SCRP

令和4年度
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

2022 JDA Student Clinician Research Program

研究発表抄録集

日本歯科医師会
人生を もっと楽しく もっと豊かに

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

歯科医師会館（定礎：昭和63年）

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※ 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。

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

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年にアメリカ歯科医師会（ADA）の100周年記念事業による歯科学生の研究発表の場としてスタートしました。その後、60年以上の長い歴史を経ながら、現在はIADR（国際歯科研究学会）の部会であるAADOCR（American Association for Dental, Oral, and Craniofacial Research）の主催により、世界各国を結ぶ歯学教育支援プログラム：SCADA（Student Competition for Advancing Dental Research and its Application）として発展し続けています。

日本は1995年から参加し、以来、本会は28年間に亘り日本代表選抜大会を主催してきました。本年度もコロナ禍の影響を受けWEBを活用した開催となりましたが、21校の歯科大学・歯学部からのご参加を得られましたことを大変喜ばしく思います。

本会は現在約64,000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、この世界大会の事務部門の会議が、3年ぶりに対面の形でスイスのジュネーブで開催され、本会からも代表団を派遣し、FDI政策声明の策定に参画すると共に、常設委員会委員選挙等を通じて日本の一層の貢献の姿勢を示しました。また、アメリカ、ドイツ、フランス、豪州、ニュージーランドを始めとする各歯科医師会との会合にも参加し、歯科医療を巡る現状と課題について活発な意見交換を行いました。

本会では歯科界の将来を担う国際感覚に優れた人材を歯科学生の時代から育成することを目的に、SCRP日本代表選抜大会を本会の重要な国際関連事業のひとつとして位置づけています。これまでのプログラム参加者の多くが現在歯科界の指導的な立場で活躍されていることからも、本年度の参加された皆様も、ご参加で得られた貴重な経験を踏まえ、卒業後は臨床・研究・教育等の様々な分野で活躍されることを期待いたします。

日本代表選抜大会に関係する各方面の方々のご理解・ご尽力に感謝申し上げますと共に、本プログラムが引き続き日本の歯学教育ならびに歯科の国際分野での発展に寄与することを祈念し、挨拶といたします。

ごあいさつ

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４年度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、本年度もCOVID-19による感染が継続していたため、3年連続でオンラインによる審査となりました。

各大学からのスチューデント・クリニシャンの選出に当たっては、学内発表会、関連委員会による推薦、学生による積極的な立候補の学内審査等を実施して大学代表の選抜を行っていただきました。各大学のご協力に厚く御礼申し上げます。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、コロナ禍で様々な制約を受けながらも立派に研究成果をまとめ上げられました。その努力に敬意を表しますと同時に、この度の貴重な経験を卒業後の進路においても活かされることを期待いたします。

本年度日本代表に選ばれたスチューデント・クリニシャンのデュアー ヒューさん（朝日大学歯学部2年生）は、2023年3月に米国オレゴン州ポートランド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。また、この研究発表抄録集には、日本代表として米国大会での発表を終えられた令和2年度（北海道大学：吉野弘菜さん）・令和3年度日本代表（岡山大学：棚井あいりさん）によるAADOCR/SCADA大会（オンライン発表）の参加報告を掲載しました。

このように、本大会で日本代表を選抜することが一つの目的ですが、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。COVID-19の感染が拡大する前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。本年もこのような場が設定できませんでしたが、SCRPの同窓会であるSCADA-Japan（代表：井田有亮先生）によるオンラインでの交流機会を設けていただきました。相互に研究発表の概要を紹介し合い、非常に有意義であったと報告を受けております。

日本歯科医師会や日本歯科医学会の専門学会などの講演会や研修会は、感染対策を行いながら対面での活動が再開されています。次年度の大会は、対面開催すなわちスチューデント・クリニシャン全員が審査員の前で発表する従来の大会運営で実施することを考えています。研究発表をするという普段経験できない緊張感を味わっていただきたいと思います。次回も更に多くの大学から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可能となっておりますので、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。

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Behavioral analysis of mice ingesting NH_4Cl: the effect of ammonia in higher brain functions

新潟大学歯学部 4年生 Niigata University Faculty of Dentistry Class of 2024

横山 望実 Nozomi YOKOYAMA

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研究指導協力者：口腔生化学分野 助教 飯田 和泉

Problem and hypothesis: High protein diets have become popular worldwide to maintain body muscle to stay healthy. However, high-protein diets can elevate blood ammonia levels, and liver failure leads to hyperammonemia that associated with psychiatric abnormalities. Even in our daily life, it is found that blood ammonia can be doubled transiently after meal and workout. However, if transient accumulation and/or intermittent elevation of body ammonia alter neuropsychiatric behaviors are ill defined. In this study, we hypothesized that *'Intermittent elevation of ammonia in the body affects brain function'* and performed behavioral experiments in mice ingesting with NH_4Cl.
Methods: Mice were given 0.28 M NH_4Cl in drinking water for 20 weeks. Body weight, water intake, and blood ammonia levels were measured and behavioral experiments were performed. 5 neuropsychiatric phenotypes; basal activity, anxiety-related behavior, sociality, memory, and spatial learning were chosen, and suitable tests for these phenotypes were performed.
Results: We found that the mice that had a free access to NH_4Cl-containing water have a trend of elevated blood ammonia levels. The elevation of body ammonia slightly reduced the body weight and increased their water intake. From the behavioral experiments, we did not identify altered spatial learning nor spatial working memory. However, we found enhanced anxiety-related behavior from the open field test and Y-maze test.
Conclusion: Elevated ammonia in the body induces anxiety-related behavior.

NH_4Cl摂取マウスの脳高次機能の解析：アンモニア蓄積がもたらす脳への影響

問題：近年、プロテインブームが来ている。しかし、高タンパク食は血中アンモニア濃度を上昇させ、肝障害による高アンモニア血症は精神異常を招く。日常生活でもまた、食後や運動後に血中アンモニアが一時的に上昇する。体内アンモニアの一過性の蓄積や断続的な上昇が脳に与える影響は不明であることから、「体内アンモニアの断続的な上昇は脳機能に影響を与える」との仮説を立て、NH_4Cl摂取マウスの行動解析を行った。
方法：マウスに0.28 M のNH_4Cl水を20週間与え、体重、飲水量、血中アンモニア濃度の計測および行動解析を行った。行動解析では基礎活動、不安関連行動、社会性、記憶、空間学習の5つの表現型に対応する試験を行った。
結果：NH_4Cl摂取マウスは血中アンモニア濃度の上昇傾向があった。アンモニアの上昇により、体重がわずかに減少し、飲水量が増加した。行動解析では空間学習や空間認識記憶に変化は確認されなかったが、オープンフィールドテストとY字型迷路から不安様行動の亢進を認めた。
結論：体内のアンモニアが上昇すると不安様行動が誘発された。

研究発表内容の紹介

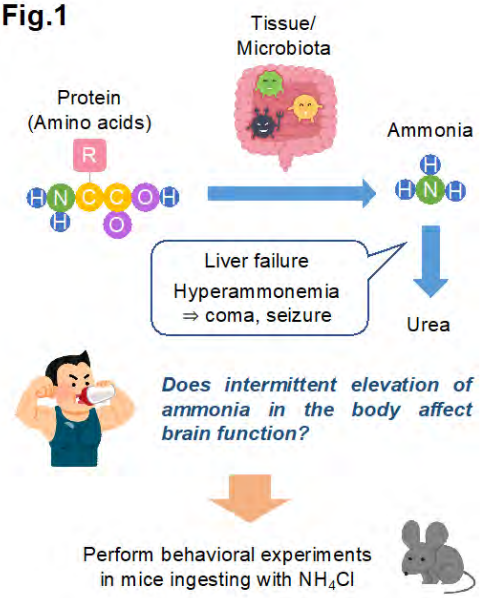
歯科臨床での栄養指導や食事指導の重要性は認識されてきているが、本研究では、最近流行しているプロテインに着目した。プロテインと体臭の関係性について取り上げる記事もあるように、タンパク質の過剰摂取はアンモニアを発生させることで体臭にも影響する。本研究は、体内でのアンモニアの蓄積が脳高次機能に与える影響を調べたものであり、歯科医学に神経科学と栄養学を融合させた新たな研究分野を展開させる重要な研究に位置づけられる。（ファカルティー・アドバイザー：照沼 美穂）

Behavioral analysis of mice ingesting NH_4Cl: the effect of ammonia in higher brain functions

【Problem】

High protein diets have become popular worldwide for the maintenance of muscle to stay healthy. The protein boom has come to Japan, and currently, the market is growing. However, protein intake may result in increased ammonia production. Ammonia is a potent neurotoxin that causes severe damage to the central nervous system. It is formed in nearly all tissues of the vertebrate organism, and is a byproduct of cellular metabolism. Deficient hepatic urea formation and bacterial infection in the gut are the major causes of pathological accumulation of ammonia, which results in hyperammonemia. Interestingly, dietary protein intake and workout also increase blood ammonia levels. However, if transient accumulation and/or intermittent elevation of body ammonia alter neuropsychiatric behaviors are ill defined (Fig 1).

Fig.1



【Hypothesis】

In this study, we hypothesized that ***'Intermittent elevation of ammonia in the body affects brain function'*** and performed behavioral experiments in mice ingesting with NH_4Cl.

【Methods】

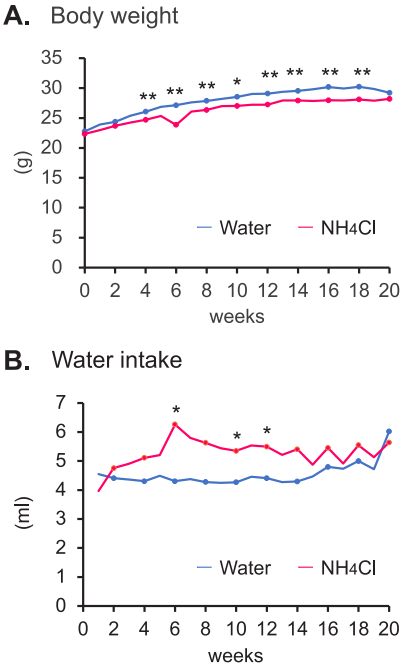
Generation of mice: Eight-week-old male C57Bl6/J mice were given 0.28 M ammonium chloride in drinking water for 20 consecutive weeks. Control mice received tap water. All mice had free access to water bottles.

Behavioral experiments: Body weight, water intake, blood ammonia level and behavioral tests were examined.

Open field test: Mice were placed into the corner of an open field apparatus (50 × 50 × 40 cm) (O'Hara & Co. Tokyo, Japan) and allowed to freely explore for 10 min with a chamber illuminated at 100 lx. During this period, the total movement distance, the time spent in the central region (25% of the total arena), and the time spent in the wall side were recorded and automatically calculated by Image OFCR software.

Y-maze test: The Y-maze apparatus consisted of three arms made of plastic (Fig. 5A) (O'Hara & Co. Tokyo, Japan). Mice were placed into one of the arms of the maze and allowed to explore the maze with one of the arms closed for 15 min (Trial 1). After a 1.5-h interval, mice were returned to the maze by placing them in the start arm. Then, the mice were allowed to explore all three arms for 5 min (Trial 2). Total number of entries, time spent in the novel arm that was closed in Trial 1, and % alternation, the percentage of sequential entries into all three arms, were calculated.

Fig.2



【Results】

1. Ammonia intake resulted in a slight reduction in body weight and an increase in water consumption

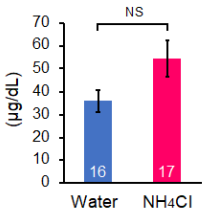
We examined body weight in both control (water) and NH_4Cl-treated mice for 20 weeks. We found that ammonia intake slightly reduced their body weight (Fig. 2A, Water: n = 16, NH_4Cl: n = 18, *p<0.05, **p<0.01, Unpaired t-test).

We also examined their water intake and found that ammonia-treated mice

drink more water than the control mice (Fig. 2B, Water: n = 16, NH_4Cl: n = 18, *p<0.05, **p<0.01, Unpaired t-test).

2. Elevated blood ammonia levels were observed in ammonia-treated mice

Fig.3



After 20 weeks of ammonia ingestion, we found that these mice have a trend of elevated blood ammonia (Fig. 3, Water: n = 16, NH_4Cl: n = 17, NS = not significant, Unpaired t-test). We think mice have some phenotypic variation, such as differences in the amount of water intake or protein metabolism. We are planning to increase the number of animals used.

3. Anxiety-related phenotype was observed in ammonia-treated mice

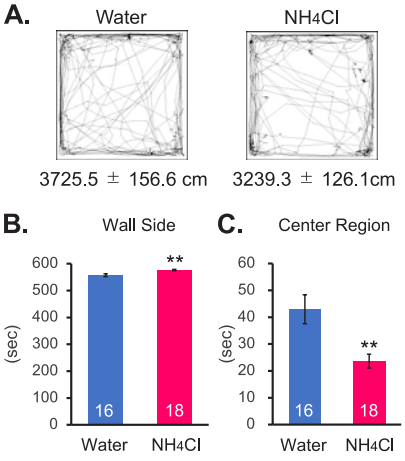
We then performed behavioral tests in these mice. We chose 5 neuropsychiatric phenotypes (basal activity, anxiety-related behavior, sociality, memory, and spatial learning), and examined suitable tests studying these behaviors (Table 1). We identified behavioral phenotypes from the open field test and Y-maze test.

In the open field test, we found that the total distance travelled was slightly reduced in ammonia-treated mice (Fig. 4A). As for anxiety-related behavior, we found that ammonia-treated mice spent more time on the wall side, and less time in the center (Fig. 4B, C, Water: n = 16, NH_4Cl: n = 18, **p<0.01, Unpaired t-test). These data suggested that elevated body ammonia may induce anxiety in mice.

Table1. Behavioral experiments performed

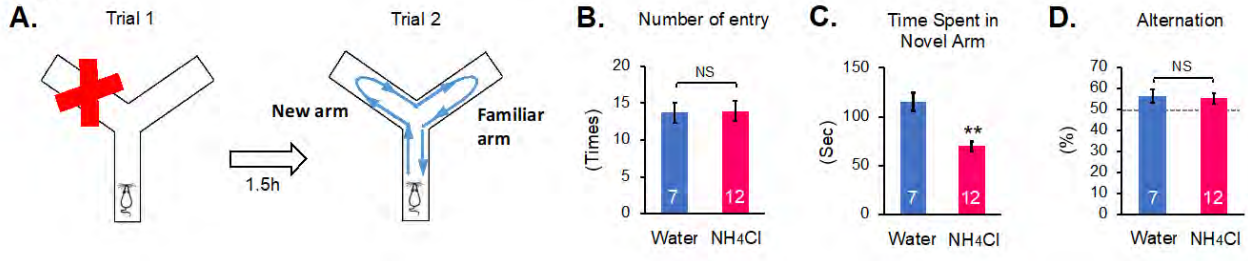
Phenotypes	Test
① basal activity	Home cage monitoring, Open field
② anxiety-related behavior	Open field
③ sociality	Social interaction (Crawley version)
④ memory	Y maze, Cued and contextual fear conditioning
⑤ spatial learning	Y maze

Fig.4



In the Y-maze test, the total number of entries in the arms were similar in both mice (Fig. 5B, Water: n = 7, NH_4Cl: n = 12, NS = not significant, Unpaired t-test). The time spent in the novel (new) arm was shorter in ammonia-treated mice than the control mice (Fig. 5C, **p<0.01, Unpaired t-test). A percentage of alternation were above chance level (50%) in both mice, suggesting that the spatial working memory in ammonia-treated mice are intact (Fig. 5D, NS = not significant, Unpaired t-test). Then why ammonia-treated mice are avoiding to stay in the novel arm? We think that this is due to an elevated anxiety in these mice.

Fig.5



【Conclusion】

Elevated ammonia in the body induces anxiety-related behavior.

In this study, we found that the mice that had a free access to NH_4Cl-containing water have a trend of elevated blood ammonia levels. The elevation of body ammonia slightly reduced the body weight and increased their water intake. From the behavioral experiments, we did not identify altered spatial learning nor spatial working memory. However, we found enhanced anxiety-related behavior from the open field test and Y-maze test.

For the future study, we would like to perform neurochemical experiments to identify the cause of these behaviors.

Application for preventing COVID-19 infection by CO2 measurement in a class room and a dental clinic

北海道医療大学歯学部　4年生　Health Sciences University of Hokkaido　Class of 2024

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研究指導協力者：生体材料工学分野 准教授　根津 尚史

COVID-19 strongly restricted our educational and medical activities. A most valid way to continue these activities without infection might be ventilation of a room by opening a window according to the CO2 environmental standard of a class room and a clinic. However, the ventilation management system using CO2 has not been established yet. While opening window is an only means of ventilation in the most of facilities, it is uncomfortable to keep opening windows during winter season in cold regions and summer season in warm regions. Thus, I fabricated a new device to monitor and record CO2 concentration and developed a management system to predict an optimum ventilation timing scientifically. I analyzed the ventilation condition to keep the CO2 concentration below 1,000 ppm by a computer program based on machine learning with an AI (artificial intelligence) system using CO2 data acquired in a class room. As a result, the timing 5 min before the CO2 concentration exceeded 1,000ppm was predicted by the machine learning using the data for a certain period of time. Thus, in this study, I developed a new management system for appropriate ventilation control to prevent COVID-19 infection and to ensure sustainable educational and medical activities under the current pandemic.

室内CO2濃度測定による新型コロナウィルス対策の検討と実践

COVID-19の感染拡大によりエッセンシャル領域とされる医療・教育おいても活動が大きく制限された。このような状況下で感染を防御しながら活動持続するためには、CO2濃度を指標に換気を的確に行う事が最も有効とされた。しかし換気を的確に管理するする方法は医療・教育機関では確立されていなかった。また多くの施設では換気を窓の開閉に頼らざるを得ず、特に寒冷地の冬場や温暖地の夏場は窓の持続的な開放が難しかった。そこで、CO2濃度を持続的に測定できる測定器を自作し、換気を科学的に管理できるシステムを開発した。講義室および歯科医院でCO2の濃度を測定し、AI（Artificial Intelligence）機能の機械学習によるプログラムを用いて、室内CO2濃度を環境基準の1,000ppm以下に保つ換気条件を解析した。その結果一定期間の測定データを機械学習することにより1,000ppmを超える時間を予想できる事が明らかになった。そして今回の測定条件では5分前にその予想が可能となった。従って、この管理システムの開発により、換気によって感染防御をしながら的確に医療・教育活動を持続することが可能となった。

研究発表内容の紹介

新型コロナウイルス感染防御のためにCO2測定機を用いた換気管理システムの開発を行った。CO2濃度を継続的に測定し、AI機能の一つである機械学習を利用した解析により、有効な換気のタイミングと時間の予想を行った。その結果、CO2濃度の変化を測定し窓の開閉を的確に行うだけで、部屋の温度を維持しながら換気を適切に管理できることが明らかになった。これにより講義室や歯科医院でも簡単に、持続的に感染防御管理ができるようになった。

（ファカルティー・アドバイザー：荒川 俊哉）

Application for preventing COVID-19 infection by CO2 measurement in a class room and a dental clinic

Problem- Under COVID-19 pandemic, any kind of activities were limited in all fields including medical care and education. However, people required continuous services to medical care and education because these services were essential and could not stop for our life. The ventilation based on the CO2 environment standard was the most effective way to keep these services under preventing the infection. However, the management of the ventilation using CO2 monitoring system was not established yet. Furthermore, a window ventilation but a machine ventilation was only ventilation in the most of facilities and it was uneasy to open a window during winter season in a cold region and summer season in a warm region. Thus, the suitable ventilation system based on scientific and visible analyses was required to keep services.

Hypothesis- It becomes possible to establish the controlling system to the infection if the timing of ventilation is accurately regulated by the continuous CO2 monitoring. And to do that,

1) The CO2 monitoring devices which continuously record the CO2 data are needed.
2) The method to predict the timing of increment based on CO2 environment standard should be established using CO2 monitoring data.

Methods-Four studies were investigated to prove the hypothesis above.

1) Developing original CO_2 monitoring devices
2) Searching the optimized ventilation frequency and duration in a class room (Fig. 1)
3) Prediction for the increment of CO_2 concentration by AI
4) Applying CO_2 concentration monitering at some general clinics

Fig. 1

Result-1:

The device which had continuously monitored CO2 concentration was not made commercially available to the public, so I made the CO2 monitoring devices by myself. I designed the electric circuitry and substrate using a commercially supplied software, 'Eagle' from AutoDesk Co and the designed electric substrate were ordered to PCBGOGO Co. The casing of device was designed using Fusion 360 from AutoDesk Co and the designed case was made by 3D printer from XYZ Co. The two kinds of NDIR (non dispersive infrared)-based CO2 sensor was utilized as a recommended parts by the guideline of the Economy, Trade and Industry Ministry, Japan, Z19B (Fig.2-1-A) from Zhengzhou Winsen Electronics Technology Co and S8 (inside of the device of Fig.2-3) from SenseAir Co. The ESP32-WROOM-32D (Fig.2-1-B) was also utilized as the control unit for Wi-Fi and was able to send CO2 data through the internet. Using these electronic parts, I made two types of devices. The first type of device had Z19B CO2 sensor and LED alarm lamp +LED (Fig.2-2); the second type of device had S8 CO2 sensor and big LED display (Fig.2-3).

Fig. 2-1

Fig. 2-2

Fig. 2-3

Result-2: The verification experiment for the need and timing of ventilation was conducted under the conditions with/without ventilation in class rooms using the newly developed CO2 monitoring device. I compared the difference in three class rooms. In class 1 (Fig.3-1), the difference of CO2 concentration was little and also the value was small. This observation suggested that the capacity of the room might be large and the machine ventilation might work well in class1. In contrast, the value of CO2 was constantly high and then quickly reduced at a certain point in time in class 2 (Fig.3-2). The machine ventilation in class 2 might not work well so the CO2 concentration kept high although class 2 is larger than class 1. It seemed that the CO2 concentration was drastically down after all students leaved the class. Next the effect of ventilation was compared in class 3 (Fig.3-3). The 10 min window ventilation effectively reduced CO2 concentration below 1,000ppm compared to the condition without ventilation. Thus, this observation indicated that the constant ventilation was effective to keep CO2 environment standard.

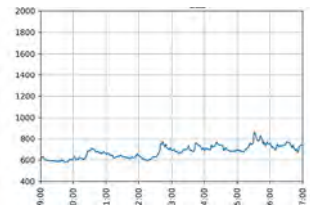

Fig.3-1

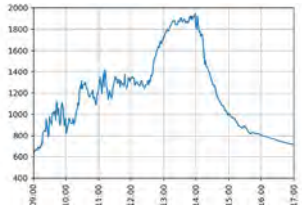

Fig.3-2

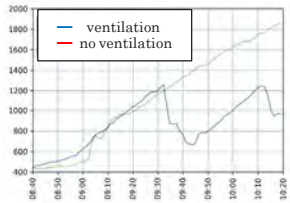

Fig.3-3

Result-3: I created a predictive model for the increase in CO2 concentration using AI. AI, artificial intelligence is a powerful tool to analyze any kinds of big data in social activities and learns a statistic relations using the vast sets of data. Using this AI system, a model to predict a future incidence of CO2 concentration was created. As a result of prediction using this model, it was possible to predict that CO2 concentration would exceed 1,000 ppm 5 minutes in advance (Fig. 4-1, 4-2).

