRP日本代表選抜大会が開催されましたことを、SCADA Associates in Japanを代表してお慶び申し上げます。

各校代表として研究発表に臨まれたStudent Clinician・Co-Clinicianの皆様は、大変多忙な学生生活の中にあって研究活動に精励され、発表に至る貴重な経験を蓄積されたことと拝察いたします。さらに、本年は長く続く感染症流行下において、例年に比べて一段と研究活動に制約が加わる状況でありながら研究活動を継続されたことに、心から敬意を表します。そして、皆さんを大会参加者の同窓会であるSCADAの新会員としてお迎えできることを大変嬉しく思います。

私は2007年に開催された第13回大会において、大学の代表として発表する機会を得ました。10年以上たった今でも緊張感と達成感がありありと蘇ってまいります。大会参加を通じて得られた最大の成果は、知識や技術以上に、リサーチマインドや研究に注ぐ情熱を持った同世代との出会いだと思っております。今回は集合しない新しい形式での開催となりましたが、従来の大会における交流の側面をSCADAがサポートさせていただくことができました。多くの皆さんに参加いただき、充実した時間になったものと考えております。これをきっかけとして、交流の輪を所属学外へと広げていただきたいと考えております。

末筆ながら、20年余にわたってSCRP日本代表選抜大会を継続的に主催してこられた公益社団法人日本歯科医師会、そして、ご多忙の中にあっても熱心に学生の研究指導に当たられている各校のファカルティーアドバイザーの先生方に、当会員一同よりあらためて感謝申し上げます。

いだ　ゆうすけ
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SCADAについて

SCADAは各国のSCRP参加経験者で構成される国際的な同窓会組織（本部：アメリカ）であり、世界各地で学術交流の場を広げています。日本では、1999年に日本人のSCADA会員により、日本におけるSCRP大会同窓会組織としてSCADA Associates in Japan (通称：SCADA-Japanスカダジャパン)が発足しました。当会は①今後の歯科医療の発展を担う歯科学生の育成及び研究意欲の向上を目的としたSCRPへの参加を全国歯科学生に呼びかけ、その参加学生に適切な助言を与えること、②世界のSCADA会員と連携して、あらゆるレベルで実施される歯科に関する研究・医療等への参加を推進・奨励し、会員相互の交流を深めること、の2つを基本理念として掲げております。SCRP参加者は、SCADAおよびSCADA-Japanに自動入会となり、Membership Certificateを得ることができます。

当会は、基礎・臨床、大学・開業、さらには地域・年齢といった様々な壁を越え、会員同士が交流しています。今後もSCRPに参加した歯科学生たちがこのSCADA-Japanという組織を最大限活用し、歯科界での活躍の場を広げていただければと思っています。

令和4年度 日本歯科医師会
スチューデント・クリニシャン・リサーチ・プログラム
日本代表選抜大会（予定）

開催日：2022年8月26日（金）※

場　所：歯科医師会館

※令和4年度 SCRP日本代表選抜大会は2022年8月26日（金）に開催予定ですが、新型コロナウイルス感染症の状況を考慮し、開催形式（審査方法）が「手引き」記載の方法から変更になる場合がありますので、ご了承ください。

【お願い】
本研究発表抄録集には、個人情報が含まれておりますので、取り扱いにはくれぐれもご配慮くださいますようお願い申し上げます。

公益社団法人 日本歯科医師会

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- 都営地下鉄新宿線市ヶ谷駅からA4出口徒歩2分

あとがき

令和3年度SCRP大会は、前回に引き続きコロナ禍対応を鑑みて、ビデオ並びに書類審査による一次審査、さらに上位4名によるオンラインでのプレゼンテーションと質疑応答による二次審査により順位を決めさせていただきました。昨年の18校から3校増えて21校と、多くの学校にご協力を賜り開催できましたことを感謝申し上げます。

二次審査当日は、図らずも緊急事態宣言下での開催となり、Student Clinicianも審査員もオンラインでの参加となりました。昨年の経験を生かし、事前にシミュレーションをしていたこともあって、ウィズコロナへ少し対応できたかと感じております。

応募開始来、種々の困難な状況下においても多くの優れた学生諸君により勉学や研究生活が継続して行なわれていることを感じ、大変頼もしく審査させていただきました。Student Clinicianにおかれましては、この経験を今後のキャリアに是非生かしていただきたいと思います。

末尾になりますが、滞りなく大会を遂行できたことは、関係各位のご協力の賜物であります。感謝の意を表してあとがきに代えさせていただきます。

審査員長　平野　裕之

SCRP日本代表選抜大会 研究発表抄録集
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