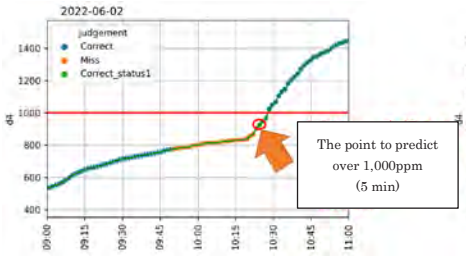

Fig.4-1

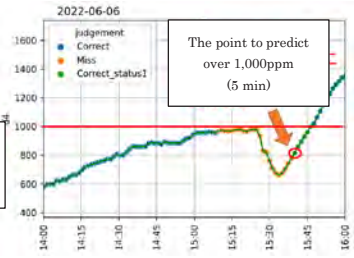

Fig.4-2

Result-4: The CO_2 monitoring devices were set in three medical facilities (Fig.5-1, 5-2, 5-3). CO2 concentration was always below 1,000ppm in all day. These results indicated that the ventilation were sufficient in all facilities. Thus, the contenius CO2 measurement were important to keep safety environment in medical facilities.

Fig.5-1

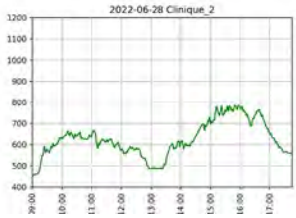

Fig.5-2

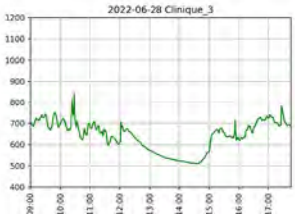

Fig.5-3

Conclusion- It was revealed that opening a window was sometime more effective in a certain condition to keep CO2 concentration below 1,000ppm although the continuous machine ventilation was also important. The timing that CO2 concentration would exceed 1,000 ppm was predicted by AI system. The ability of ventilation would be sufficient in surveyed medical facilities in which CO2 concentration was always below 1,000ppm in all day. Thus, in this study, we developed the new management system for appropriate ventilation control to prevent COVID-19 infection using CO2 sensor and it was able to ensure sustainable educational and medical activities during infection.

Development of novel 3D-printable resin composite for tooth restorative material

九州歯科大学 3年生 Kyushu Dental University Class of 2025

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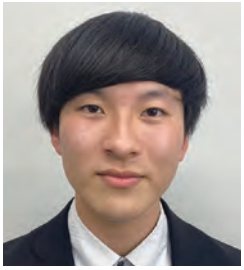

Additive manufacture (3D-print) materials have been growing attention for dental applications. However, 3D-printable resin composite for a tooth restoration has not been established yet. This study aimed to develop novel 3D-printable resin composite for tooth restorative material. The 3D-printable resin composite was prepared in our laboratory. Appropriate amount of silanized silica particles, resin monomers, photo-initiator, and photo-absorber were mixed to prepare the 3D-printable resin composite. The prepared 3D-printable resin composite was printed by using SLA 3D-printer. The 3D-printed resin composite was characterized SEM observation, mechanical property, physicochemical properties, and printing accuracy. The SEM result indicated that the fillers were well dispersed in the resin matrix without any aggregates up to 70 wt% filler content. The mechanical properties (Vickers hardness, elastic modulus, and flexural strength) increased with increasing filler content. The mechanical properties of the sample with 70wt% filler were significantly higher than those of commercial CAD/CAM resin composite. The physicochemical properties (water sorption and water solubility) of the 3D-printed resin composite were superior than those of commercial CAD/CAM resin composite. The printing discrepancy of the 3D-printed crown was relatively low. The 3D-printable resin composite was demonstrated to apply the 3D-printed crown for tooth restoration.

3Dプリント可能な新規歯冠修復用コンポジットレジンの開発

近年、3Dプリント（積層造形または付加造形）によって造形できる歯科材料が注目されている。一方、3Dプリントできる材料は少なく、CAD/CAM切削加工用ブロックと比べると機械的性質や物理化学的性質は低いなどの課題がある。本研究では、3Dプリント冠の実現を目指し、3Dプリント可能な新規歯冠修復用コンポジットレジンの開発に取り組んだ。3Dプリント用コンポジットレジンは独自に作製した。シリカ粒子、UDMA、TEGDMA、光重合開始剤、光吸収剤を所定の重量比で混合することで新規3Dプリント用コンポジットレジンを得た。調製した新規3Dプリント用コンポジットレジンを市販のSLA-3Dプリンターを用いて所定の形状に造形した。造形したコンポジットレジンは、機械的性質、物理化学的性質、および造形精度について評価した。フィラーの含有量が70wt%までは、フィラー同士が凝集することなく均一に分散したコンポジットレジンが得られた。3Dプリントした新規コンポジットレジンの機械的性質（ビッカース硬さ、弾性係数、および曲げ強さ）はフィラー含有量が増加するに従って増加し、70wt%のフィラーを添加した試料の値は、比較試料として用いた市販のCAD/CAM冠用コンポジットレジンと同等または有意に大きな値であった。3Dプリントした新規コンポジットレジンの物理化学的性質（吸水量と溶解量）は、市販のCAD/CAM冠用コンポジットレジンより優れていた。3Dプリント冠とSTLデータとの形状誤差は比較的低く、多くの箇所で0.1mm以下であった。以上の結果より、新規3Dプリント用コンポジットレジンを開発し、3Dプリント冠への適用可能性が示された。

研究発表内容の紹介

3Dプリント（積層造形または付加造形）は、歯冠修復物の作製に適していると考えられていますが、暫間修復を除き、実用的な材料がないことが課題です。本研究では、3Dプリントに適した新規コンポジットレジンを開発しました。この新規コンポジットレジンは優れた機械的性質と物理化学的性質をもっており、歯冠修復物として用いることが可能と考えられます。したがって本研究では、3Dプリント冠の実現に大きく貢献できる研究であると考えています。（ファカルティー・アドバイザー：池田 弘）

Development of novel 3D-printable resin composite for tooth restorative material

(Problem)

Additive manufacture (3D-print) materials have been growing attention for dental applications. Among various 3D-printing processes, stereolithography (SLA) 3D-printing is popular for producing dental prosthesis because of its highly accuracy of printed objects. On the other hand, there are few practical materials for the SLA 3D-printing to use as a tooth restorative material. Mechanical and physicochemical properties of the SLA 3D-printable materials are inferior than those of CAD/CAM milled material. Therefore, 3D-printed materials for permanent crowns have not been established yet.

(Hypothesis)

This study aimed to develop novel 3D-printable resin composite for tooth restorative material. The research hypothesis is that practical 3D-printable resin composite with adequate mechanical and physicochemical properties can be prepared from appropriate amounts of silica fillers and acrylic resin.

(Methods)

1. Preparation of 3D-printable resin composite

The 3D-printable resin composite was prepared in our laboratory. Silanized silica particles (mean diameter 0.5 μm, Admstechs), urethanedimethacrylate (UDMA, Fujifilm Wako Pure Chemical), Triethyleneglycoldimethacrylate (TEGDMA, Fujifilm Wako Pure Chemical) were mixed using a centrifugal mixer with different filler contents (0, 20, 40, 60, 70, and 80wt%). The resultant mixture was further mixed with photo-initiator (BAPO, Tokyo Chemical Industry) and photo-absorber (TBT, Tokyo Chemical Industry). Subsequently, micro-bubbles in the mixture were eliminated by centrifugal separation. As a result, the 3D-printable resin composite was obtained.

2. SLA 3D-printing

STL data (bar-shape and crown shape) was created using a 3D-CAD software. The prepared 3D-printable resin composite was printed by using SLA 3D-printer (ELEGOO MARS, λ = 400–410 nm 24 W LED laser, ELEGOO). The printed object was cleaned with alcohol by ultrasonication to eliminate unpolymerized resin. Subsequently, the cleaned object was post-cured by UV irradiation for 5 min. Finally, the 3D-printed object consisted with the resin composite was obtained.

3. Evaluation

The bar-shaped objects were used for SEM observation, mechanical and physicochemical tests (ISO6872: 2015). The mechanical properties were characterized by Vickers hardness, elastic modulus, and flexural strength. The physicochemical properties were characterized by water sorption and solubility. For control sample, commercial CAD/CAM resin composite block (Shofu block HC, Shofu) was used. The mechanical properties were compared by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$). The physicochemical properties were compared with student's t test ($p < 0.05$). The 3D-printed crown was evaluated by printing accuracy test. The STL data and 3D-printed crown was superimposed using a software to confirm a discrepancy between each shape.

(Results)

Fig. 1 shows SEM images of the 3D-printed resin composites. It was clearly seen the dispersed-filler microstructure consisted with spherical silica and resin matrix. Up to 70 wt% of filler content, the silica fillers are well dispersed in the resin matrix without any aggregates. For the 80 wt% filler content, however, the sample could not be prepared because the fillers were aggregated with the resin matrix.

The mechanical properties were characterized in each sample with filler content below 70 wt% (Fig. 2). Each mechanical property increased with increasing the filler content. The mechanical properties of the sample with 70 wt% filler content were comparable or significantly higher than those of the commercial CAD/CAM resin composite. The 3D-printed resin composite with 70 wt% filler was further characterized by the physicochemical properties with comparison of those of the commercial CAD/CAM resin composite (Fig. 3). The water sorption and water solubility of the 3D-printed resin composite were significantly lower than those of the commercial CAD/CAM resin composite, indicated that the physicochemical properties superior than those of the CAD/CAM resin composite.

By using the 3D-printable resin composite, the 3D-printed crown was fabricated via SLA 3D-print. Fig. 4 shows the superimposed images of STL data and measured 3D-printed crown. Shape difference between the STL data and the 3D-printed crown were almost below 0.1 mm. On the other hand, the shape of the 3D-printed crown had a relatively larger discrepancy on the inner surface and occlusal area, with a maximum difference of about 0.5 mm.

The 3D-printable resin composite with filler content of 70 wt% has potential to be used as an indirect tooth restorative material since the mechanical and physicochemical properties are superior than those of commercial CAD/CAM resin composite. Further study is needed to determine long-term durability of the 3D-printable resin composite. In addition, the 3D-printing accuracy of the crown is insufficient for clinical use. The printing accuracy should be improved by optimizing printing conditions such as printing direction and layer thickness.

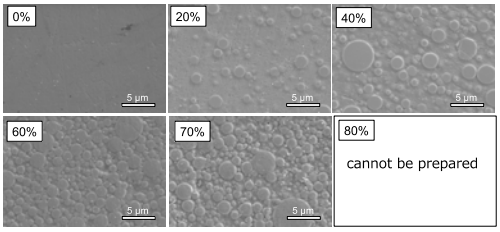

Fig.1 SEM images of 3D-printed resin composites with given filler contents.

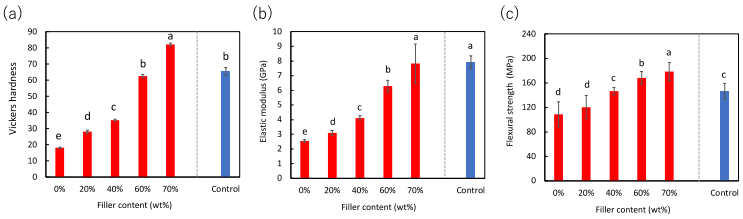

Fig. 2 Mechanical properties of the 3D-printed resin composites: (a) Vickers hardness, (b) elastic modulus, (c) flexural strength.

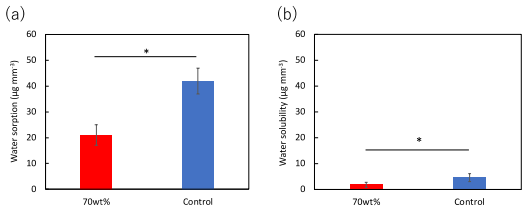

Fig. 3 Physicochemical properties of the 3D-printed resin composite: (a) water sorption, (b) water solubility.

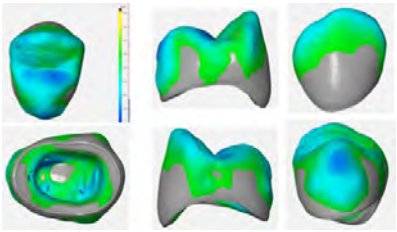

Fig. 4 Printing accuracy of 3D-printed crown. The color map was obtained by superimposing the STL data and measured surface profile.

(Conclusion)

Within the limitation of this study, we concluded that:

- Novel 3D-printable resin composite was prepared from 70 wt% filler and 30 wt% resin.
- The mechanical and physicochemical properties of the 3D-printable resin composite were higher than those of commercial CAD/CAM resin composite.
- The 3D-printed crown can be fabricated using the resin composite via SLA 3D-printing.

Zoledronate negatively regulates T cell-mediated immune responses

東京歯科大学　4年生　Tokyo Dental College　Class of 2024

冨秋 智博　Chihiro TOMIAKI

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研究指導協力者：口腔科学研究センター 講師　大野 建州
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Problems: Medication Related Osteonecrosis of the Jaw（MRONJ）is a disease caused by invasion for the jawbone under the use of bone resorption inhibitors or angiogenesis inhibitors. The pathogenesis of MRONJ remains unclear. Prevention methods for the development of MRONJ have not yet been established. In addition, risk factors for the development of the disease have not yet been elucidated.
Methods: We performed our experiments on MRONJ model mouse based on previous reports. Briefly, MRONJ was induced by ZOL administration, ligature-induced periodontitis, and subsequent tooth extraction. Jawbone was collected to evaluate for osteonecrosis induction by H-E staining. In addition, T cell activity in the regional lymph nodes was evaluated by flow cytometry and cytokine expression in the gingiva was measured by PCR. Finally, the effect of ZOL on T cell and DC activity was evaluated based on the effect of the addition of ZOL on T cell proliferation and co-stimulatory molecule CD86 expression on DCs.
Results: Osteonecrosis of jawbone was not induced in our setting. In addition, ZOL treatment did not affect cytokine expression in gingiva. ZOL treatment significantly reduced the percentages of IFN-γ and IL-17 expressing $CD4^+$ T cells and IFN-γ expressing $CD8^+$ T cells. Moreover, ZOL does not directly inhibit T cell activity, but ZOL directly suppress DC antigen presenting capacity.
Conclusion: These results suggest ZOL directly attenuates T cell activity by inhibiting the antigen presenting capacity of DCs, and suppresses immune responses.

ゾレドロン酸はT細胞依存的な免疫応答を負に制御する

問題点：Medication Related Osteonecrosis of the Jaw（MRONJ）は骨吸収阻害薬や血管新生阻害薬の使用下に顎骨に侵襲が加わることによって発症する疾患である。その病態誘導機構に関しては不明な点が多く残されており、予防方法の確立や発症リスク因子の解明には至っていない。
方法：MRONJマウスモデル作成は過去の文献を一部改変して行った。マウスへのゾレドロン酸、結紮による歯周炎誘導および抜歯によりMRONJを誘導した。顎骨は病理組織標本を作製し腐骨形成を、歯肉はPCRによるサイトカイン発現を、所属リンパ節中のT細胞活性はフローサイトメトリーによって、評価した。
結果：すべてのマウスにおいて腐骨形成は誘導されなかった。ZOLは抜歯窩周囲のサイトカインの発現に影響を与えなかった。ZOL投与はCD4陽性T細胞中のIFN-γおよびIL-17陽性率およびCD8陽性T細胞中のIFN-γ陽性率を有意に減少させていた。また、ZOLは細胞の増殖には影響を与えず、樹状細胞のCD86発現を減少させた。
結論：これらの結果から、ZOLは樹状細胞の抗原提示を抑制することによりT細胞の活性化を阻害することで、抜歯後の免疫反応が抑制されることが示唆された。

研究発表内容の紹介

MRONJの発症機序は不明な点が多く、診断や治療に苦慮する機会が多い。本研究はBP製剤投与環境下における免疫応答に着目し、特にBP製剤の免疫細胞に与える影響について研究を行った。その結果、MRONJモデルマウスにおいて、BP製剤は樹状細胞の抗原提示機能を阻害することでT細胞活性を低下させることを明らかにした。これらの知見は、BP製剤は従来の骨代謝の阻害に加え、直接的に抜歯後の顎骨免疫を抑制していることが示唆され、MRONJ発症機序解明の一助になると考える。
（ファカルティー・アドバイザー：片倉 朗）

Zoledronate negatively regulates T cell-mediated immune responses

(Problem)

Medication Related Osteonecrosis of the Jaw (MRONJ) is a disease caused by invasion for the jawbone under the use of bone resorption inhibitors or angiogenesis inhibitors. One of the most common drugs that induce MRONJ are bisphosphonates (BPs). BPs are used in the treatment of osteoporosis and bone metastases of malignant tumors. BPs are localized on bone and are taken by osteoclasts to induce osteoclast apoptosis. On the other hand, apoptosis of osteoclast affects bone metabolism by inhibiting osteoblast activity. Although there have been many reports on MRONJ, the detail of pathogenesis of MRONJ remains unclear. Prevention methods for the development of MRONJ have not yet been established. Moreover, risk factors for the development of the disease have not yet been elucidated.

(Hypothesis)

MRONJ is known to be caused not only by additional jawbone involvement such as tooth extraction, but also by inflammation such as chronic periodontitis. In addition, immune-related disease states and therapeutic agents for them, are risk factors for MRONJ. So, we hypothesized that inflammatory response may trigger for MRONJ in a state of impaired bone metabolism. Furthermore, since osteoclasts are differentiated from monocyte-macrophage lineage, we also hypothesized that BPs may regulate immune cell function.

(Methods)

In vivo

We generated MRONJ murine model following previous report. Mice were treated with or without (125 μg/kg) of Zoledronate (ZOL) twice a week. Maxillary second molar of mice was ligated with 5-0 silk threads 1 week after the first ZOL treatment. Three weeks after the first ZOL treatment, ligated the teeth was extracted. We collected gingiva, jawbone, and reginal lymph nodes (submandibular LNs) for measuring cytokine expression by qRT-PCR, analysis of T cell activity by FACS, respectively (Fig. 1).

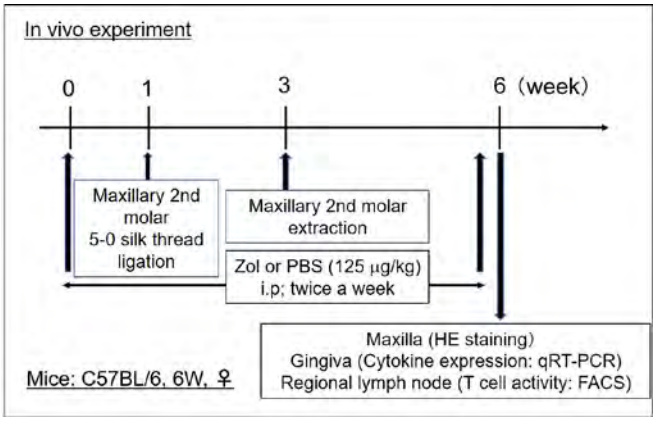

Figure 1. *In vivo* experiment

In vitro

1. **We isolated T cells from murine spleen and lymph nodes, stimulated with anti-CD3/CD28 antibody in the presence or absence of ZOL, and evaluated T cell proliferation by BrdU uptake assay.**

2. **We isolated dendritic cells from spleen, stimulated with LPS in the presence or absence of ZOL, and evaluated the expression levels of DC activation markers, CD86, B7-DC, and MHC class II by using FACS.**

(Results)

① ZOL treatment did not induce osteonecrosis in this model

Osteonecrosis of jawbone was not induced in our setting. In addition, ZOL treatment did not affect both pro-inflammatory (IL-1β, IL-6, IL-33, TNFα, IFN-γ) and anti-inflammatory (IL-10, TGF–β) cytokine expression in gingiva.

② ZOL treatment decreased effector T cell numbers in regional lymph nodes

We analyzed T cell activation status in the regional lymph nodes 2 weeks after tooth extraction by FACS. ZOL treatment significantly reduced the percentages of IFN-γ and IL-17 positive $CD4^+$ T cells and IFN-γ positive $CD8^+$ T cells. These results suggest that ZOL treatment suppresses T cell activity after tooth extraction (Fig. 2).

③ The addition of ZOL does not directly affect T cell proliferation in vitro

T cells were isolated from spleen and lymph nodes of mice, and T cell proliferation was evaluated after anti-CD3/CD28 antibody stimulation in the presence or absence of ZOL. The addition of ZOL had no effect on T cell proliferation. This result suggests that ZOL does not directly inhibit T cell activity.

④ The addition of ZOL directly inhibits dendritic cell activation in vitro

Dendritic cells were isolated from spleen and stimulated with LPS in the presence or absence of ZOL. The co-stimulatory molecules CD86 and B7-DC and MHC class II expression were evaluated by FACS. (Fig. 3).

The addition of ZOL decreased CD86 and MHC class II expression on dendritic cells, suggesting that ZOL directly suppress DC antigen presenting capacity.

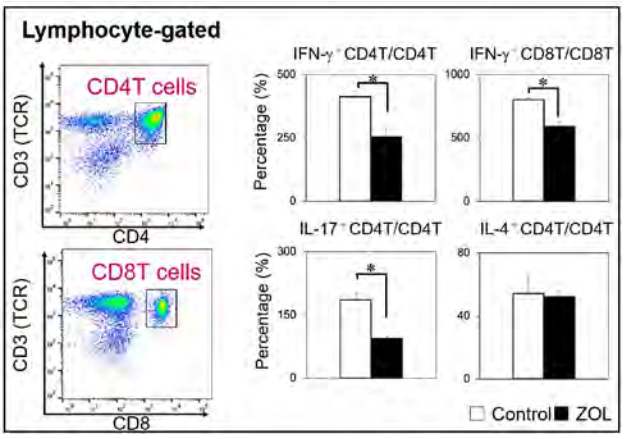

Figure 2. ZOL suppresses T cell activity

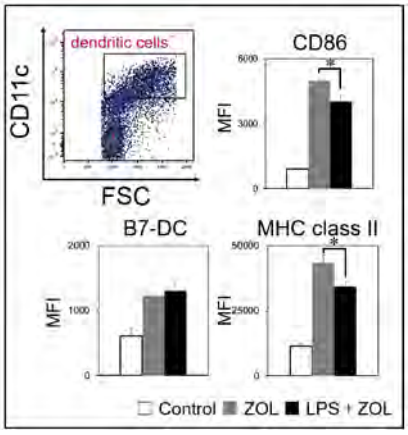

Figure 3. ZOL suppresses DC activity

(Conclusion)

① ZOL treatment impaired T cell activity in reginal lymph nodes after tooth extraction.

② Addition of ZOL did not affect T cell activity in vitro.

③ Addition of ZOL directory decreased CD86 and MHC II expression in vitro.

These results suggest ZOL directly attenuates T cell activity by inhibiting the antigen presenting capacity of DCs to suppress immune responses after tooth extraction (Fig. 4).

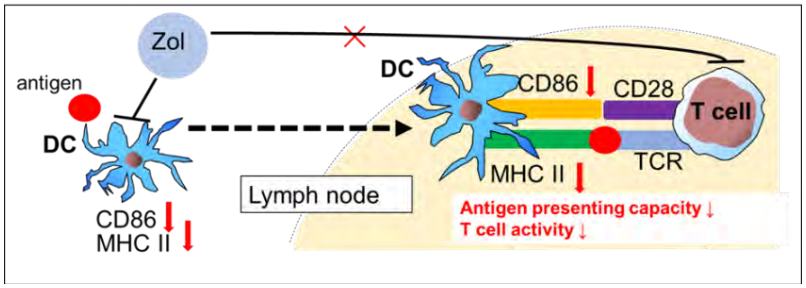

Figure 4. Summary

The effect of iberin on CXCL10 production in human oral epithelial cells

徳島大学歯学部 4年生 Faculty of Dentistry Tokushima University Class of 2024

佐藤 朱里 Juri SATO

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CXC chemokine ligand 10（CXCL10）plays a pivotal role in the recruitment of Th1 cells, and it is certain that Th1 cells are involved in the pathogenesis of periodontitis. Iberin, the bioactive substance derived from cabbage, has some beneficial effects, but the effects of iberin on CXCL10 production in human oral epithelial cells is uncertain. In this study, we investigated the mechanisms by which iberin inhibit TNF-α-induced CXCL10 production in TR146（human oral epithelial cell line）. TR146 was stimulated with TNF-α for 24 hours. CXCL10 concentration of the culture supernatants was measured with enzyme-linked immunosorbent assay（ELISA）. In some experiments, we pre-incubated TR146 with iberin prior to the stimulation.NF-κB p65, IκB-α, or signal transducer and activator of transcription（STAT）3 phosphorylation, which were related to CXCL10 production from TNF-α-stimulated TR146, were detected by western blot analysis. Iberin treatment prevented TNF-α-mediated CXCL10 production in TR146. Iberin pre-treatment significantly inhibited TNF-α-induced phosphorylation of NF-κB p65, IκB-α, and STAT3. These data provide a novel mechanism through which iberin can provide direct benefits in periodontal disease to inhibit CXCL10 production.

イベリンがヒト口腔上皮細胞のCXCL10産生に及ぼす影響の解析

【目的】CXCL10は歯周炎の病態進行に関与しているTh1細胞浸潤に関与しているケモカインであり、TNF-α刺激されたヒト口腔上皮細胞から産生される事が明らかとなっている。イベリンはブロッコリーなどの緑黄色野菜に含まれている生理活性物質であるが抗炎症作用に関する報告は少なく不明な点が多い。そこで我々はイベリンがTNF-αが口腔上皮細胞に誘導するCXCL10産生に及ぼす影響を解析した。

【材料および方法】TR146（口腔上皮細胞株）をTNF-αで刺激し、CXCL10産生をELISA法にて、CXCL10産生に関与しているシグナル伝達経路であるNF-κBとSTAT3の活性化に及ぼす影響をwestern blot法にて解析した。

【結果および考察】イベリンはTR146のNF-κBおよびSTAT3の活性化を抑制することによりTNF-α誘導CXCL10産生を抑制する事が明らかとなった。イベリンは歯周炎病変局所でCXCL10産生を抑制する事によりTh1細胞浸潤を減少させ歯周炎の増悪を抑制できる可能性が示唆された。

研究発表内容の紹介

歯周炎治療において抗菌薬の局所投与は炎症を抑えるのに有効な方法である。しかしながら、耐性菌や副作用の問題があり新たな抗炎症作用を持つ生理活性物質の使用が望まれている。本研究で用いたイベリンはブロッコリーなどの緑黄色野菜に含まれる生理活性物質であり、今回の研究により歯周組織構成細胞において抗炎症作用を示すことが明らかとなった。ゆえにイベリンは抗菌薬に代わる歯周炎治療薬になりうる可能性が本研究で示されたと考えている。（ファカルティー・アドバイザー：細川 義隆）

The effect of iberin on CXCL10 production in human oral epithelial cells

(Problem)

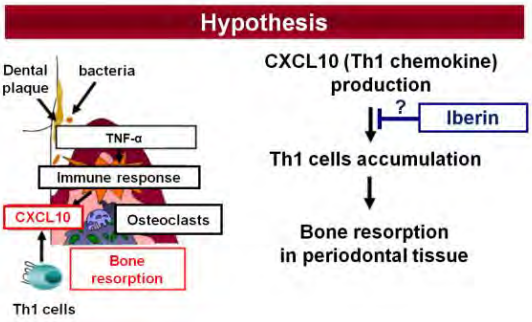
Periodontitis is a chronic bacterial infection of tooth-supporting structures. It causes the destruction of periodontal connective tissues and alveolar bone. The initiation and progression of periodontal disease result from the host response to periodontopathic bacteria. Immunohistochemical studies have demonstrated dense inflammatory cells infiltration, including T cells, B cells and macrophages in periodontal lesion. It has been reported T cells and B cells in periodontally diseased tissues expressed receptor activator of NF-κB ligand (RANKL), which is an osteoclast differentiation factor. Therefore, it is believed that T cells and B cells are related to bone destruction in periodontal tissues, and several studies demonstrated that Th1 cells are especially involved in bone resorption in periodontal disease.

CXCL10 is a chemokine that was discovered as an IFN-gamma-inducible protein of 10kDa in the monocytes. It is known that CXCL10 attracts activated Th1 cells through interaction with CXC chemokine receptor 3 (CXCR3). In vivo, enhanced levels of CXCL10 have been reported in human periodontally diseased tissues. It is reported that inflammatory cytokines, such as TNF-alpha or IL-27, induced CXCL10 production in human oral epithelial cells.

Recently, it has been reported bioactive substances in green and yellow vegetables have anti-inflammatory activity. Iberin, which is included in broccoli and cabbage, has some bioactive effects such as anti-cancer, anti-microbial. However, it is uncertain about the bioactive effects of iberin on periodontal resident cells, and there are no attempts to use iberin for periodontal disease treatment.

(Hypothesis)

We hypothesized iberin might suppress inflammatory reaction in periodontal lesion. We focused on CXCL10 production in this study. The aim of this study was to examine the effect of iberin on CXCL10 production in TNF-α-stimulated-human oral epithelial cells. Moreover, we investigated whether iberin treatment modified NF-κB or STAT3, which are related to CXCL10 production in TNF-α-stimulated-human oral epithelial cells, pathways activation.

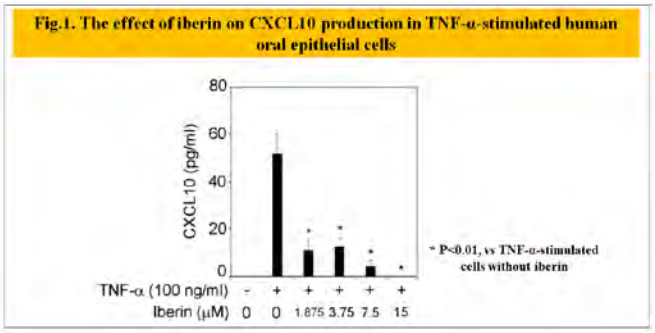


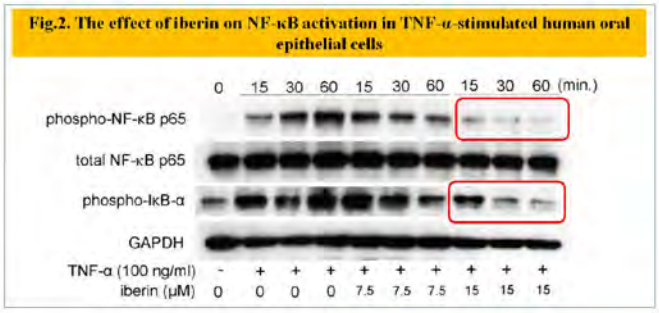
(Methods)

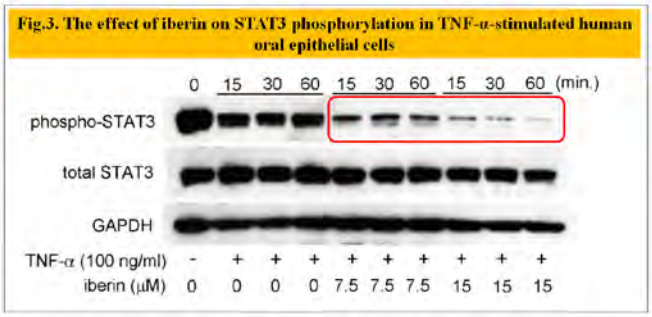
TR146 cells, which are a human oral epithelial cell line, were kindly provided by Dr. Mark Herzberg (University of Minnesota, MN, USA). TR146 cells were cultured in Ham's F12 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified air with 5% CO_2. When the cells reached subconfluence, they were harvested and subcultured. TR146 cells were stimulated with TNF-α (100 ng/ml) with or without iberin for 24 hours. The culture supernatants were collected, and CXCL10 concentration was measured with enzyme-linked immunosorbent assay (ELISA). In some experiments, TR146 cells were stimulated by TNF-α (100 ng/mL) for 15, 30, or 60 min. with or without iberin (7.5 or 15 μM) pretreatment for 1 hour. Then, we collected total proteins from the samples, and NF-κB p65, IκB-α, and STAT3 phosphorylations in TR146 cells were detected by western blot analysis.

(Results)

TNF-α (100 ng/ml) stimulation induced CXCL10 production in TR146 cells. ELISA analysis revealed iberin significantly suppressed CXCL10 production in TNF-α-stimulated TR146 cells (Fig.1). Moreover, western blot analysis showed iberin (15 μM) inhibited NF-κB p65 and IκB-α phosphorylation in TNF-α-stimulated TR146 cells (Fig.2). Furthermore, we found that iberin (7.5 and 15 μM) treatment inhibited STAT3 phosphorylation in TNF-α-stimulated TR146 cells (Fig.3).

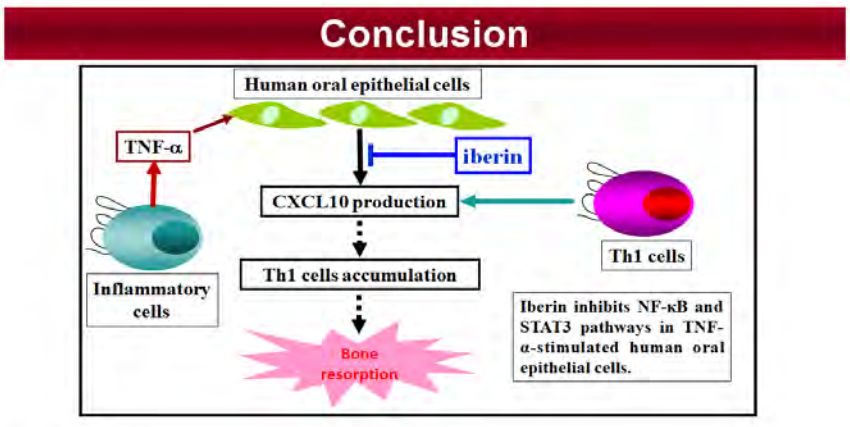






(Conclusion)

In conclusion, the current study demonstrates that TNF-α induces CXCL10 release by cultured TR146 cells. Iberin suppressed TNF-α-induced CXCL10 production in TR146 cells. In addition, we revealed iberin inhibited TNF-α-induced NF-κB and STAT3 activation in TR146 cells. These data show that iberin treatment, such as periodontal pocket irrigation or local drug delivery system, might inhibit Th1 cells migration in periodontally diseased tissues. That means that iberin could suppress periodontal bone destruction to inhibit Th1 cells activation in periodontal lesion. These data provide a novel mechanism through which the bioactive substance in green and yellow vegetables could be used to provide direct benefits in periodontal disease.



Periostin is a critical factor for tooth and bone development

昭和大学歯学部　5年生　Showa University School of Dentistry　Class of 2023

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研究指導協力者：歯学教育学講座 准教授　坂井 信裕
歯科薬理学講座 教授　高見 正道

The question "What mechanisms are involved in tooth development and maintenance of homeostasis?" lies at the core of basic dentistry and also has important disease-related implications in clinical settings. The present study was conducted to identify novel factors involved in tooth formation and homeostasis. Examinations of periostin-knockout (KO) medakas with various gene modifications (transgenic) maintained in the "gene-modified medaka library" of our institution revealed those with abnormal pharyngeal teeth, which are a collection of small teeth located in the posterior part of the pharynx. Pharyngeal teeth of periostin-KO medakas were smaller as compared to those in the wild-type, including shorter size and a reduced enamel area, whereas attachment bone supporting the teeth was increased. Based on those results, periostin-KO mice were analyzed and found to have shorter incisors, loss of pulp with increased dentin, and discontinuous enamel formation as compared to the wild-type. In addition, roots of molars were enlarged and alveolar bone thickness was markedly increased. These results indicate that morphological abnormalities in teeth and surrounding bone tissue are similar in periostin-KO medakas and mice, suggesting that periostin plays an important role in tooth and bone homeostasis in vertebrates.

ペリオスチンは歯と骨の形成に必須の因子である

「歯はいかなるメカニズムで発生し、その恒常性を維持しているのか?」これは、基礎歯科医学の中核に位置する問いであり、歯科臨床においても疾患に関連する重要な意味をもつ。本研究では歯の形成やその恒常性維持に関わる新規因子を同定することを目的とした。我々は「遺伝子改変メダカライブラリー」と呼ばれる様々な遺伝子を改変（ノックアウト：KOおよびトランスジェニック）したメダカの中から歯（咽頭歯）に異常が生じるペリオスチン-KOメダカを見出した。咽頭歯はメダカの咽頭後部に存在する小さな歯の集合体である。このメダカの咽頭歯は野生型よりサイズが小さく、歯が短く、エナメル質領域が減少したが、歯を支持する歯足骨は増加した。この結果に基づいてペリオスチン-KOマウスを解析したところ、野生型より切歯が短く、象牙質の増加に伴う歯髄の消失と、エナメル質の不連続形成が認められた。また臼歯の歯根部が肥大化し、歯槽骨の厚さが著しく増加した。以上の結果は、歯と周辺の骨組織に生じた形態異常がペリオスチン-KOメダカおよびマウスで類似していることを示しており、ペリオスチンが脊椎動物の歯と骨の恒常性に重要な役割を担っていることが示唆された。

研究発表内容の紹介

歯の発生やその恒常性維持のメカニズムを解明することは基礎歯科医学の中核的な課題であり、歯科臨床においても疾患に関連するため重要な意味をもつ。本研究では遺伝子改変メダカおよびマウスを用いた方法により、新規因子としてペリオスチンを同定した。ペリオスチン-KOメダカの咽頭歯は小さく、エナメル質領域が減少し、歯を支持する歯足骨が増加した。ペリオスチン-KOマウスも切歯が短く、象牙質の増加、歯髄の消失、エナメル質形成不全が認められた。以上より、ペリオスチンが脊椎動物の歯と骨の恒常性に重要な役割を担っていることが示唆された。（ファカルティー・アドバイザー：畔津 佑季）

Title

Periostin is a critical factor for tooth and bone development

Problem

A core question in basic dentistry is; "What mechanisms are involved in tooth development and maintenance of homeostasis?", which also has important disease-related implications in clinical settings. While parathyroid hormone-related protein (PTHrP) is a factor known to be involved in tooth formation and homeostasis, few findings regarding its role have been reported, and details related to tooth formation and homeostasis mechanisms remain largely unknown.

Hypothesis

Identification of factors involved in tooth formation and homeostasis will lead to elucidation of regulatory mechanisms of tooth morphology and quality, as well as improved care for dental diseases such as dental caries and malocclusion, which cause abnormalities in tooth development and homeostasis. Based on that hypothesis, we aimed to identify such novel factors using an original research method.

Mice as well as gene-modified [knockout (KO) and transgenic] medakas were used as experimental model animals. It is easy to produce and breed transgenic medaka, as only a small facility is required for testing of tens of thousands. At our institution we maintain what is termed the “gene-modified medaka library”. In the present study, that library was screened for medaka with abnormal (pharyngeal) teeth and selection of candidate genes for determining their involvement in regulation of tooth morphology. Next, mice with genetic modification were also obtained, and used to analyze tooth development and morphology for elucidation of the functions of candidate genes.

Methods

I. Experimental animals

1) Periostin-KO medakas

Periostin-KO medakas were obtained by screening with use of the targeting induced local lesions in genomes (Tilling) method, which revealed replacement of the 712th base of periostin coding DNA from A to T and conversion of the 238th lysine to a stop codon, causing a dysfunction. Four such medaka were used, each about 13 months old.

2) Periostin-KO mice

Periostin-KO mice, in which the first exon of the periostin gene was replaced with a drug resistance gene (Neo) and subsequently removed, causing periostin dysfunction, were obtained. No more than six were used, with ages ranging from approximately 12-18 months.

II. Hard tissue analysis

1) Analysis of medaka pharyngeal teeth and bone

Pharyngeal teeth are a collection of small teeth aligned on the surface of the bone and present in the posterior part of the pharynx in medakas (Figure 1A). Following euthanasia, those were removed with tweezers, then fixed with paraformaldehyde and subjected to microcomputed tomography (μCT) scanning, then three-dimensional images of teeth and bone were constructed using image analysis software. Following μCT analysis, pharyngeal teeth were treated with 0.5% KOH to promote transparency and stained with

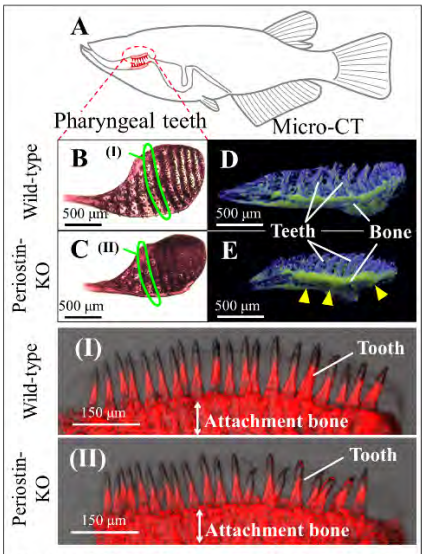

Figure 1. Effects of periostin knockout on pharyngeal teeth of medakas.
(A) Schematic diagram of medaka showing parasagittal sectional plane. Pharyngeal teeth are located at the posterior end of the pharynx. (B, C) Pharyngeal teeth stained with Alizarin red. (I, II) Frontal views of teeth and attachment bone shown in green highlighted area. (D, E) Micro-CT analysis of pharyngeal teeth. Periostin-KO medakas had increased bone and highly compacted dentition with closely spaced teeth.

alizarin red. The dentition was also trimmed with a scalpel to further separate teeth from the attachment bone providing support and morphology was analyzed.

2) Analysis of mouse teeth and alveolar bone

Following euthanasia, mice were decapitated and then the head was fixed with 70% ethanol and scanned with μCT. Three-dimensional images were constructed using image analysis software to analyze the morphology of the teeth and alveolar bone.

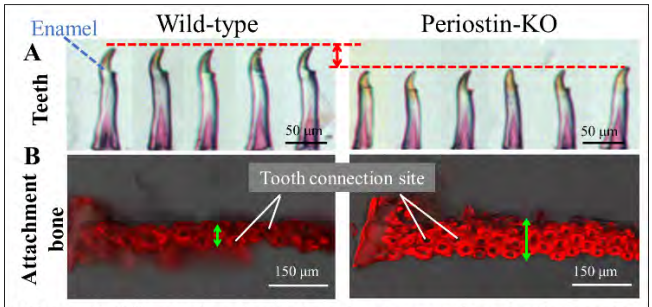

Figure 2. Effects of periostin knockout on teeth and attachment bone of medakas.
Teeth and attachment bone were stained with Alizarin red. (A) In periostin-KO medakas, enamel was limited and teeth were smaller as compared to the wild-type. (B) Top views of attachment bone. Attachment bone size was increased in periostin-KO medakas.

Results

Periostin is a secreted protein produced by osteoblasts, fibroblasts, and cancer cells, and known to be involved in tissue regeneration, wound healing, inflammation, atopic dermatitis, asthma, and other types of fibrosis by binding to integrins and type 1 collagen. Screening of the gene-modified medaka library revealed the following abnormalities in pharyngeal teeth of periostin-KO medakas.

1) Results of periostin KO medakas analysis

1-1) Pharyngeal teeth: Pharyngeal tooth size was smaller and bone density was greater in some instances on the side of the bone providing support for the dentition as compared to the wild-type (Figure 1B, C).

1-2) Teeth and attachment bones: Teeth were shorter than in the wild-type and the size of the enamel area forming the apical portion was also reduced (Figure 2A). The width of attachment bones supporting the teeth was increased as compared to the wild-type (Figure 2B).

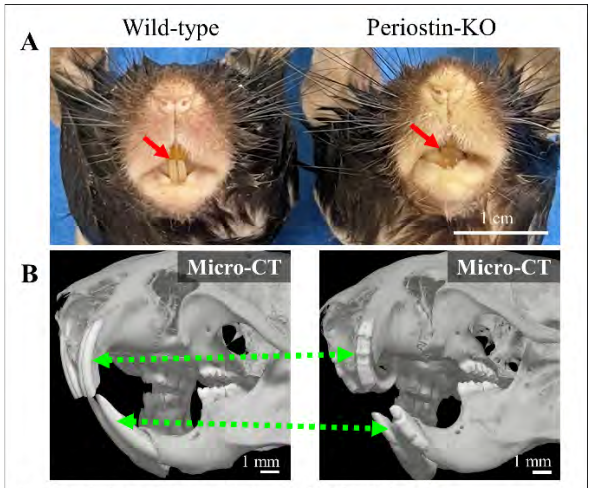

Figure 3. Impaired teeth in periostin-KO mice
(A) As compared to the wild-type, periostin-KO mice were found to have smaller teeth. (B) Micro-CT analysis of head region of mice. The incisors of periostin-KO mice were shorter and the surface was not smooth, but actually jaggy.

Based on these results, we analyzed periostin-KO mice and found the following abnormalities in teeth and alveolar bone.

2) Results of periostin KO mice analysis

2-1) Tooth size/surface: Teeth were smaller as compared to the wild-type and showed malocclusion (Fig. 3A). Also, tooth surfaces had a saw-blade shape (Figure 3B).

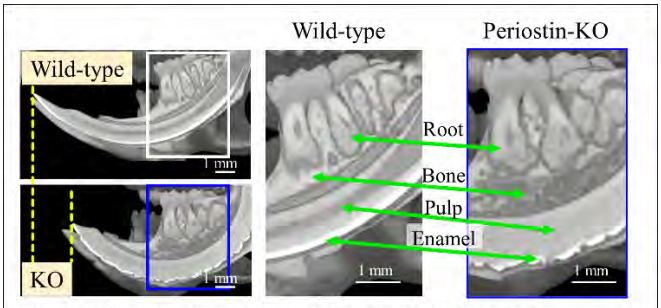

Figure 4. Images of inside lower jaw of periostin-KO mice.
In periostin-KO mice, tooth roots were enlarged and alveolar bone thickness was markedly increased. Furthermore, dental pulp had disappeared and enamel was irregularly developed.

2-2) Tooth and alveolar bone morphology: As compared to the wild-type, ① root enlargement, ② increased dentin and associated pulp loss in the incisors, and ③ discontinuous enamel formation in the incisors were observed in the periostin-KO mice. In addition, ④ a marked increasin in alveolar bone thickness was observed (Figure 4).

Conclusion

Morphological abnormalities in teeth and surrounding bone tissue were similar in periostin-KO medakas and mice. These results suggest that periostin plays an important role in tooth and bone homeostasis in vertebrates. To reveal the function of periostin in tooth development, future studies will be necessary to identify the target cells of periostin and analyze its effects on those cells.

Exacerbation of inflammation in periodontitis: *Porphyromonas gingivalis* fimbriae induce synergistic interleukin-6 production in human monocytes

東北大学歯学部 6年生 Tohoku University School of Dentistry Class of 2022

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ファカルティー・アドバイザー：口腔分子制御学分野 講師 多田 浩之
研究指導協力者：口腔分子制御学分野 教授 菅原 俊二

Problem: It has been shown that most periodontal pathogenic bacteria that invade into blood vessels through periodontal pockets are dead, suggesting that live periodontal pathogenic bacteria may not be necessary for periodontal disease to exacerbate inflammation of systemic organs. It is unknown how individual virulence factors of *P. gingivalis* affect the pathogenesis of bacterial inflammatory diseases. In this study, we analyzed the effect of sensitization of human monocytes with *P. gingivalis* bacterial components on the induction of IL-6 production by LPS. **Methods:** The human acute monocytic cell line THP-1 was differentiated into monocyte-like cells (THP-1 monocytes) with OCT. *P. gingivalis* fimbriae was isolated from bacterial culture by agitation, ammonium sulfate precipitation, and dialysis. THP-1 monocytes were primed with *P. gingivalis* fimbriae following stimulation with *Escherichia coli* LPS. **Results:** Priming of THP-1 monocytes with *P. gingivalis* fimbriae synergistically enhanced IL-6 production by LPS. IL-6 was the only cytokine that showed a synergistic effect of *P. gingivalis* fimbriae priming. IL-6 production was also synergistically enhanced by *P. gingivalis* fimbriae priming and with LPS of *P. gingivalis*, *Fusobacterium nucleatum* and *Salmonella enterica*. THP-1 monocytes sensitized to *P. gingivalis* fimbriae synergistically produce IL-6 via the NF-κB, ERK, and IκB-ζ pathways upon LPS stimulation. **Conclusion:** Our results suggest that *P. gingivalis* fimbriae diffusing from periodontal tissues of patients with chronic periodontitis may sensitize blood monocytes and may play exacerbation of bacterial inflammatory diseases.

歯周炎による炎症増悪機構: *Porphyromonas gingivalis*線毛はヒト単球におけるインターロイキン-6産生を相乗的に誘導する

【問題点】歯周病罹患者では全身の臓器で*P. gingivalis*が検出されるが、同菌の病原因子が細菌性炎症性疾患の病態に及ぼす影響は不明である。本研究は、*P. gingivalis*菌体成分で感作させたヒト単球がLPS刺激によるIL-6産生へ及ぼす影響について解析した。**【方法】**ヒト単球系THP-1細胞株を活性型ビタミンD3誘導体（OCT）で分化した（THP-1単球）。*P. gingivalis*線毛は攪拌、硫安沈殿と透析にて精製した。**【結果】**THP-1単球を*P. gingivalis*線毛で感作すると、NF-κB、ERKおよびIκB-ζ経路を介してLPS刺激によるIL-6産生が相乗的に亢進した。**【結論】***P. gingivalis*線毛に感作された単球は、LPS刺激によりIL-6を相乗的に産生する。歯周組織から*P. gingivalis*線毛が全身の臓器に拡散すると、細菌感染による単球のIL-6産生を介した炎症誘導が増悪することが推察される。

研究発表内容の紹介

歯周病は誤嚥性肺炎や炎症性腸疾患など細菌による炎症性疾患の病態に関わるが、歯周組織から血管内に侵入した歯周病原細菌の多くは死滅することから、死菌の状態でも炎症が誘導されることが推察される。本研究は、ヒト単球が*Porphyromonas gingivalis*線毛の感作を受けると、リポ多糖によるインターロイキン-6産生が相乗的に亢進することを示した。本知見は、歯周病が全身における細菌性炎症性疾患を増悪させる可能性を示唆する。（ファカルティー・アドバイザー：多田 浩之）

Exacerbation of inflammation in periodontitis: *Porphyromonas gingivalis* fimbriae induce synergistic interleukin-6 production in human monocytes

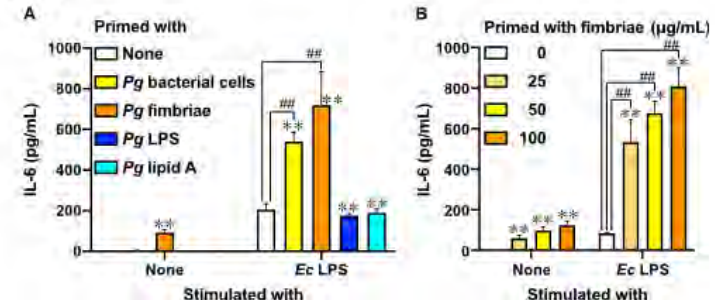
[Problem] There is a growing consensus that periodontal pathogenic bacteria affect the pathogenesis of a variety of diseases throughout the body. However, it has been shown that most periodontal pathogenic bacteria that invade into blood vessels through periodontal pockets are dead, suggesting that live periodontal pathogenic bacteria may not be necessary for periodontal disease to exacerbate inflammation of systemic organs. *Porphyromonas gingivalis*, the major pathogenic bacterium of chronic periodontitis, possesses a variety of virulence factors such as fimbriae, lipopolysaccharide (LPS), lipoproteins, and gingipain. On the other hand, *P. gingivalis* has been detected in other systemic organs in patients with chronic periodontitis, indicating that the presence of the bacterium is involved in the pathogenesis of systemic diseases. However, it is unknown how individual virulence factors of this organism affect the pathogenesis of bacterial inflammatory diseases such as sepsis, aspiration pneumonia and inflammatory bowel disease.

[Hypothesis] In this study, we focused on interleukin-6 (IL-6), which plays an important role in the pathogenesis of chronic inflammatory diseases, to determine the impact of *P. gingivalis* virulence factors on systemic bacterial inflammatory diseases. We analyzed the effect of sensitization of human monocytes with *P. gingivalis* bacterial components on the induction of IL-6 production by LPS.

[Methods] The human acute monocytic cell line THP-1 was induced to differentiate into monocyte-like cells (hereafter referred to as THP-1 monocytes) with an active vitamin D3 analog, maxacalcitol (OCT). For isolation of *P. gingivalis* fimbriae, bacterial culture was agitated by pipetting and sonication then ultracentrifuged. The supernatant containing fimbriae was purified by ammonium sulfate precipitation and DEAE-Sepharose FAST Flow column. The fimbriae fraction was confirmed to be free of endotoxin by a limulus assay. The experimental procedures for this study were as follows: THP-1 monocytes were primed with *P. gingivalis* fimbriae for 24 h, and cells were washed with PBS, following stimulation with *Escherichia coli* LPS for 16 h. IL-6 levels in the supernatants were measured by ELISA.

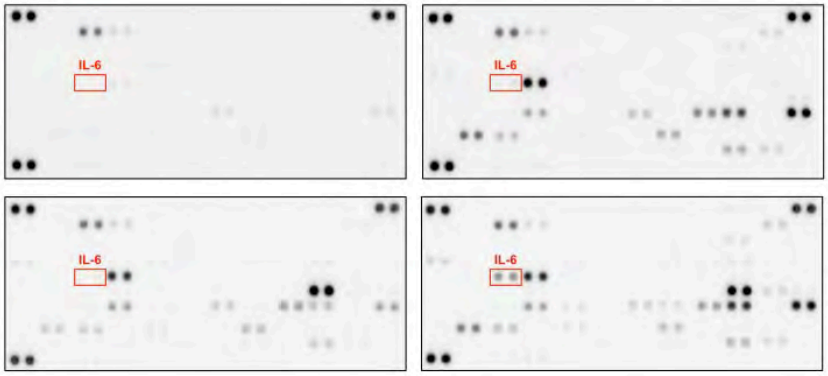
[Results] Priming of THP-1 monocytes with lyophilized *P. gingivalis* whole bacterial cells as well as with *P. gingivalis* fimbriae synergistically enhanced IL-6 production by *E. coli* LPS (Fig. 1A). However, cells primed with *P. gingivalis* LPS and lipid A showed similar IL-6 levels by *E. coli* LPS as control cells (Fig. 1A). The synergistic IL-6 production by priming of *P. gingivalis* fimbriae was shown to be a concentration-dependent manner (Fig. 1B). These results indicate that *P. gingivalis* fimbriae synergistically enhance LPS-induced IL-6 production by human monocytes.

Fig. 1



Next, we investigated the possibility that priming of THP-1 monocytes with *P. gingivalis* fimbriae could synergistically induce cytokines production other than IL-6 using a membrane array (Proteome Profiler Cytokine Array, R&D Systems). The IL-6 expression was increased about 14-fold by *P. gingivalis* fimbriae and 10-fold by *E. coli* LPS, whereas it was increased about 113-fold by *P. gingivalis* fimbriae priming and *E. coli* LPS stimulation compared to untreated cells. Interestingly, IL-6 was the only cytokine that showed a marked synergistic effect of *P. gingivalis* fimbriae priming and *E. coli* LPS stimulation (Fig. 2).

Fig. 2



Furthermore, IL-6 production by *P. gingivalis* fimbriae priming was synergistic with *E. coli* LPS as well as with LPS of *P. gingivalis*, *Fusobacterium nucleatum* and *Salmonella enterica* serovar Typhimurium (Fig. 3).

Fig. 3

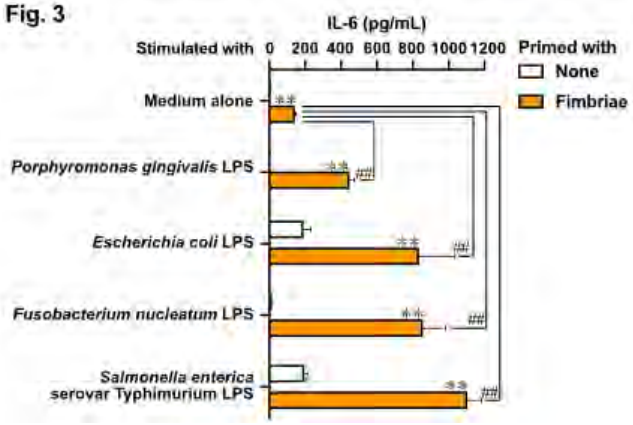
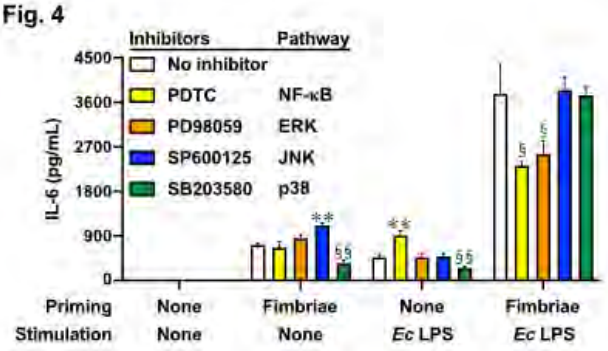
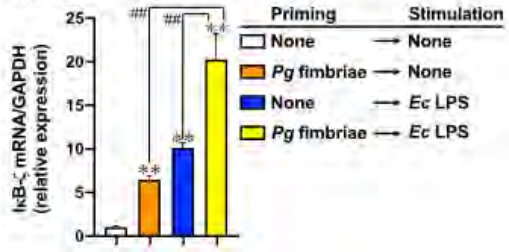


Fig. 4



The signaling pathways involved in synergistic IL-6 production by *P. gingivalis* fimbriae were focused on NF-κB and MAP kinase. THP-1 monocytes were pre-treated with the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC), the MAP kinase ERK inhibitor PD98059, the JNK inhibitor SP600125, and the p38 inhibitor SB203580 for 1 h, cells were primed with *P. gingivalis* fimbriae and then stimulated with *E. coli* LPS. The IL-6 production in the cells of *P. gingivalis* fimbriae-primed *E. coli* LPS-stimulated were partially and significantly suppressed when pre-treated with PDTC and PD98059, indicating that NF-κB and ERK are involved in the synergistic IL-6 production (Fig. 4). Next, we focused on IκB-ζ, an IκB family protein whose expression is enhanced by TLR ligands and responsible for IL-6 induction. IκB-ζ mRNA expression in THP-1 monocytes was significantly upregulated by *P. gingivalis* fimbriae priming + *E. coli* LPS stimulation compared to *P. gingivalis* fimbriae and *E. coli* LPS stimulation (Fig. 5).

Fig. 5



[Conclusion] Human monocytes sensitized to *P. gingivalis* fimbriae synergistically produce IL-6 via the NF-κB, ERK, and IκB-ζ pathways upon LPS stimulation. Since this effect is independent of the LPS species, we speculate that *P. gingivalis* fimbriae exacerbate IL-6-mediated inflammation induced by bacterial infections not only in oral but also in other systemic organs. Our results suggest that *P. gingivalis* fimbriae diffusing from periodontal tissues of patients with chronic periodontitis may sensitize blood monocytes and play a part in the underlying mechanisms that exacerbate the pathogenesis of bacterial inflammatory diseases such as sepsis, aspiration pneumonia, and inflammatory bowel disease.

Bone metastatic mammary tumor cell-derived extracellular vesicles inhibit osteoblast mineralization

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Osteolytic bone metastases are characterized by osteoclast activation and suppression of osteoblast function, which are related to intercellular communication via various factors released by the tumor cells. Extracellular vesicles (EVs) are lipid bilayer membrane vesicles that are shed by most cells and key mediators of intercellular communication. In this study, the effects of EVs derived from 4T1 bone metastatic mammary tumor cell line (4T1-EVs) on osteoblast differentiation and function were examined. We observed that 4T1-EVs significantly inhibited mineralization of osteoblastic cells (ST2, MC3T3-E1 and MLO-A5). Interestingly, 4T1-EVs markedly inhibited upregulation of osteoblast differentiation markers at late stage of differentiation. Our findings indicated that bone metastatic mammary tumor cell-derived EVs function as an important factor mediating the cross-talk between tumor cells and osteoblasts. The inhibition of osteoblast mineralization by bone metastatic mammary tumor cell-derived EVs may be involved in the pathogenesis of bone destruction in osteolytic bone metastases.

骨転移性乳癌細胞由来細胞外小胞を介した骨芽細胞石灰化抑制

溶骨性骨転移は、破骨細胞の活性化と骨芽細胞機能の抑制を特徴とする。これらの現象には、癌細胞由来液性因子を介した細胞間コミュニケーションが深く関与している。本研究では、4T1-EVsの骨芽細胞の石灰化ならびに分化関連遺伝子群発現への影響について、アリザリンレッド染色およびqPCR解析により検討した。その結果、骨芽細胞系細胞株（ST2、MC3T3、MLO-A5）において、4T1-EVs存在下では顕著な石灰化抑制が観察された。興味深いことに、4T1-EVs刺激により、石灰化誘導後期において骨芽細胞分化関連遺伝子(*Runx2, osterix, Atf4, Alkaline phosphatase, bone sialo protein, osteocalcin*）の顕著な発現低下がみられた。以上の結果から、4T1-EVsは骨芽細胞成熟を制御する重要な細胞間コミュニケーション因子として機能することが明らかとなった。くわえて、骨転移腫瘍細胞由来のEVを介した骨芽細胞の石灰化阻害は、溶骨性骨転移における骨破壊の病態に寄与する可能性が考えられた。

研究発表内容の紹介

癌の骨転移において、癌細胞と骨微小環境に存在する細胞との相互作用は重要である。本研究では、骨転移性乳がん細胞由来細胞外小胞（EVs）が、骨芽細胞の石灰化を抑制することを明らかにした。今後、がん細胞由来EVを介した骨芽細胞機能制御メカニズムの解明により、がん骨転移の病態制御に有用な知見が得られるとともに、口腔癌における顎骨浸潤の制御法開発への応用も考えられる。（ファカルティー・アドバイザー：上原 範久）

Bone metastatic mammary tumor cell-derived extracellular vesicles inhibit osteoblast mineralization

(Problem/Hypothesis)

Bone homeostasis is maintained by the orchestrated actions of bone-forming osteoblasts and bone-resorbing osteoclasts. However, once tumor cells colonize and grow in the bone microenvironment, physiological bone metabolism is disrupted. Osteolytic bone metastases are characterized by osteoclast activation and suppression of osteoblast function, which are related to intercellular communication via various factors released by the tumor cells (Figure 1).

Extracellular vesicles (EVs) are lipid bilayer membrane vesicles that are shed by most cells and key mediators of intercellular communication. EVs contain microRNAs (miRNAs), proteins, and other signaling molecules that propagate to neighboring and distant cells, thereby regulating cellular functions and participating in various pathological processes.

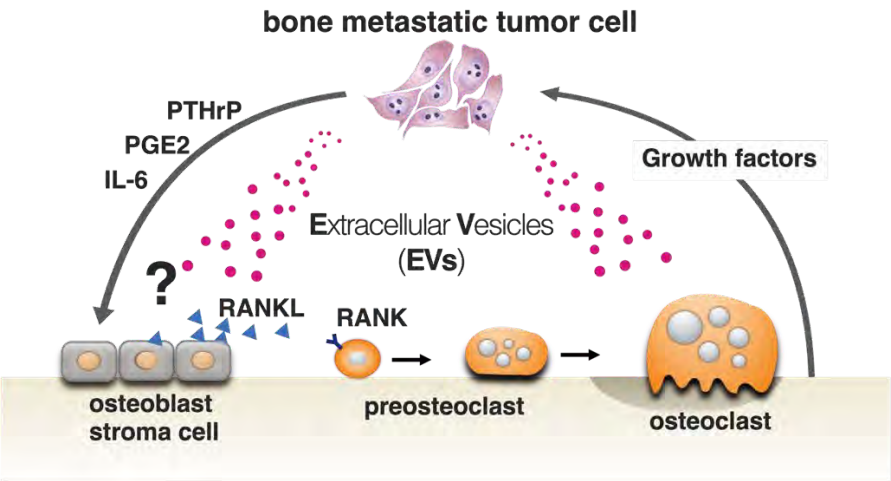

Figure 1

We have previously reported that EVs derived from bone metastatic mouse mammary tumor cells promoted osteoclastogenesis and bone resorption. However, the roles of bone metastatic mouse mammary tumor cells-derived EVs in osteoblast differentiation and function have not been fully elucidated.

(Methods)

Cell lines

Mouse non-metastatic and metastatic mammary tumor cell lines (67NR and 4T1) were obtained from Karmanos Cancer Institute and Peter MacCallum Cancer Center, respectively. Mouse cranial crown-derived osteoblasts (MC3T3-E1) and mouse bone marrow stromal cells (ST2) were purchased from RIKEN BRC and MLO-A5 was obtained from Kerafast.

EV isolation

67NR and 4T1 cells were cultured in DMEM containing 10% Exosome-depleted FBS (Thermo) for 48 h. The culture media were centrifuged using ExoQuick-TC (System Bioscience). Protein concentration was measured using a DC protein assay (Bio-Rad). The size distribution of the EVs was analyzed using a NanoSight LM10 instrument (NanoSight) equipped with the nanoparticle tracking analysis (NTA) 2.0. software.

Real-time PCR

Total RNA was isolated and purified using RNeasy Mini kit (Qiagen), and cDNA synthesis was performed using SuperScript VILO (Thermo) as a template for PCR reaction. Thunderbird qPCR mix (TOYOBO). Expression was calculated using the comparative threshold cycle (CT) method

Alizarin Red Staining

Osteoblasts were cultured for 8-21 days in a medium containing beta-glycerophosphate (10 mM), ascorbic acid (50 μg/ml) and EVs (100 ng/ml) and culture medium were changed every 3 days. The cells were then fixed in 10% formalin and stained with Alizarin Red S to evaluate mineralization.

(Results)

1) Effects of bone metastatic mammary tumor cell-derived EVs on osteoblast mineralization.

The effects of EVs derived from 4T1 bone metastatic mammary tumor cell line (4T1-EVs) on osteoblast mineralization were evaluated by Alizarin red staining. (Figure 2). In the presence of EVs derived from 67NR mouse non-metastatic mammary tumor cell line (67NR-EVs), mineralization level was similar to that observed in EVs untreated control. In contrast, 4T1-EVs markedly inhibited mineralization in either ST2, MC3T3-E1 or MLO-A5 cells.

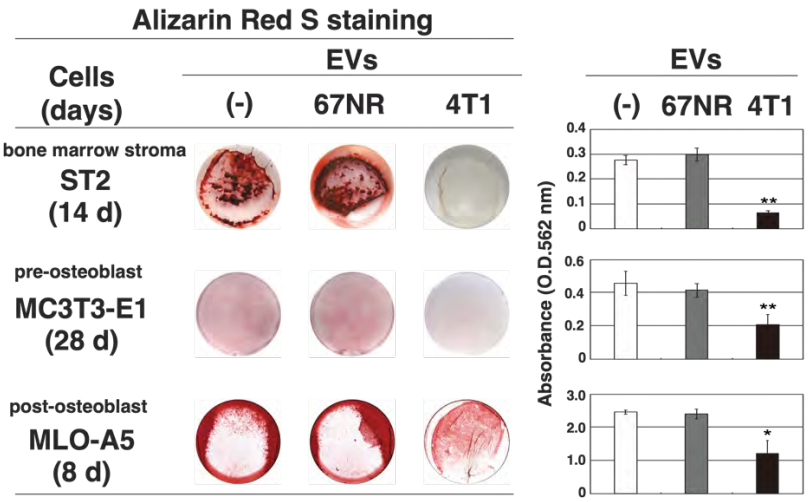

Figure 2

(2) Bone metastatic mammary tumor cell-derived EVs inhibited induction of osteoblast differentiation markers.

The effect of 4T1-EVs on the expression of osteoblast differentiation markers in ST2 cells was examined by qPCR (Figure 3). 67NR-EVs and 4T1-EVs treatment showed no differences in the expression levels of osteoblast differentiation markers, such as *Runx2 (runt-related transcription factor 2), osterix (osx), Atf4, Alkaline phosphatase (Alp), bone sialo protein (Bsp), and osteocalcin (Ocn)* until day 6. Interestingly, 4T1-EVs treatment markedly inhibited upregulation of osteoblast differentiation markers at day12 and day 18, which was accompanied by the inhibition of mineralization as demonstrated by Alizarin Red S staining (Figure 2). These results suggest that 4T1-EVs are involved in the regulation of osteoblast maturation.

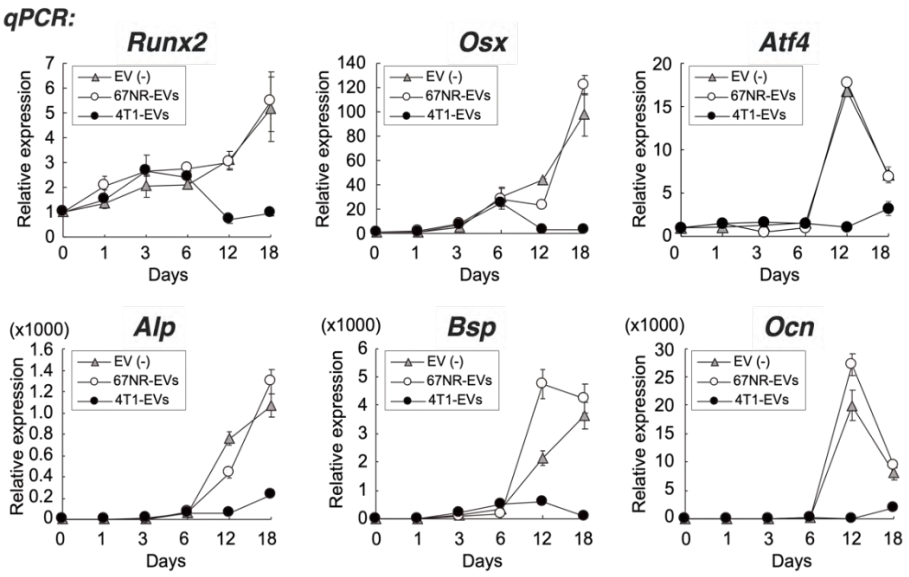

Figure 3

(Conclusion)

Our findings indicated that bone metastatic mammary tumor cell-derived EVs function as an important factor mediating the cross-talk between tumor cells and osteoblasts. The inhibition of osteoblast mineralization by bone metastatic mammary tumor cell-derived EVs may be involved in the pathogenesis of bone destruction in osteolytic bone metastases. Further investigations are needed to elucidate the mechanisms how 4T1-EVs block mineralization of osteoblasts, which may provide useful insights into the regulation of bone metastasis.

Original development of cancer chip and discovery of immunoresistance of oral cancer

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歯科薬理学分野 教授 岡元 邦彰

Although oral cancer has a good prognosis in early detection, metastasis and recurrence are unsolved problems. In metastatic cases, macrophages accumulate around/in the tumor; however, cancer progression is not suppressed by these immune cells. Therefore, we hypothesized that cancer cells secrete exosomes that adversely affect macrophages. We originally developed "cancer chip" designed to separate cancer cells and macrophages by a porous membrane with 200-nm pores, so that 100-nm exosomes can pass through it, whereas cells with a diameter of 30–50 μm cannot. Oral cancer cells and their exosomes were labeled with red fluorescent proteins, while macropahges and their exosomes were labeled with green fluorescent proteins for analysis in confocal laser microscopy. Oral cancer exosomes were found to pass through the membrane, reach macrophages, then replace the macrophage components, and accumulate inside the cell, called "hijacking" by cancer exosomes. In the hijacked cells, cellular swelling and blebbing were observed. "Hijacking of macrophages by oral cancer exosomes" is considered a new mechanism of immunoresistance of oral cancer.

癌チップの独自開発と口腔癌の免疫抵抗性の発見

口腔癌は、早期発見では予後が良いものの、転移や再発が課題となっている。転移や再発の症例では、腫瘍周囲にマクロファージが集積するが、癌の進行は抑制できていない。このことから、癌細胞がエクソソームを分泌してマクロファージに悪影響を及ぼしていると仮説を立てた。癌細胞とマクロファージを200 nmの穴をもつ膜で区切って培養し、直径100 nmのエクソソームが通過でき、直径30〜50 μmの細胞は往来できない「癌チップ」を開発した。口腔癌細胞とそのエクソソームを赤色蛍光タンパク質で標識し、マクロファージとそのエクソソームを緑色蛍光タンパク質で標識し、共焦点レーザー顕微鏡で解析した。口腔癌エクソソームは、膜を通過してマクロファージに到達後、もとの細胞成分に取って代わって細胞内に集積する現象が見られ、これを癌エクソソームによる「ハイジャック」と名付けた。ハイジャックされた細胞では、膨化や細胞表面の小胞形成を認めた。「口腔癌エクソソームによるマクロファージのハイジャック」は、口腔癌がもつ免疫抵抗性の新しいメカニズムと考えられる。

研究発表内容の紹介

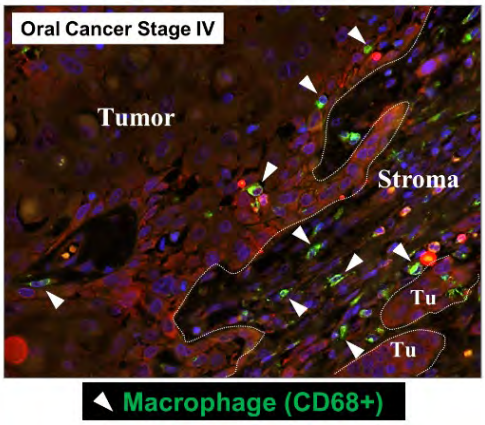
この研究は、口腔癌の転移性・再発性という臨床上の未解決問題に取り組んだものであり、独自に「癌チップ」を開発して、難治性口腔癌の病態解明や治療法開発を目指し、患者予後の向上に繋げようというものです。「口腔癌がエクソソームを分泌してマクロファージをハイジャックする現象」は、癌が免疫系に対して抵抗しつつ難治性の微小環境を構築していく過程と考えられ、歯科医学において新しい領域を開拓しようとするものです。（ファカルティー・アドバイザー：江口 傑徳）

Original development of cancer chip and discovery of immunoresistance in oral cancer

【Problem】

Oral cancers of the tongue, gingiva, buccal mucosa, and floor of the mouth account for 40% of head and neck cancers and are increasing each year. The recurrence rate after treatment is 24-48%, but metastasis and recurrence are problems in cases of advanced cancer. When patient-derived tissues from stage IV oral cancer were examined, CD68-positive macrophages were clustered around the tumor (**Fig. 1**). However, even these macrophages have not been able to cure the cancer. Although refractory oral cancer is thought to be resistant to immunity, the lack of effective methods to investigate immunoresistance in oral cancer is a limitation in clinical practice and research.

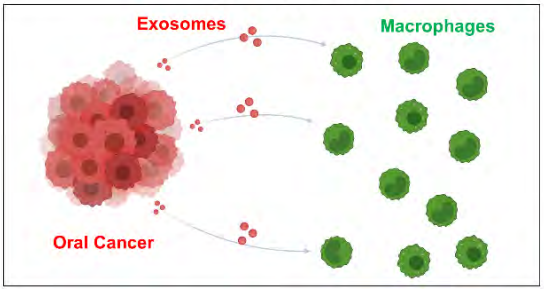
Fig. 1 Macrophages are associated with oral cancer in patients



【Hypothesis】

We hypothesized that refractory oral cancers secrete exosomes which are about 100 nm in diameter and exert a detrimental effect on macrophages (**Fig. 2**).

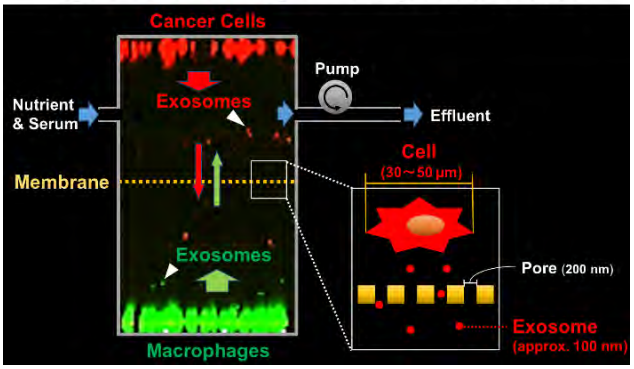
Fig. 2 Hypothesis



【Methods】

1. Development of Cancer Chip - We independently developed a "cancer chip" for co-culturing oral cancer cells and macrophages (**Fig. 3**). Oral cancer cells labeled with red fluorescent proteins were cultured on the top room of the cancer chip, and macrophages labeled with green fluorescent proteins were cultured on the bottom room of the chip. The top and bottom rooms were separated by a porous membrane with 200-nm pores, which allowed exosomes to pass through but not cells, which are 30-50 μm in diameter.

Fig. 3 Cancer chip to analyze exosomes



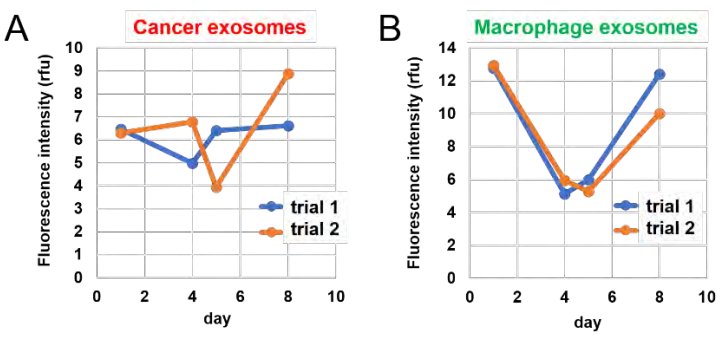
2. Qualitative analysis using confocal laser microscopy - Qualitative analysis of cells and exosomes was performed using confocal laser microscopy starting the day after co-culture, and nuclei were stained using Hoechst 33342.

3. Quantitative analysis of exosome secretion - Co-culture effluent was filtered through a 220 nm filter for quantitative analysis of fluorescent exosomes.

【Results and Discussion】

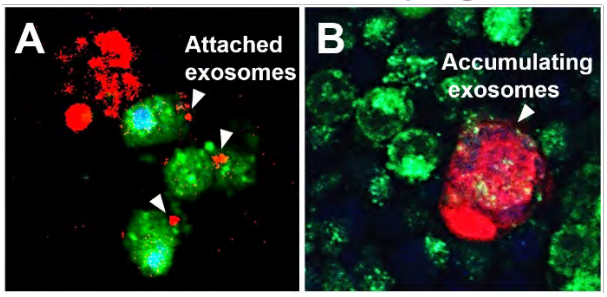
1. Detection of fluorescent exosomes - Red fluorescent exosomes secreted by cancer cells and green fluorescent exosomes secreted by macrophages were detected on the cancer chip (**arrowheads in Fig. 3**). Red fluorescent exosomes and green fluorescent exosomes were also detected in the effluent (**Fig. 4**).

Fig. 4 Detection of Exosomes in Effluent



2. Oral cancer exosomes reached macrophages - Red fluorescence was observed at the rim of macrophages between day 2 and day 6 after the co-culture in the cancer chip (**Fig. 5A**), indicating that oral cancer exosomes passed through the thin membrane and reached macrophages.

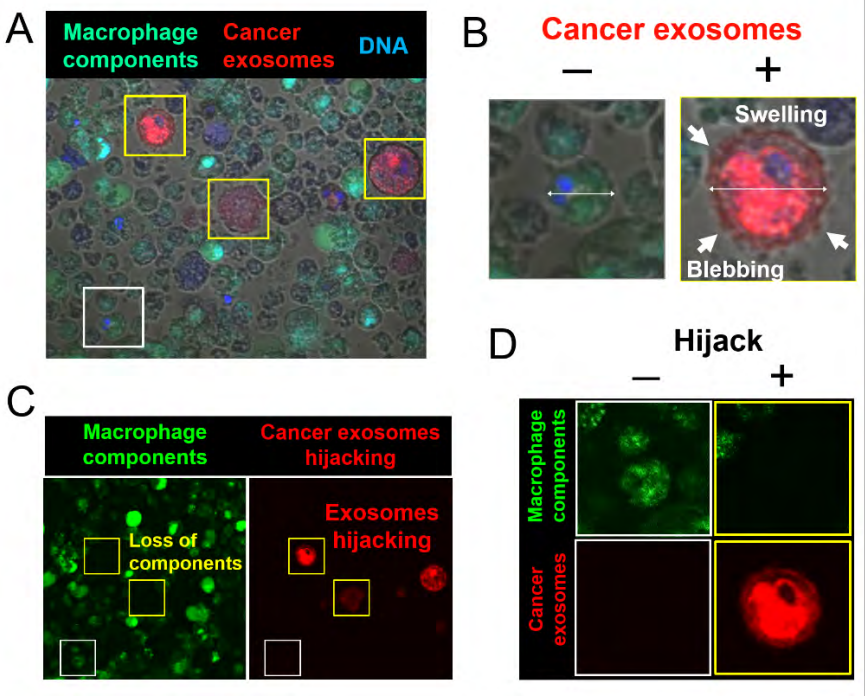
Fig. 5 Cancer exosomes reached and accumulated in macrophages



3. Accumulation of oral cancer exosomes on macrophages – Attachment of oral cancer exosomes to macrophages increased day by day, and between 7 and 10 days of co-culture, a reticulated image of cancer exosomes accumulating inside macrophages was observed (**Fig. 5B**).

4. Hijacking of macrophages by oral cancer exosomes - On day 7 of co-culture, cells lost in the green fluorescent macrophage component and filled with red fluorescent cancer exosomes were observed (**Fig. 6A yellow box**). These cells were greatly swollen compared to green fluorescent macrophages and membrane blebbing was observed on the cell surface (**Fig. 6B**). When analyzed separately for green and red fluorescence, these cells had completely lost the green fluorescence that was macrophage components and had been replaced by red fluorescent cancer exosomes (**Fig. 6C**). In magnified images, control macrophages without cancer exosome accumulation maintained their cellular components, whereas cells with cancer exosome accumulation lost their macrophage components (**Fig. 6D**). These data indicate that cancer exosomes hijacked macrophages and affected their cellular components.

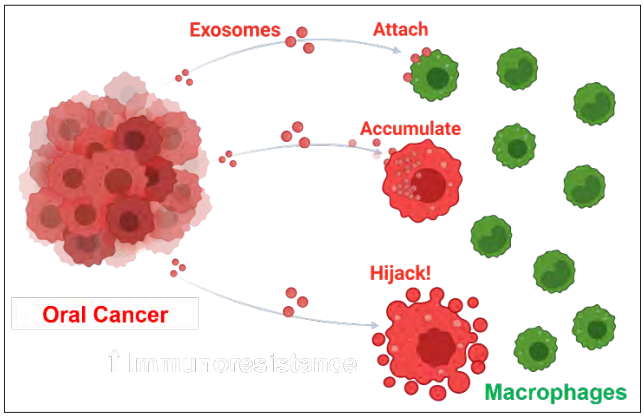
Fig. 6 Cancer exosomes hijacked macrophages



【Conclusion】

I have originally developed a new co-culture system of cancer cells and macrophages, which I have named the "Cancer Chip". The Cancer Chip has made it possible to visualize the interaction between cancer and immunity. In this study, we found that exosomes secreted by refractory oral cancer attach to, accumulate on, and eventually take over macrophages (**Fig. 7**). This may represent a novel mechanism for the immunoresistance of refractory oral cancers. The cancer chip will be useful for elucidating novel interactions between cancer and the immune system and for developing new drugs.

Fig. 7 Oral Cancer Cells Release Exosomes to Hijack Macrophages



Investigation of the role of vascular endothelial cells in severe COVID-19 using a SARS-CoV-2 infected mouse model

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血管生物分子病理学教室 助教　松田 彩

The establishment of methods for the prevention and treatment of severe COVID-19 is essential to overcome this pandemic, and an understanding of the pathogenesis of the severe disease is an urgent issue. Although vascular pathology is known to be deeply involved in lethal COVID-19, the role of vascular endothelial cells, which constitute the vessel wall, in severe disease is not well known. In this study, we examined the validity of a mouse model of severe COVID-19 established by our collaborators as an evaluation system for human severe disease by scoring histopathological features. We then focused on CD41 (platelets marker) -positive thrombus formation among vascular lesions and examined it in conjunction with qualitative changes in vascular endothelial cells. The results suggest that infected aged mice can be models of human severe COVID-19. The pulmonary vessels of infected aged mice have a strong platelet aggregating effect, and gene expression analysis showed that the coagulation-related genes were included in a group of up-regulated genes specific to pulmonary vascular endothelial cells of the infected aged mice. There is the possibility that vascular endothelial cells trigger lethal pathogenesis by inducing thrombus formation via the promotion of platelet aggregation, which will be further investigated.

SARS-CoV-2感染マウスモデルを用いたCOVID-19重症化における血管内皮細胞の役割の検討

COVID-19のパンデミックの克服には重症化の予防・治療方法の確立が必須であり、その基盤となる重症化病態の詳細な理解が喫緊の課題である。致死的COVID-19には血管病態の深い関与が知られるが、血管壁を構成する血管内皮細胞の重症化に対する役割はよく知られていない。本研究では、共同研究者が樹立した重症COVID-19マウスモデルについてヒト重症化病態評価系としての妥当性を病理組織学的所見のスコアリングとウイルス感染細胞面積の定量によって検証したのち、血管病変のうちCD41（血小板マーカー）陽性の血栓形成に着目し、感染肺血管内皮細胞の質的変化と合わせて検討した。結果、重症化モデルである感染加齢マウス肺血管は血小板を凝集させる作用が強いことが示唆され、また、遺伝子発現解析では感染加齢マウス肺血管内皮細胞特異的な発現亢進遺伝子群に血液凝固関連遺伝子が含まれていた。CD41陽性血小板の凝集亢進をきたしている重症化モデルの血管内皮細胞において血液凝固関連遺伝子の特異的な発現亢進を認めたことから、血管内皮細胞が血小板の凝集促進を介して血栓形成を惹起することで致死的な病態形成のトリガーの一つとなっている可能性が示唆され、今後更なる検討を行う予定である。

研究発表内容の紹介

COVID-19は唾液から発生するマイクロ飛沫を介し感染するため、歯科診療現場に大きな影響を及ぼした。また、超高齢社会の我が国においてウイルスを含む唾液の誤嚥は重症化をきたし、医療崩壊に直結する。したがって、重症化病態の解明と治療法の確立は喫緊の課題である。本研究では、COVID-19による死亡に深く関わる血管病態が、どのような機序でおこるのかを重症化マウスモデルを用いて詳細に検討している。ヒト剖検検体だけでは明らかにし得ない重症化メカニズムを感染後の経時的な病理組織学的解析により明らかにしようという挑戦的な研究である。（ファカルティー・アドバイザー：樋田 京子）

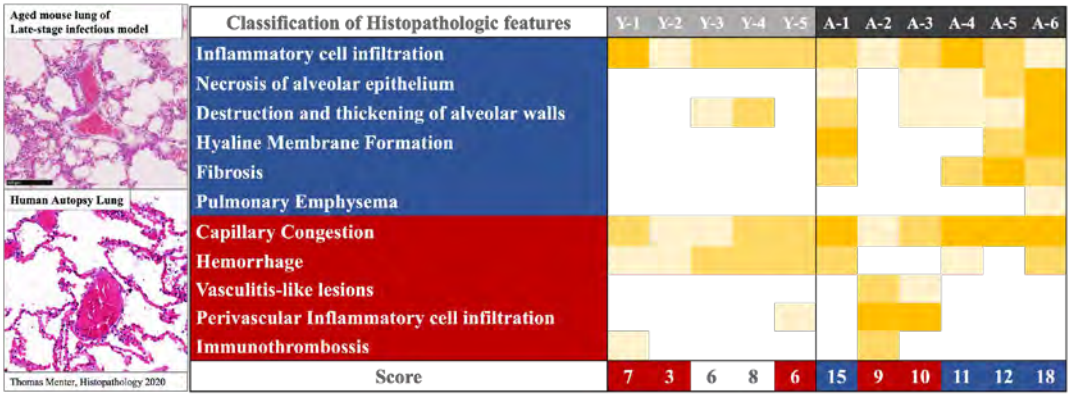
Investigation of the role of vascular endothelial cells in severe COVID-19 using a SARS-CoV-2 infected mouse model

(Problem)
The COVID-19 outbreak that began in 2019 continues to cause unprecedented damage around the world. From a social and economic perspective, the establishment of prevention and treatment methods for severe COVID-19 and the elucidation of the pathogenesis of severe COVID-19 for these purposes remain urgent issues. A major problem in the study of severe COVID-19 has been the lack of appropriate animal models that reproduce the severe disease well. In fatal COVID-19, vascular pathology such as thrombus formation and vasculitis have been reported in human autopsy in previous papers. Thrombosis has also been noted as a direct cause of death, and it is now understood that blood vessels are deeply involved in severe COVID-19. Although the role of vascular endothelial cells in severe COVID-19 has been debated in various ways, how the qualitative changes in vascular endothelial cells and their behavior are involved in the formation of lethal vascular pathology is still an open debate.
(Hypothesis)
Thrombus formation in severe COVID-19 is generally believed to be caused by an inflammatory response in alveolar tissue that damages and breaks away vascular endothelial cells and activates an extrinsic coagulation pathway with tissue factor as an upstream factor. Endothelial cells play a passive role in this mechanism. In malignant tumors, however, vascular endothelial cells, which are stromal cells, are known to acquire abnormalities and play a tumor-promoting role in the tumor microenvironment. This led us to hypothesize that in severe COVID-19, inflammatory vascular endothelial cells may actively promote thrombus formation in addition to promoting blood coagulation due to vessel wall damage. To approach this hypothesis, we performed the following tests.
Verification A Validation of SARS-CoV-2 mouse adaptive strain-infected aged mice as a model of human severe COVID-19
Verification B Investigation of thrombus formation and the involvement of vascular endothelial cells using the severe COVID-19 mouse model studied in Verification A
(Methods)
Our collaborators established a SARS-CoV-2 infected mouse model by nasal inoculation of BALB/c mice with a mouse adaptive strain of SARS-CoV-2. This model has been shown to induce severe pneumonia and death only in aged mice, so it is expected to serve as an appropriate evaluation system for severe cases in humans. The group of infected aged mice whose lungs were collected 4 days after virus inoculation, the period just before death, was used as the late-stage infectious model. Young infected mice were used as a control group, and the following analyses were performed using microscopic images of HE staining and immunohistochemical staining of N protein(nucleocapsid protein of SARS-CoV-2), CD41 (platelets marker), and CD31 (vascular endothelial cells marker).
Verification A
1. Scoring of histopathologic features by HE-stained images
Lung lesions were classified by referring to " NEJM, 2020", " Histopathology, 2020", " Histopathology, 2020", " Emerging Infectious Diseases, 2020", "The Lancet Microbe, 2020" with at least three research mentors and collaborators.
2. Quantitative analysis of Virus-positive Area
We evaluated the dynamics of infected mouse lungs, time-dependently, by sampling whole lung tissue for 1 day (early-stage infectious model) and 4 days (late-stage infectious model) after virus inoculation.
Verification B
To evaluate the state of thrombus formation, we calculated the number of vessels containing CD41-positive regions in the lumen of all pulmonary arteriovenous in infected lung tissue. CD41-positive regions were classified according to their morphology and whether they adhered to the vessel's inner wall. In addition, RNA sequencing was performed using highly purified CD31-positive cells isolated from the lungs of young/aged infected mice, and qualitative changes were examined in pulmonary vascular endothelial cells by pathway analysis and gene expression analysis.

(Results of Verification A)

A representative example is shown in Figure 1 and the results of scoring of histopathologic features are shown in Figure 2. The lungs of infected aged mice showed more extensive lesions than those of young mice, with findings similar to those reported at human severe COVID-19 autopsy. The total score of lung lesions was significantly higher than that of the young, with a variety of lesions depending on the individual and the location of the lung. Quantitative analysis of virus-positive cells showed that N protein-positive cells consistently reached higher levels in infected aged mice, although they decreased from early to late stage. Analysis of the area of virus-positive cells in the lung respiratory area shown in Figure 3 indicates that a large amount of virus enters the alveolar tissue in infected aged mice from the early stage, suggesting that vascular endothelial cells in the microvasculature of infected aged mice are exposed to more virus for a longer time than in younger mice.

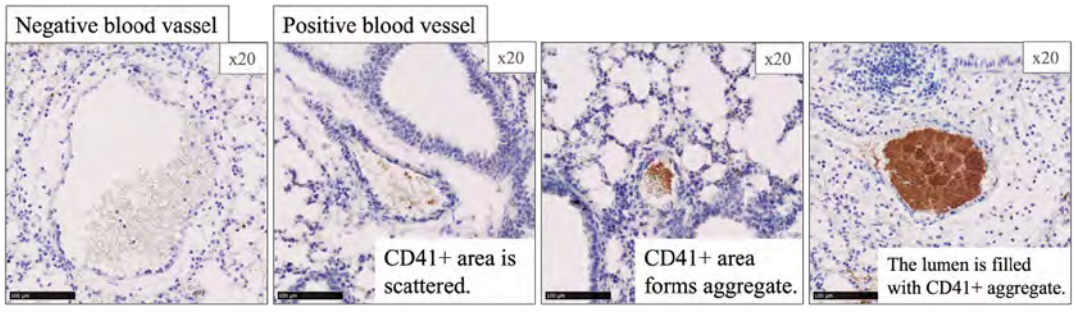
Fig. 1 Fig. 2 Heatmap of Histopathologic features Fig. 3 Percentage of Virus entering alveoli

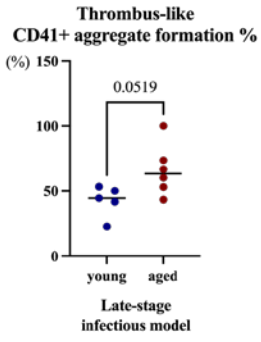


(Results of Verification B)

The identification and classification of CD41-positive regions in the pulmonary arteriovenous lumen on CD41 immunohistochemical staining images are shown in Fig. 4. There was no difference in the percentage of CD41-positive pulmonary arteriovenous in the total pulmonary arteriovenous between infected young and aged mice. On the other hand, as shown in Fig. 5, the percentage of CD41-positive platelets forming thrombus-like aggregates among CD41-positive arteriovenous vessels was significantly higher in infected aged mice than in young mice.

Fig. 4 Classification of Pulmonary Arteriovenous Lumen CD41-positive Areas Fig. 5





Pathway analysis and gene expression analysis of bulk RNA sequencing of vascular endothelial cells isolated from infected lungs revealed increased production of inflammatory cytokines in both young and aged mice, and coagulation system-related genes were specifically seen among the upregulated genes in infected aged mice.

(Conclusion)

Aged mice infected with a mouse adaptive strain of SARS-CoV-2 had more severe lung lesions and more extensive viral distribution than young mice, suggesting that this mouse model is a valid evaluation system that well recapitulates the severe COVID-19 pathogenesis in humans. The results also suggest that infected aged mice have a strong ability to activate platelets and form aggregates. Gene expression analysis of infected pulmonary vascular endothelial cells suggested that vascular endothelial cells in infected aged mice are altered to be procoagulant. Since vascular endothelial cells are close to thrombus-like aggregates, vascular endothelial cells may be involved in lethal thrombus formation via promoting platelet aggregation, which will be investigated in the future.

Induction of limb bud cells from human pluripotent stem cells (hPSCs)

長崎大学歯学部 6年生 Nagasaki University School of Dentistry Class of 2022

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細胞生物学分野 技術職員 松尾 友紀
細胞生物学分野 客員教授 大庭 伸介

Some cells in the sclerotome and limb bud, which are derived from paraxial mesoderm and lateral plate mesoderm, respectively, differentiate into skeletal cells including chondrocytes and osteoblasts. Induction of the skeletal cell populations from pluripotent stem cells (PSCs) will contribute to understanding of the skeletal formation process and regenerative therapies. However, reliable induction methods have not been established yet. This study was aimed to develop protocols for inducing PSCs particularly into limb bud cells, which were origins of skeletal progenitors, by manipulating activities of the developmentally critical signaling pathways *in vitro*. Limb bud cells were induced from PSCs in stepwise manners with combinations of small compounds (CHIR99021, C-59, and A83-01) and bone morphogenetic protein (BMP)-4. The 3-day limb bud induction culture achieved upregulation of posterior primitive streak, lateral plate mesoderm, and limb bud markers in a stepwise manner. Thus, PSCs were induced into limb bud populations, although the induction efficiency remains to be investigated. The induction protocols will lead to the development of reliable methods for inducing skeletal cells from PSCs.

ヒト多能性幹細胞から肢芽の細胞を誘導する

沿軸中胚葉、側板中胚葉に由来する椎板や肢芽を構成する細胞の一部は、骨芽細胞や軟骨細胞といった骨格系細胞となる。ヒト多能性幹細胞から骨格系細胞集団を誘導できれば、骨格形成過程の理解や再生医療に貢献すると考えられる。しかしながら、効率的な誘導方法は未だ確立されていない。そこで本研究は、発生学的に重要なシグナル経路を調節することで、ヒト多能性幹細胞から骨格前駆細胞の由来となる肢芽の細胞を*in vitro*で誘導する手法の開発を目指した。低分子化合物（CHIR99021, C-59, A83-01）と骨形成タンパク質（BMP）-4を組み合わせてヒト多能性幹細胞を培養することで、肢芽への誘導を段階的に行った。その結果3日間の肢芽誘導培養において、後方原始線条のマーカー、側板中胚葉のマーカー、肢芽のマーカーが段階的に上昇することを確認した。遺伝子発現のデータより、ヒト多能性幹細胞から肢芽の細胞へ誘導できたと考えられたものの、誘導効率についてはさらなる検討が必要だと思われる。本法は、ヒト多能性幹細胞から骨格系細胞を誘導する手法の開発に貢献すると考えられた。

研究発表内容の紹介

発生過程に組織再生のヒントを得ることは論理的なアプローチである。このアプローチにより、多能性幹細胞から骨や軟骨の前駆細胞を誘導できれば、顎顔面領域の組織再生法の開発、ヒト骨格発生過程のメカニズムの解明、そして骨関連疾患の発症メカニズムの理解と治療法開発に貢献すると考えられる。また本発表の方法は、3日間で多能性幹細胞から肢芽を誘導することが可能であり、今後の展開が期待される。（ファカルティー・アドバイザー：松下 祐樹）

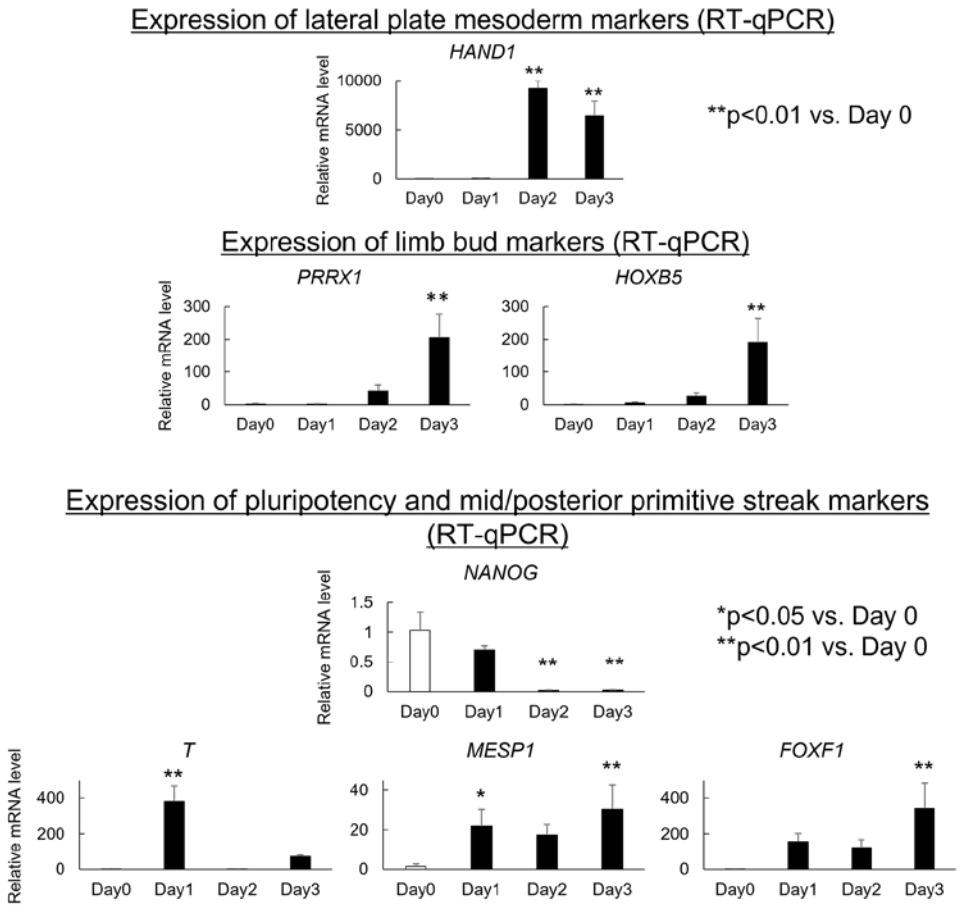
Induction of limb bud cells from human pluripotent stem cells (hPSCs)

Problem: During the third week of gestation, all three germ layers are established. The mesoderm differentiates into paraxial mesoderm, intermediate mesoderm, and lateral plate mesoderm. The paraxial mesoderm develops into the sclerotome, and the lateral plate mesoderm forms the limb bud. Some cells in the sclerotome and limb bud differentiate into skeletal cells including chondrocytes and osteoblasts. Induction of the skeletal cell populations from pluripotent stem cells (PSCs) will contribute to understanding of the skeletal formation process and regenerative therapies. However, reliable induction methods have not been established yet.

Hypothesis: Developmentally critical signaling pathways for limb bud formation (Wnt, TGFb, and BMP signals) have been clarified from basic researches. Therefore, we hypothesized that we would be able to reproduce the developmental process by recapitulating the pattern of the signaling activities in PSCs using small compounds even under zeno-free and serum-free conditions. Thus, this study was aimed to develop protocols for inducing PSCs particularly into limb bud cells, which were origins of skeletal progenitors, by manipulating activities of the developmentally critical signaling pathways in vitro.

Methods: Limb bud cells were induced from PSCs in stepwise manners with combinations of small compounds (CHIR99021, C-59, and A83-01) and bone morphogenetic protein (BMP)-4. Total RNA was extracted from the induced cells, and mRNA expressions were analyzed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Results: In the 3-day limb bud induction culture, posterior primitive streak markers (*T*, *MESP1*, and *FOXF1*) were upregulated on day 1. The lateral plate mesoderm marker *HAND1* was subsequently upregulated on day 2. Limb bud markers, *PRRX1* and *HOXB5*, were upregulated on day 3.



Conclusion: PSCs were induced into limb bud populations, although the induction efficiency remains to be investigated. The induction protocols will lead to the development of reliable methods for inducing skeletal cells from PSCs.

Controlling the growth of hydroxyapatite nanocrystals on lipid bilayers with the guide of molecular dynamics simulation

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【Problem】 Nano-sized crystal of hydroxyapatite (nano-HAp) has potential for many medical application, including dentistry; however, controlling the shape and surface, by which the properties and performance are highly affected, remains a problem.
【Aim】 The student clinician was intrigued by the growth of nano-HAp on a lipid bilayer and the possibility that the type of lipid molecule may control the growth surface. This research aims to investigate what character is needed for lipids to control the growth surface using computer simulation.
【Method】 Using a computational technique called molecular dynamics, the affinity of a- and c-surfaces for dipalmitoylphosphatidylcholine (DPPC) bilayer was simulated and analyzed at an atomic level.
【Result】 The calculation of energy by molecular dynamics simulation revealed that the a-surface of nano-HAp is easy to grow on DPPC bilayer. The simulation also showed that selective removal of the c-surface is enabled by heating. This temperature control will facilitate the refinement of nano-HAp. The analysis of bond angles between atoms demonstrated that the growth surface is determined by matching of the sizes between the crystallographic periodicity of nano-HAp and the hydrophilic group of lipid: the surface with a periodicity that has better match to the hydrophilic group is easier to grow.
【Conclusion】 An optimal condition for controlling the growth surface of nano-HAp on lipid bilayers was uncovered using molecular dynamics simulation.

分子動力学法を利用して脂質二重膜上で成長するハイドロキシアパタイトナノ結晶を制御する

【問題】 ハイドロキシアパタイトのナノサイズ結晶（nano-HAp）は、歯科をはじめ医療への応用的価値の高い材料だが、その特性と実用性を決める表面状態を制御して製造する技術は未だ発展途上である。
【目的】 nano-HApが脂質二重膜で成長するとき、脂質の分子種を変えると結晶の特定の表面が成長することに気づいた。コンピュータシミュレーションにより、脂質分子と、nano-HApの2つの結晶面、*a*面と*c*面との吸着性を原子レベルで調べることで、意図した結晶表面を成長させるために必要な脂質分子の条件を明らかにしようとした。
【方法】 分子動力学法と呼ばれる計算手法を用いて、脂質の1つであるDPPCの二重膜上にnano-HAp*a*面と*c*面を吸着させ、両者の親和性の違いを原子レベルでシミュレートし解析した。
【結果】 シミュレーション計算により、DPPC上ではnano-HApのa面が成長しやすく、温度を上げることでc面のみを選択的に脱離させ結晶をさらに精製できるとわかった。原子間結合角の解析から、nano-HApの成長表面として、吸着を司る脂質親水基のサイズに対し、結晶周期間隔の合致度が高い面が成長することを解明した。
【結論】 nano-HApの脂質二重膜上での成長を制御するための条件を分子動力学により明らかにした。

研究発表内容の紹介

原子1個1個をシミュレートする分子動力学はこれまでシンプルな物質の性質を調べるのに使われてきましたが、近年のPC性能の向上とともに大きな系、中でも生体現象を理解するのに使われ始めています。本研究はハイドロキシアパタイトのナノ結晶という歯科材料と脂質二重膜の親和性を左右する要因を明らかにし、結晶成長だけでなく、細胞と硬組織が関与する基本的現象を原子レベルで理解できる点でも多くの発展性があると考えています。（ファカルティー・アドバイザー：佐藤 衆一）

Controlling the growth of hydroxyapatite nanocrystals on lipid bilayers with the guide of molecular dynamics simulation

【Problem】

Nano-sized crystals of hydroxyapatite (nano-HAp) have various uses in medical fields, including dentistry, such as repairing caries and coating catheters. Its quality performance heavily depends on the shape and surface of the nanocrystal. Regarding the surfaces, HAp has two crystallographically-defined surfaces (*a*- and *c*-surfaces; Fig. 1). These surfaces show quite different physical properties when they are exposed outside. Controlling the surfaces is therefore needed for applications, although it remains a technical problem.

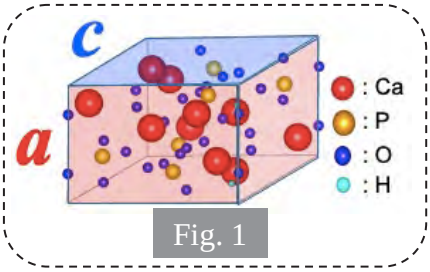


Fig. 1

【Hypothesis】

On the subject of surface control, I was intrigued by the phenomena that static deposition on lipid bilayers in water solvents composed of ions constructing HAp, that is, Ca^{2+}, PO_4^{3-}, and OH^- grows the specific surface of nano-HAp. In addition, the growth surface differs according to the type of lipid molecule (Fig. 2). I thought that appropriate selection of the lipid molecule will enable us to control the specific growth surface selectively.

In this research, I thought that making use of molecular dynamics will provide us with the most suitable lipid molecule for the intended growth surface. Molecular dynamics is a computer simulation of molecular motions according to each force working on each atom. For the pursuit of this research, the situation that nano-HAp crystal adsorbing on lipid bilayer as the scaffold for growth should be reproduced on molecular dynamics. Then the following problems may be investigated at the atomic level:

1. Which surface grows on a certain lipid bilayer?
2. What is the important factor for the growth of a specific surface?

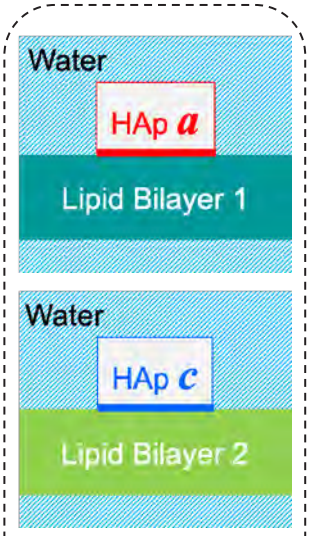


Fig. 2

【Method】

As a model of lipid molecules for this research, dipalmitoylphosphatidylcholine (DPPC) was selected because it has been used in various experiments. A 5 × 5 × 5 unit cell-sized nano-HAp crystal was set on DPPC bilayer in water (Fig. 3). The condition was set at 37°C and 1 atm.

Open-source software Lammps was used for the molecular dynamics simulation. Winmostar (X-ability) and open-source software Vesta were used for model construction and analysis.

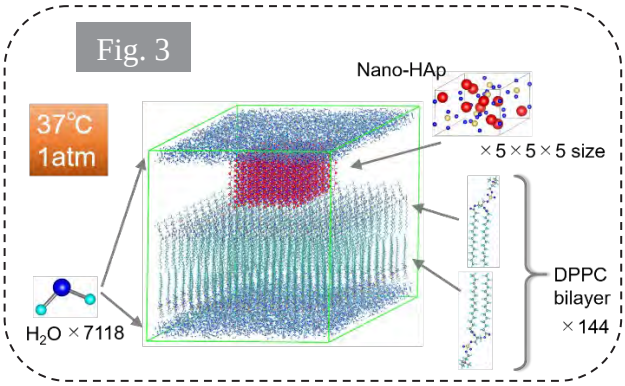


Fig. 3

【Result】

1) The affinity of nano-HAp *a*- and *c*-surfaces for DPPC

To find the answer to the question on ‘‘Which surface grows?’’ I compared the affinity of nano-HAp *a*- and *c*-surface for DPPC by calculating the energy. Energy is a measure of stability because things with large energy will move so hard, which means they are unstable.

I found that the *a*-surface has smaller energy, which indicates that the *a*-surface is easier to grow on DPPC (Fig. 4).

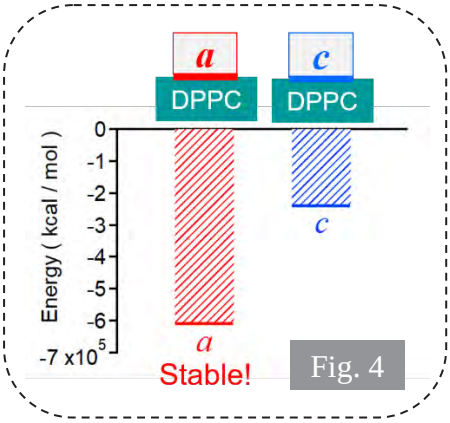


Fig. 4

2) Temperature dependence of the affinity

Temperature is one of the controllable parameters for crystal fabrication. I heated the system from 37℃ to 57℃ to study the temperature dependence of the affinity. As a result, the *a*-surface continues to adsorb DPPC, whereas the adsorption on the *c*-surface decreases to 60%. This result suggests that we may be able to refine the crystal with a specific surface by controlling temperature (Fig. 5).

3) Matching of sizes: hydrophilic group in lipid and periodicity of HAp surface

I found an important factor which influence the growth surface of nano-HAp. That is the matching of sizes between the hydrophilic group of lipid and crystallographic periodicity of the HAp surface.

The hydrophilic group of DPPC, and Ca on the HAp surface have a key role in the adsorption (Fig. 6a). In the adsorption on the *c*-surface, the size of DPPC hydrophilic group is too large for the periodicity of the HAp *c*-surface, which constrains the hydrophilic group to distort itself in order to adsorb on the surface with the same interval. This stress of DPPC induces instability of the *c*-surface adsorption. While in the adsorption on the *a*-surface, the size of DPPC hydrophilic group is similar to the size of the periodicity of the HAp *a*-surface. Therefore, DPPC can adsorb on the *a*-surface without much stress (Fig. 6b).

The distortions were shown by calculating the atomic bond angles in the hydrophilic group. Compared with the hydrophilic group which was not adsorbed on HAp, the one adsorbed on the *a*-surface showed little change in the angles. Whereas the one adsorbed on the *c*-surface showed a distinguished change in the small-angle region, indicating large distortion of the hydrophilic group (Fig.6c).

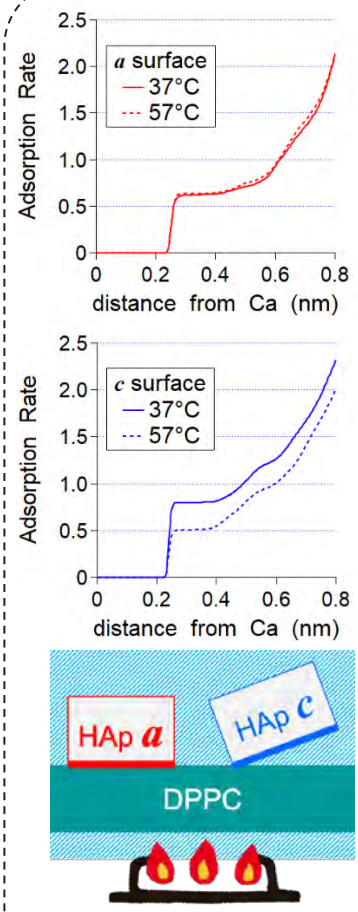
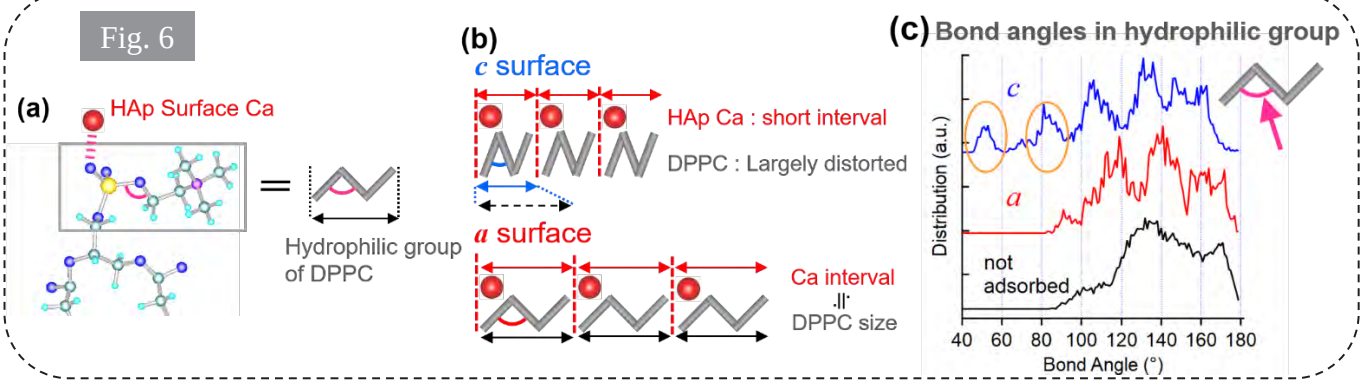


Fig. 5

Fig. 6



【Conclusion】

Using molecular dynamics, I was able to predict the growth surface, and able to find that matching of sizes between the hydrophilic group in lipid and the periodicity of HAp crystal is the important factor for controlling the growth surface.

I believe we can aim at the growth of the *c*-surface, with which enamel layer is covered. We can try many additional elements on computers, such as substituting the hydrophilic or hydrophobic group and mixing cholesterol. The trial will provide the most suitable lipid bilayer for the intended growth surface (Fig. 7).

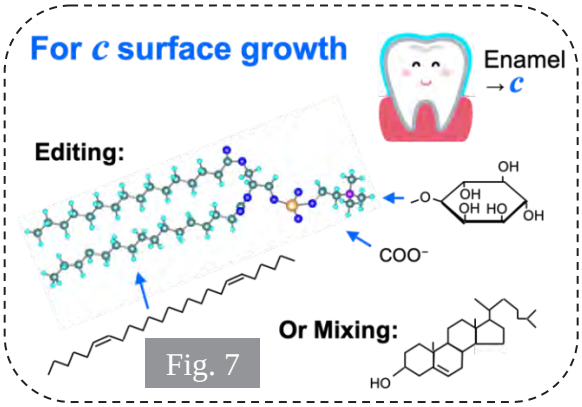


Fig. 7

Investigation of parasympathetic nerve fiber to dominate the inferior labial gland

奥羽大学歯学部 3年生 Ohu University School of Dentistry Class of 2025

國分 瑚楠 Konan KOKUBUN

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口腔組織学分野 教授 安部 仁晴

Objective The pathway of parasympathetic fibers to dominate the labial gland remains to be elucidated. The purpose of this study was to investigate the pathway of parasympathetic fibers to dominate the inferior labial gland.
Methods The inferior alveolar nerve from mandibular foramen to mental foramen was extracted from the cadavers for anatomical practice preserved at the Department of Anatomy. The nerve was divided into five groups, embedded in paraffin wax and made into transvers sections. The sections were stained with hematoxylin and eosin and observed with a light microscope.
Results There were no ganglion cells in the inferior alveolar nerve in the mandible.
Conclusion As parasympathetic nerve has the autonomic ganglion near the target organ, there is a possibility that the mental nerve has ganglion cells. We shall observe histologically the neural fibers of the mental nerve from the mental foramen to the inferior labial glands.

口唇腺からの唾液分泌に関与する副交感神経線維の探索

目的 大唾液腺は上唾液核と下唾液核からの節前線維が、それぞれ顎下神経節と耳神経節でニューロンを交換し、節後線維がそれぞれ顎下腺・舌下腺と耳下腺に到達することが知られているが、口唇腺については節前線維、神経節、節後線維は不明である。本研究では口唇腺からの唾液分泌を支配する副交感神経線維の経路を探索した。
方法 歯学部解剖実習体の下顎管内の構造物を確認した後、下顎管内の下歯槽神経を摘出した。下顎孔からオトガイ孔までを5つの領域に分けて薄切切片を作成し、ヘマトキシリンエオジン染色して光学顕微鏡にて観察した。
結果 下歯槽神経の5つの領域いずれにおいても神経細胞体は観察されなかった。
結論 耳神経節でニューロンを交換した節後線維が下歯槽神経に含まれているという動物実験の報告と、ヒトの舌神経で顎下神経節とは異なる部位に神経細胞体が存在するとの報告から、解剖実習体から下歯槽神経を採取して組織学的検索を行ったが、神経細胞体はみられなかった。副交感神経の神経節は効果器の近傍に存在するという特徴があるので、オトガイ孔から口唇腺までの検索を行う予定である。

研究発表内容の紹介

唾液はその約95%が三大唾液腺でつくられており、残りの数%が小唾液腺から分泌されている。口唇腺は口唇粘膜を湿潤に保つ役割を担っているが、唾液分泌を支配する自律神経の経路については不明である。下歯槽神経に副交感性の神経線維が含まれているという動物実験の報告に接する機会があり、ヒトでも可能性があるのではないかと考えて探索を行っているところである。
（ファカルティー・アドバイザー：柴田 達也）

Investigation of parasympathetic nerve fiber to dominate the inferior labial gland

(Problem) Saliva is produced by the salivary glands and is secreted in the oral cavity. The water secretion of the saliva is dominated by parasympathetic nerve. Preganglionic parasympathetic fibers from the inferior salivatory nucleus project the otic ganglion and postganglionic fibers from the ganglion innervate the parotid gland. Preganglionic parasympathetic fibers from the superior salivatory nucleus project the submandibular ganglion and postganglionic fibers from the ganglion innervate the submandibular and sublingual glands. The labial gland, one of the minor salivary glands, is located to the mucosal epithelium of the upper and lower lip. The pathway of parasympathetic fibers to dominate the labial gland remains to be elucidated.

(Hypothesis) The presence of the postganglionic fibers originating in the otic ganglion is reported in the inferior alveolar nerve in the guinea pig. The inferior labial glands are also reportedly innervated by the inferior labial branch of the mental nerve. We hypothesized that the labial branch contains postganglionic parasympathetic fibers to dominate saliva secretion of the inferior labial gland although this branch of the trigeminal nerve dominates general sense in the lower labial and mental regions. In this study, we have investigated the presence of parasympathetic fibers in the inferior alveolar fiber which is obtained from bodies for anatomy by histological observation in order to investigate the pathway of parasympathetic fibers to dominate the inferior labial gland.

(Materials and Methods) This study was performed using 6 cadavers for anatomical practice preserved at the Department of Anatomy. The inferior alveolar nerve from mandibular foramen to mental foramen was extracted and divided into five groups (Fig 2E). These specimens were post-fixed for 24 hrs with formalin and then embedded in paraffin wax. Transverse sections 10 μm in thickness were mounted on glass slides and then stained with hematoxylin and eosin. Histologic observations were performed with the aid of a light microscope. Moreover, it was observed how the inferior labial branch of the mental nerve innervated the inferior labial glands (Fig 1).

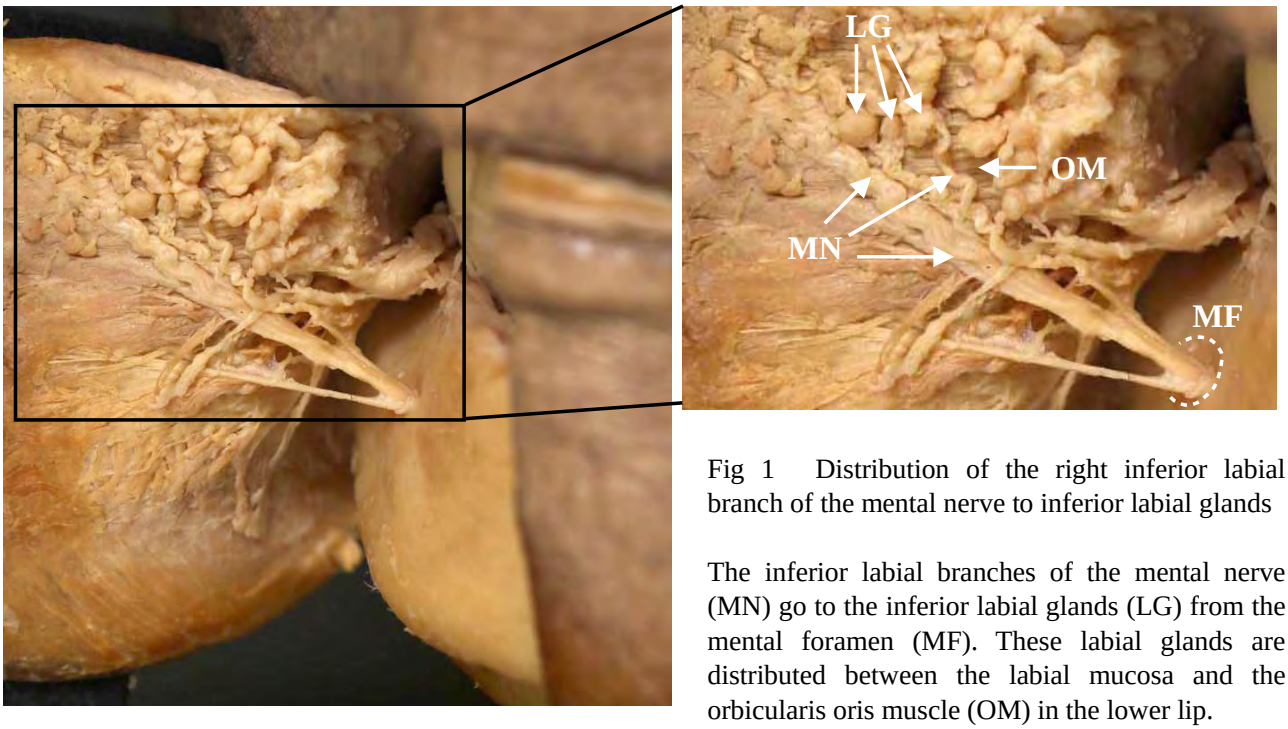


Fig 1 Distribution of the right inferior labial branch of the mental nerve to inferior labial glands

The inferior labial branches of the mental nerve (MN) go to the inferior labial glands (LG) from the mental foramen (MF). These labial glands are distributed between the labial mucosa and the orbicularis oris muscle (OM) in the lower lip.

(Results) The inferior labial glands were innervated by the inferior labial branches of the mental nerve. These labial glands were distributed between the labial mucosa and the orbicularis oris muscle in the lower lip (Fig 1). In the region near the mandibular foramen, the large neural fiber was observed (Fig 2A and 2B). In the central part of the mandibular canal, the number of neural fibers increased and the diameter of the neural fibers became smaller (Fig 2C and 2D). In the region near the mental foramen (Fig 2E), the number of neural fibers decreased and the diameter of the neural fibers became much smaller. No ganglion cell bodies were found in the five regions (Fig 2).

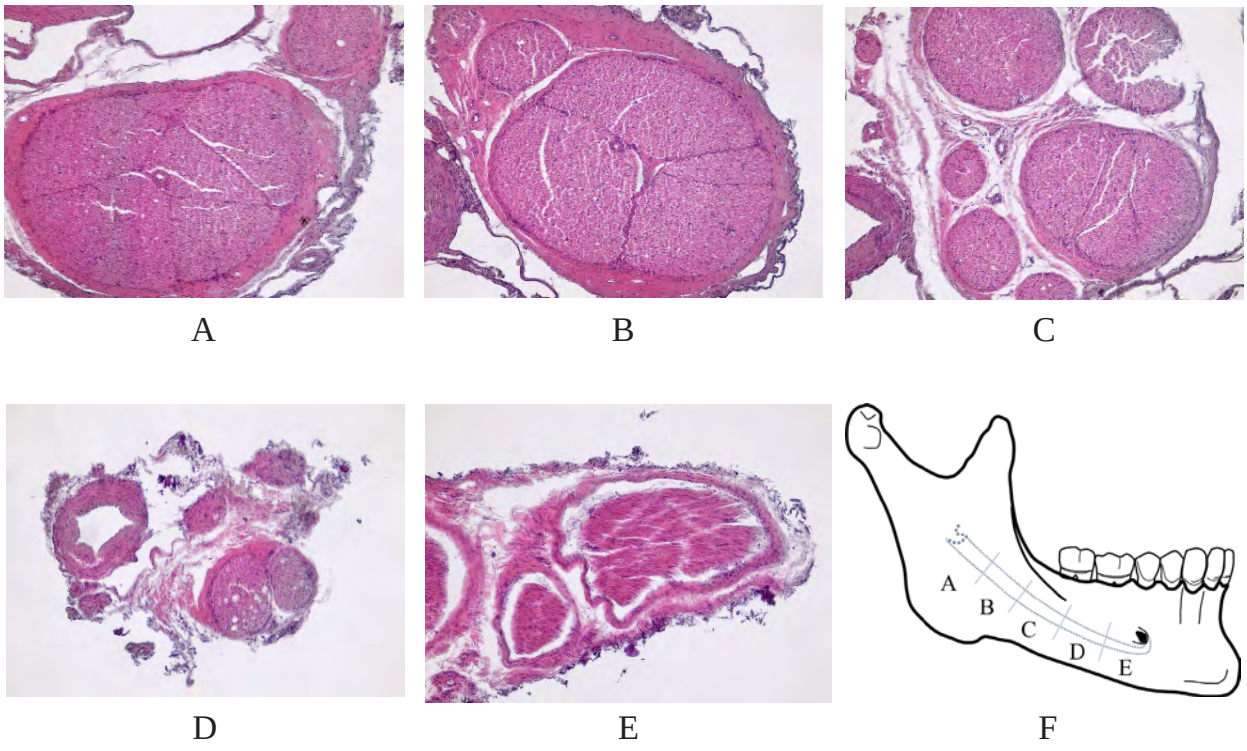


Fig 2　Histological sections of the inferior alveolar nerve with hematoxylin and eosin staining

(Conclusion) There is no description of the autonomic nerve pathway to innerve the labial glands in the textbooks. The postganglionic parasympathetic fibers originating in the otic ganglion were reported to be distributed in the inferior alveolar nerve in the guinea pigs. Another human study reported the presence of ganglion cells within the lingual nerve. Then, we investigated if there were ganglion cells within the inferior alveolar nerve in the mandibular canal. There were no ganglion cells in the inferior alveolar nerve which we observed. Although the postganglionic fibers from the otic ganglion might innervate the inferior labial glands, the ganglion is far from those glands. In general, parasympathetic nerve has the autonomic ganglion near the target organ. There is also a possibility that the mental nerve has ganglion cells. We shall observe histologically the neural fibers of the mental nerve from the mental foramen to the inferior labial glands and clarify if the mental nerve has parasympathetic ganglion cells.

Effects of the number of ethylene glycol units on the antitumor function of 9bw

日本大学歯学部　5年生　Nihon University School of Dentistry　Class of 2023

関本 和祥　Kazuaki SEKIMOTO

ファカルティー・アドバイザー：解剖学第Ⅰ講座 准教授　藤原恭子
研究指導協力者：理工学部 物質応用化学科 教授　大月穣

Nonaethylene glycol mono（4′-iodo-4-biphenyl）ester（9bw）induce cell death by inhibiting the activity of respiratory complex I in cancer cells including oral cancer cells. As its toxicity is lower in normal cells than in cancer cells, 9bw has potential as a new anti-tumor agent with low adverse effects. However, the exact molecular mechanisms by which 9bw inhibits complex I are not yet known. As 9bw is composed of nine ethylene glycol（EG）units, I hypothesized that the number of EG units may be critical for its function. To verify this hypothesis, the function of 9bw was compared with those of 9bw derivatives, 3EG, 6EG, and 12EG containing 3, 6, and 12 EG units, respectively. All compounds suppress the viability of human tongue cancer-derived cell line HSC4 in a dose-dependent manner, however, the effective concentration was higher in 3EG and 6EG than that of 9bw and 12EG. Only 9bw and 12EG reduced ATP concentration in HSC4 cells significantly. When the effects on respiratory complex I were analyzed, only 6EG and 9bw showed significant inhibitory effect. Based on these results, I concluded that the number of EG units in the molecule affects the function of 9bw.

分子内エチレングリコールの数が9bwの抗腫瘍効果に与える影響

Nonaethylene glycol mono（′4-Iodo-4-biphenyl）ester（9bw）は呼吸鎖複合体Ⅰの活性を阻害することで、口腔がんをはじめ様々ながん細胞を殺傷する。正常細胞への毒性は低いため、9bwは副作用の少ない新規抗腫瘍薬として有望だが、その詳細な分子機序は不明である。9bwは分子内に9個のエチレングリコール（EG）を持つが、このEGの数が9bwの機能を左右する可能性について検証するため、本研究ではEG数を3、6、12に変化させた化合物3EG、6EG、12EGと9bw の間で機能比較を行った。ヒト舌がん細胞株HSC4の生存率は、どの化合物によっても濃度依存的に低下したが、9bwや12EG は3EGと6EGと比べて低濃度で効果を示した。また、9bwと12EGのみがHSC4の細胞内ATP濃度を有意に抑制した。一方、呼吸鎖複合体Ⅰに対しては、9bwと6EGのみが有意な阻害効果を示した。以上の結果から、分子内EGの数は9bwの機能に影響すると結論付けた。

研究発表内容の紹介

口腔がんの治療法は外科的切除が第一選択であるが、顔貌や飲食、発話機能に影響する場合も多く、高いQOL維持のために、有効で副作用の少ない抗腫瘍薬の開発が求められている。解析対象とした9bwは呼吸鎖複合体Ⅰを阻害して細胞を殺傷するが、その詳細な分子機序や9bwががん細胞特異的に殺細胞効果を示す理由については全く不明である。これらを明らかにし、9bwを抗腫瘍薬として開発する上で、今回得られた知見は大変重要であると考える。（ファカルティー・アドバイザー：藤原 恭子）

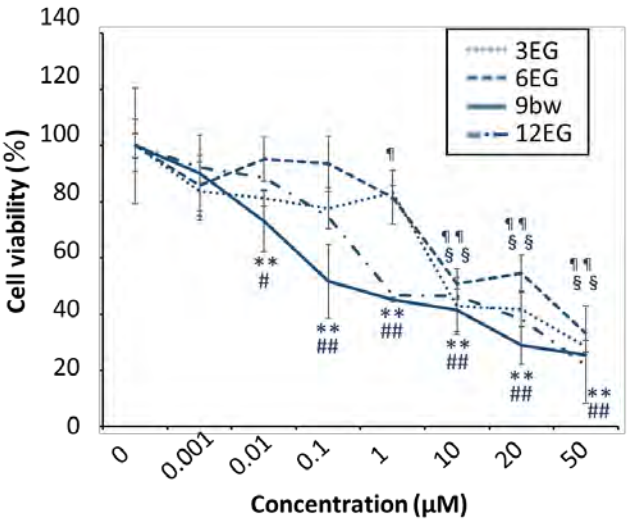
Effects of the number of ethylene glycol units on the antitumor function of 9bw

（Problem） Surgery is the first-line treatment for oral cancer. However, radiation therapy and/or chemotherapy are also applied depending on stage of the disease and/or the patient's condition. As surgery affects facial appearance and oral function, such as masticatory ability and articulation, there is an urgent need to develop novel therapeutic agents for oral cancer. Nonaethylene glycol mono(4′-iodo-4-biphenyl) ester (9bw), a novel polyethylene glycol molecule derived from soil bacteria, was recently shown to induce cell death by inhibiting respiration in mitochondria, suppressing ATP production in cancer cells. As the toxicity of 9bw is lower in normal cells than in cancer cells, this compound has potential as a new therapeutic agent for malignancies, including oral cancer, with low adverse effects. The exact molecular mechanisms by which 9bw inhibits the function of respiratory complex I in mitochondria is not yet known, and there is little similarity in chemical structure between 9bw and known complex I inhibitors.

（Hypothesis）9bw is composed of nine ethylene glycol (EG) units, and it was hypothesized that the number of EG units may be critical for its antitumor function.

（Methods） To verify the above hypothesis, the function of 9bw was compared with those of a number of 9bw derivatives, 3EG, 6EG, and 12EG with 3, 6, and 12 EG units, respectively. The human tongue cancer-derived cell line HSC4 was seeded into 96-well plates (n = 4) to test cell viability and ATP concentration. Cell viability was examined by WST8 assay. ATP concentration in cells was analyzed by measuring luciferase activity using Intracellular ATP measurement kit (IC2-200; TOYO Be-net). The activity of respiratory complex I was measured by monitoring the oxidation rate of NADH by isolated bovine heart mitochondria, using a MitoCheck Complex I Activity Assay Kit (Cayman) (n = 3).

（Results） Experiment 1 HSC4 cells were cultured in the presence of various concentrations of 3EG, 6EG, 9bw, and 12EG for 3 days, and cell viability was measured. As shown in Figure 1, the viability of the cells treated with ≥ 0.01 μM 9bw or 12EG was decreased in a dose-dependent manner. Treatment with ≥ 1 μM 3EG or 6 EG also decreased cells viability in a dose-dependent manner. However, cells treated with 3EG and 6EG at concentrations < 1 μM showed almost the same viability as control cells.



The viability of the cells treated with 1 μM 9bw or 12 EG was about 45% that of control cells, while cells treated with 1 μM 3EG or 6EG showed viability of about 80%. The reduced cell viability was confirmed by microscopic analysis, although no clear differences in morphology were found among cells treated with or

Figure 1. Cell viability 3 days after the addition of test compounds Cell viability is shown relative to control cells cultured in the absence of test compounds. Statistical significance vs. controls: 3EG, $^{\S}P < 0.05$, $^{\S\S}P < 0.01$; 6EG, $^{\P}P < 0.05$, $^{\P\P}P < 0.01$; 9bw, $^{*}P < 0.05$, $^{**}P < 0.01$; 12EG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

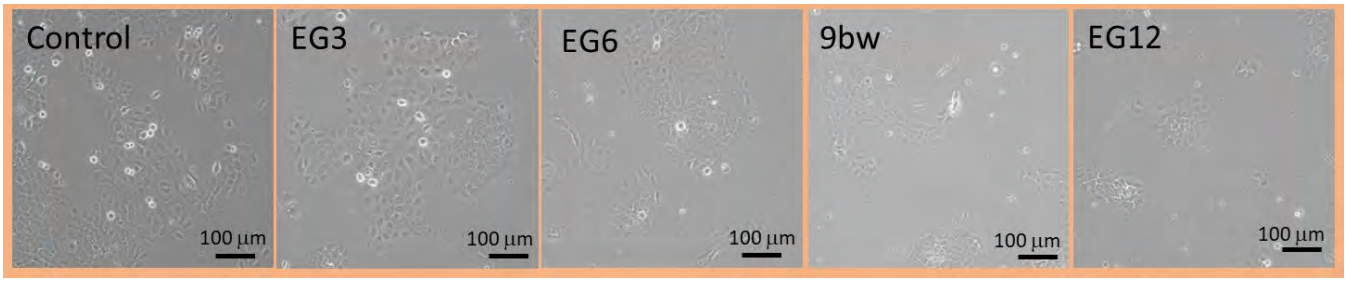


Figure 2. Images of the cells cultured in the presence or absence of compound (1 μ M)for 3 days

Experiment 2 Next, HSC4 cells were cultured with or without each test compound at 1 μM for 12 h, and the cells were then lysed in buffer containing detergent followed by measurement of intracellular ATP concentration (Figure 3). ATP concentration was reduced to around 60% of the control in cells treated with 9bw or 12EG. In 6EG-treated cells, ATP concentration was 80% of control, but the difference was not significant. There were no clear differences in ATP concentration between 3EG-treated and control cells.

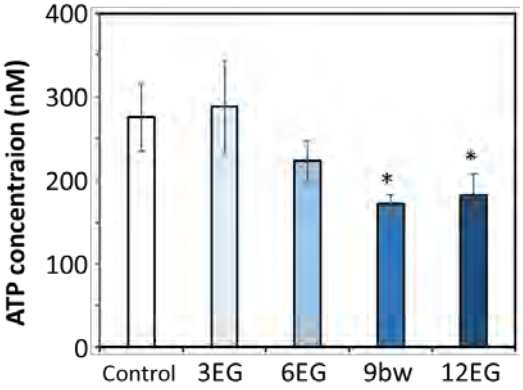


Figure 3. Concentration of ATP in the cells treated with each compound (1 μ M)for 12h

*$P < 0.05$

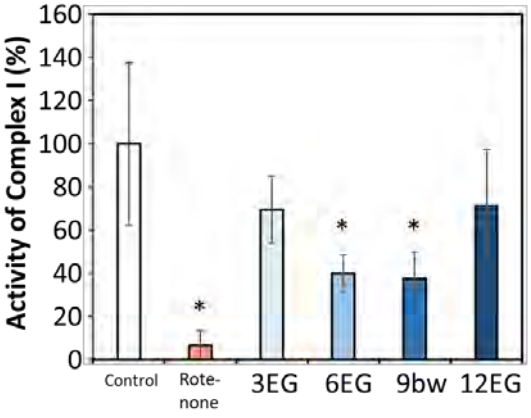


Figure 4. Activity of respiratory complex I in the presence of each compound (2 μ M)

*$P < 0.05$

Experiment 3 Finally, the inhibitory effects of each compound on mitochondrial respiratory complex I were examined (Figure 4). Test compounds at 2 μM or medium alone as a control was applied to reaction medium containing bovine heart mitochondria, and the oxidation rate of NADH was measured by monitoring the OD_{340}. In the presence of 6EG and 9bw, the activity of complex I was around 40% of control, and the differences were statistically significant. The activity of complex I was slightly reduced in the presence of 3EG and 12EG, but the differences were not statistically significant.

（Conclusion） The results of this study showed that the number of EG units in the molecule affects the function of 9bw. That is, a greater number of EG units showed a greater effect on cell viability and intracellular ATP concentration. Both 3EG and 6EG showed less effect on cell viability and ATP concentration than 9bw and 12EG. However, only 6EG and 9bw showed significant inhibitory effects on respiratory complex I.

Although the discrepancies are difficult to explain at present, there are several possible explanations for these observations. First, the number of EG units may be critical for the uptake of the compounds into cells, which would explain why the results of the third experiment in which purified mitochondria were used were different from the other two. Alternatively, 12EG may target respiratory complexes other than complex I.

To verify these hypotheses, it will be necessary to analyze the intracellular distributions of the compounds using fluorescently labeled 9bw, 3EG, 6EG, and 12EG. In addition, further studies to analyze the inhibitory effects of these compounds on respiratory complexes other than complex I are required.

Epigallocatechin gallate involves in inhibition of histamine H1 receptor expression and cell proliferation and upregulation of histamine synthesizing enzyme in human oral epithelial cancer cell line HSC4

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ファカルティー・アドバイザー：病態制御学分野 教授 小笠原 正人

Histamine production and expression of histamine receptors have been reported in tumors such as malignant melanoma, small cell lung cancer. In recent studies, it is reported that epigallochatechin gallate (EGCG) have potentials to potently inhibit cancer cell proliferation *in vivo* using animal administration study. However, the mechanism of inhibitory effects has not been fully understood. Furthermore, knowledge of histamine production and receptor systems in oral epithelial carcinoma were not comprehensibly clarified to date. Using the human oral epithelial cancer cell line, HSC4 cells, scratch assay was performed to evaluate the effects on proliferation/migration. Next, histamine synthesizing enzyme, and histamine H1R expression were analyzed with western blot analysis in the presence or absence of EGCG. Histamine concentration in cell culture media were measured with histamine assay kit. EGCG inhibited cell proliferation/migration, the expression of H1R and showed the increases of HDC expression and production of histamine. These results suggested that EGCG have effects on regulation of histamine producing enzyme and histamine receptors, possibly resulting in inhibition of cell proliferation/migration in oral epithelial cancer cell line. EGCG might have potentials to present novel molecular targets for oral epithelial cancer cell inhibition.

エピガロカテキンガレートは口腔扁平上皮癌細胞株HSC4のヒスタミンH1受容体発現と細胞増殖を抑制し、ヒスタミン合酵素の誘導を促進する

悪性黒色腫や小細胞肺がんなどでヒスタミンの産生とヒスタミン受容体の発現が報告されている。最近の研究で、エピガロカテキンガレートの投与によって癌細胞の増殖を強力に抑制することが動物実験で示されている。しかしながら、その作用機序は十分に理解されていない。また、口腔扁平上皮癌でのヒスタミン産生、ヒスタミン受容体の発現に関しては十分に検討されていない。口腔扁平上皮癌細胞株の一つであるHSC4を用い、EGCG影響下でのスクラッチアッセイ、ヒスタミン産生能、ヒスタミンH1受容体の発現をウエスタンブロットにて解析した。EGCGは口腔扁平上皮癌に対しヒスタミン産生を促進し、一方、ヒスタミンH1受容体の発現を低下させた。さらに、スクラッチアッセイで細胞の増殖/浸潤を抑制した。EGCGは口腔扁平上皮癌細胞に対して新たな分子標的を提示している可能性を示した。

研究発表内容の紹介

癌化学療法の進歩には目覚ましいものがあり、癌全体での予後改善に大きく貢献してきているが、口腔扁平上皮癌の予後は十分な改善が認められない。新たな分子標的の探索が必要である。最近、腫瘍細胞でもヒスタミンの産生、ヒスタミン受容体の発現が報告され、悪性黒色腫では増殖因子として働き、抗腫瘍薬としての抗ヒスタミン薬が見直されている。さらに、最近、緑茶成分の一つでカテキンの中のepigallochatechin gallate (EGCG) が抗腫瘍効果を示すことが報告されている。本研究はEGCGが口腔扁平上皮癌のヒスタミン合成・受容体システムに対する効果を明らかにしたもので、臨床応用への期待ができると考えられる。
（ファカルティー・アドバイザー：小笠原 正人）

Epigallocatechin gallate involves in inhibition of histamine H1 receptor expression and cell proliferation and upregulation of histamine synthesizing enzyme in human oral epithelial cancer cell line HSC4

[Problem]

Squamous epithelial cell carcinoma is reported as the most frequent type of oral cancer. Although recent cancer chemotherapy has been making novel strategies and showing significant improved prognosis in overall analysis, unfortunately, the prognosis of oral cancer has not been improved over years. Therefore, a novel strategy for oral cancer treatment including chemotherapy is required. Recent studies demonstrated that malignant melanoma, small cell lung cancer produce and release histamine, which is involved in proliferation and extension of cancer cells as a growth factor in autocrine or paracrine modes. However, investigation of histamine production and histamine receptor expressions in oral epithelial cancer remains to be elucidated.

Recent study shows that epigallocathechin gallate (EGCG), one of catechins from green tea, inhibits cancer cells. In the current study, we investigated the effects of EGCG on histamine production, histamine receptor expression in oral epithelial cancer cell lines.

[Hypothesis]

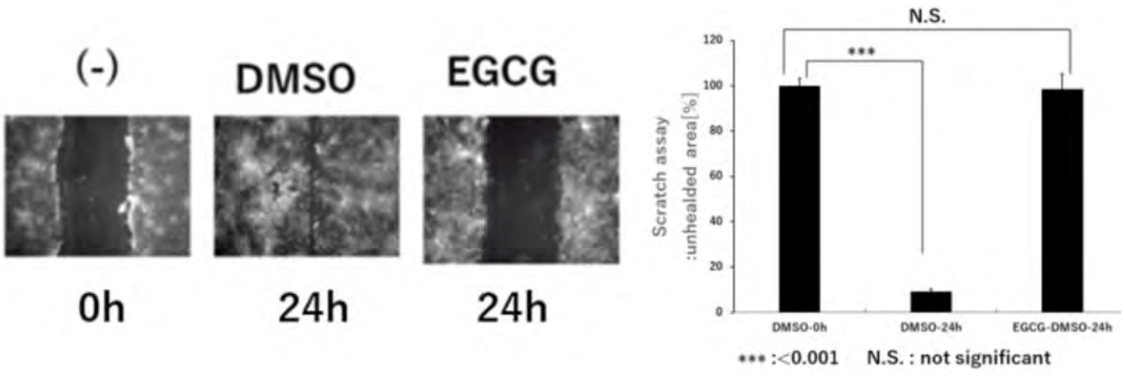
We hypothesized that histamine is produced from L-histidine via L-histidine decarboxylase (HDC) and that histamine receptors are expressed in oral epithelial cancer cell line, HSC4. Based on the recent studies that the usage of histamine H1 receptor antagonist showed inhibition of tumors such as malignant melanoma, we also hypothesized that EGCG can confer inhibition of the cancer cell lines through downregulation of histamine production and histamine receptors.

[Materials and methods]

1. Cell treatment: HSC4 cells were exposed to EGCG (100μM) for 24 hours.
2. Wound healing assay: The cultured cells were scratched for 24 hours in the presence or absence of EGCG to evaluate the remaining areas compared with initial scratched areas, using J-image area calculation methods.
3. Western blot analysis: cell pellets obtained from oral cancer cell line, HSC4 were lysed with RIPA buffer supplemented with a protease inhibitor cocktail and phosphatase inhibitors. The total soluble proteins(30mg) were separated via 10% SDS-polyacrylamide gel electrophoresis. After protein blotting to PVDF membranes, protein expressions of histidine decarboxylase (HDC) and histamine H1 receptor (H1R) were investigated HDC antibody (1:1000) and H1R antibody (1:1000), respectively.
4. Measurements of histamine concentration in cell culture medium: The cell culture media were collected and analyzed using a histamine test kit (Bertin Pharma, France).

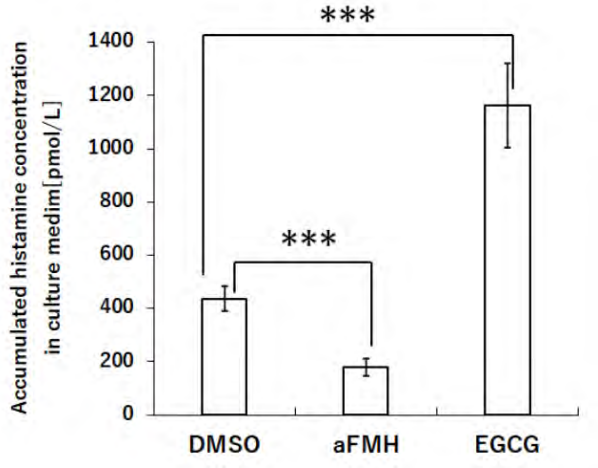
[Results]

Figure 1 **Epigallochatechin gallate inhibited proliferation/migration of HSC4 cells by scratch assay.**



HSC4 cell were exposed with DMSO or EGCG (100μM) for 24 hours. The proliferation/migration in HSC4 cells were significantly inhibited.

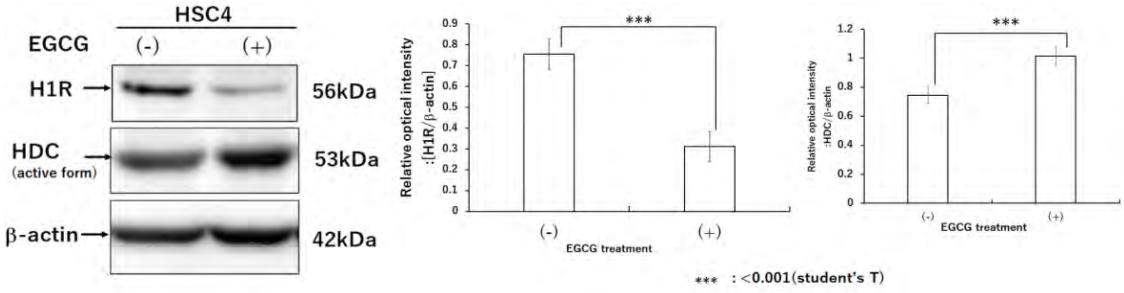
Figure 2 **Histamine production was induced by EGCG treatment in HSC4 cells.**



aFMH: a strong inhibitor for L-histidine decarboxylase

HSC4 cells produced histamine. Histamine production was inhibited in the presence of aFMH and was increased in the presence of EGCG for 24 hours.

Figure 3 **EGCG involved in downregulation of histamine H1 receptor and upregulation of histidine decarboxylase in HSC4 cells**



Protein expressions of Histamine H1 receptor (H1R) and L-histidine decarboxylase (HDC) were analyzed by western blot. β-actin was used as a loading control. H1R expression was significantly decreased and HDC expression was significantly increased.

[Conclusion]

In the current study, we concluded the following three points.

1. Oral epithelial cancer cell line, HSC4 produced and released histamine, consistent with induction of histamine synthesizing enzyme, HDC.

2. Proliferation/migration of HSC4 cells was inhibited by EGCG.

3. EGCG downregulated histamine receptor H1

EGCG is a strong inhibitor for HDC in vitro and shows difficulty in entering the cells. In our experiment, EGCG induced HDC in vivo, suggesting that EGCG has potentials to interact membrane proteins such as receptors. It is reported that EGCG can activate bitter taste receptor 14(TAS2R14) and 39(TAS2R39). In the future study, we need to elucidate the mechanism using cells with genetically deleted TAS2R14 or TAS2R39.

Effects of site-directed mutagenesis of the *mfa1* gene on fimbriation in *Porphyromonas gingivalis*

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The periodontal pathogen, *Porphyromonas gingivalis*, possesses Mfa1 fimbriae, which regulate biofilm formation. Mfa1 fimbriae are composed of major Mfa1 proteins, which form a fimbrial structure on the cell surface. However, little is known about the biogenesis mechanism. Recently, a new morphogenetic mechanism of fimbriae was proposed for the phylum Bacteroides, which includes *P. gingivalis*, in which the N-terminal region of a fimbrial protein is cleaved by a protease（gingipain）and the C-terminal region binds to and polymerizes another fimbrial protein. In this study, we used mutant strains by site-directed mutagenesis of the N- or C-terminal regions of the *mfa1* gene and examined the effects on Mfa1 fimbriation. The N- and C-terminal mutants showed lower levels of fimbriae on the cell surface compared to the parent strain. The amount of Mfa1 protein polymerization was reduced in the N-terminal and C-terminal mutants. These data demonstrated that both the N- and C-terminal regions play important roles in Mfa1 fimbriation.

Porphyromonas gingivalis における部位特異的変異導入によるMfa1線毛形態形成に及ぼす影響

歯周病関連細菌*Porphyromonas gingivalis*はバイオフィルム形成に強く関係するMfa1線毛を発現している。本線毛は、主要タンパク質であるMfa1が数珠状に重合し線維を形成するが、その形成メカニズムは不明な点が多い。近年、*P. gingivalis*を含むBacteroides門の線毛において、線毛タンパク質のN末端領域がプロテアーゼにより切断され、C末端の領域がもう一方の線毛タンパク質に結合し重合するという新たな形態形成機序が提唱された。本研究では、ジンジパイン（プロテアーゼ）により切断される*mfa1*のN末端領域、あるいはC末端領域に部位特異的変異を導入した変異株を作製し、Mfa1線毛の形態形成への影響を検討した。*mfa1*のN末端およびC末端の変異株では菌体表面での発現量が親株と比較して減少した。また、N末端変異株およびC末端変異株では重合型Mfa1の形成量が減少した。以上の結果から、*mfa1*のN末端およびC末端領域の両方が、線毛の菌体表面での発現に重要な役割を担っていることが明らかとなった。

研究発表内容の紹介

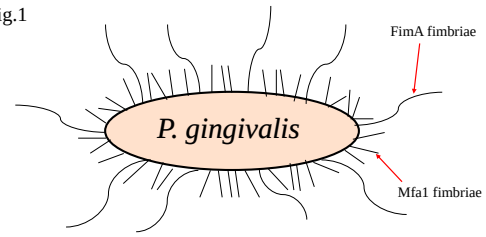
細菌の付着因子である線毛の新しい型であるV型線毛の形態形成機序の解明を目指した研究である。V型線毛に分類されるMfa1線毛を持つ歯周病原細菌*Porphyromonas gingivalis*に焦点を当て、部位特異的変異株を作製し、それらの株の表現型を解析することにより、線毛の形態形成機序の一端を明らかにした報告である。本研究が発展し、線毛の形態形成機序が明らかになれば、この機序を特異的に阻害するような新しい薬剤の開発に繋がる。（ファカルティー・アドバイザー：長谷川 義明）

Effects of Site-Directed Mutagenesis of the *mfa1* Gene on Fimbriation in *Porphyromonas gingivalis*

Problem

Periodontal disease is a chronic inflammatory disease caused by dental plaque adhering to the periodontium. The disease has also been associated with cardiovascular disease, low birth weight, diabetes, and rheumatoid arthritis. Chronic periodontitis is most often associated with three Gram-negative anaerobic bacteria, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, referred to as the "Red complex". In recent years, *P. gingivalis* has been recognized as the keystone pathogen inducing dysbiosis, an imbalanced state of the microbiota. The bacterium is of increasing importance to the prognosis of periodontal disease. It produces a variety of virulence factors, such as fimbriae, lipopolysaccharides, and proteases (Rgp and Kgp). Among these factors, the fimbriae are thought to play an important role as adhesion factor involved in attachment to host cells, biofilm formation, autoagglutination, and coaggregation with streptococci. *P. gingivalis* is known to have two distinct types of fimbriae, FimA and Mfa1 (Fig.1). Each of these fimbriae consist of five proteins encoded by *fim (fimA-E)* and *mfa (mfa1-5)* clusters, respectively (Fig.2). Recently, a new morphogenetic mechanism of fimbriae has been proposed for the phylum Bacteroides, including *P. gingivalis*, in which the N-terminal region of a fimbrial protein is cleaved by a protease and the C-terminal region binds to and polymerizes another fimbrial protein. However, it is unclear that Mfa1 fimbriation.

Fig.1



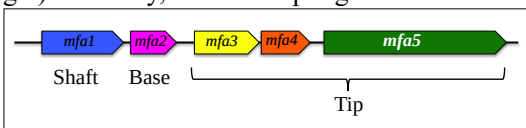


Fig.2

Hypothesis

Mfa1 fimbriae are composed of major Mfa1 proteins, forming a fimbrial structure on the cell surface. In addition, Mfa2, an outer membrane protein expressed downstream of *mfa1*, constitutes the base of the fimbriae, and serves as an anchor protein. Mfa3, Mfa4, and Mfa5 proteins expressed downstream of *mfa2* are localized at the tip of the fimbriae and are thought to function as adhesins (Fig.3). In the biogenesis of Mfa1 fimbriae, the 49th arginine in the N-terminal region of the Mfa1 protein is processed by Rgp to initiate polymerization, and six amino acid residues of the C-terminus are required for polymerization (Fig.4). In this study, we used mutant strains by site-directed mutagenesis in the N- or C-terminal regions of the *mfa1* gene and examined the effect on Mfa1 fimbriae expression on the cell surface of *P. gingivalis*. We also isolated and purified Mfa1 fimbriae from the mutant strains by ion exchange chromatography and observed them using transmission electron microscopy. This analysis would provide insights into the mechanism of Mfa1 fimbriation in *P. gingivalis*.

Fig.3

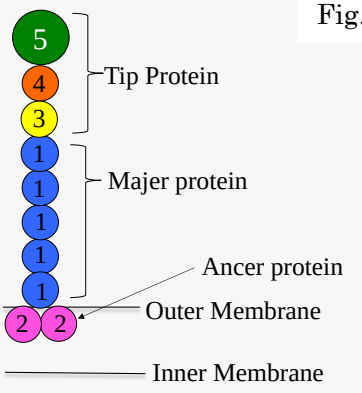
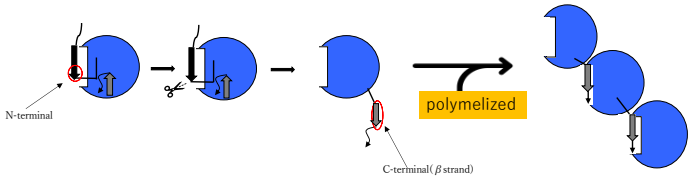


Fig.4 (Type five fimbriae polymerization mechanism)



Methods

Generation of site-directed mutant strains in the *mfa1* gene

Using the *fimA* deletion strain (JI-1) from *P. gingivalis* ATCC 33277 as the parent strain, the +mfa1R49A strain was constructed by replacing the 49th arginine, which is processed by Rgp, with alanine. In addition, the +mfa1K34R49A strain was constructed by replacing both the 49th arginine and 34th lysine, which are assumed to be processed by Rgp and Kgp, with alanines. The +mfa1ΔC strain, in which the C-terminal six amino acid residues were deleted, was also used in this study.

Filtration enzyme-linked immunosorbent assay (ELISA)

For performing filtration ELISA, *P. gingivalis* cells were initially applied over filters in a 96-well filtration plate. Anti-Mfa1 fimbriae primary antibody was used, followed by peroxidase (HRP)-conjugated anti-rabbit secondary antibody; *o*-phenylenediamine and H_2O_2 were added as substrates for HRP. The expression level of Mfa1 fimbriae on the cell surface was analyzed by absorbance at 490 nm.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

The whole cell lysates or the purified fimbriae were separated by SDS-PAGE and stained with Coomassie Brilliant Blue (CBB). In western blotting, the whole cell lysates separated by SDS-PAGE were transferred to PVDF membranes and reacted with anti-Mfa1 primary antibody followed by peroxidase-conjugated anti-rabbit secondary antibody.

Purification of Mfa1 fimbriae by ion exchange chromatography and heparin column

Mfa1 fimbriae were isolated and purified from the whole cell lysates of *P. gingivalis* using DEAE Sepharose Fast Flow chromatography. Samples with low purity and low number of fimbriae recovered by ion exchange chromatography were further purified by affinity chromatography using a heparin column.

Electron microscopy

The purified Mfa1 fimbriae were placed on a carbon-coated grid, negatively stained with 2% uranyl acetate, and observed by using a transmission electron microscope (H-7600, HITACHI).

Results

The +mfa1K34R49A and +mfa1ΔC strains showed significantly lower expression of fimbriae on the cell surface compared to the +mfa1 strain (Fig. 5). In contrast, no significant difference in fimbriae expression was observed in the +mfa1R49A strain. The amount of Mfa1 protein polymerization was reduced in the N-terminal mutant strain and it was not detected in the C-terminal mutant strain (Fig. 6). Using SDS-PAGE analysis of the purified fimbriae, bands for the tip proteins Mfa3, Mfa4, and Mfa5 were detected in the +mfa1R49A strain as in JI-1 (Fig. 7). Electron microscopy analysis revealed fibrous structures of approximately 100 nm in JI-1 (Fig. 8A), while in the +mfa1R49A strain, small number of fibers were observed alongside many round structures (Fig. 8B). Although purification of Mfa1 fimbriae from +mfa1K34R49A and +mfa1ΔC strains was attempted, no purified products were obtained.

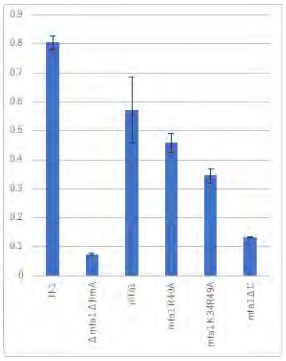


Fig. 5. Filtration ELISA. In addition to the site-specific mutant strains (+mfa1R49A, +mfa1K34R49A, and +mfa1 ΔC), JI-1 (parent strain), Δmfa1 Δ fimA (negative control strain), and +mfa1 (positive control strain) were analyzed.

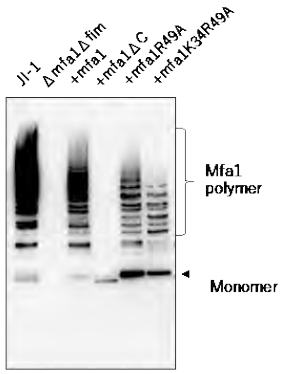


Fig. 6. Immunoblot analysis. The whole cell lysates were solubilized in SDS buffer and heated to: 70°C for 10 min. The samples were separated on SDS-PAGE, blotted to a membrane and probed with a polyclonal Mfa1 fimbriae antibody. The signal intensity of the polymerized Mfa1 band was reduced in the N- and C-terminal mutants.

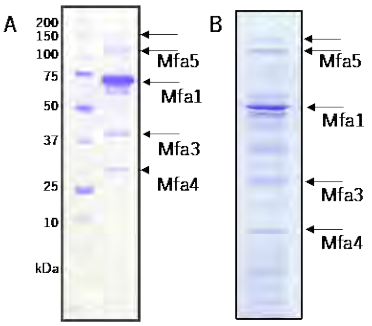


Fig. 7. SDS-PAGE and CBB staining. The Mfa1, Mfa3, Mfa4 and Mfa5 bands were observed in JI-1 (A) and +mfa1R49A strain (B).

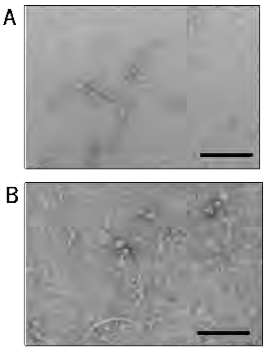


Fig. 8. Structure of the purified fimbriae. Electron micrograph of negatively stained purified from JI-1 (A) and +mfa1R49A (B).

Conclusion

Both the N-terminal and C-terminal regions play important roles in Mfa1 fimbriation. In addition, the six amino acid residues at the C-terminal region are essential for the polymerization of the Mfa1 protein and the expression of Mfa1 fimbriae on the cell surface.

Elucidation of the signal system in the oral biofilm that regulates dysbiosis of the oral flora

日本大学松戸歯学部 2年生 Nihon University School of Dentistry at Matsudo
Class of 2026

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ファカルティー・アドバイザー：感染免疫学講座 教授 泉福 英信

Oral dysbiosis may affect the immune system and affect general health. The biofilm (BF) formed on the tooth surface by *Streptococcus mutans* is involved in the onset of caries, but has the induction abilities of aggregation with various bacteria including the opportunistic bacterium *Staphylococcus aureus*. Such BF can cause oral dysbiosis, affect immunity and threaten general health. *S. mutans* has various signaling mechanisms for molecules involved in glucan production (GtfB, GtfC) and quorum sensing (QS). Therefore, in this study, I hypothesized that various signaling mechanisms of *S. mutans* are involved in dysbiosis of the oral flora. As a result, BF formation of *S. aureus* was not dependent on glucan formation by *S. mutans*, but affected salinity and QS-controlled killing. It was clarified that QS works by the aggregation of bacteria and kills the bacteria, and as a result, *S. aureus* forms BF utilizing the destroyed bacterial components in a salt concentration-dependent manner. To prevent dysbiosis of oral flora, it is important to control salt intake rather than sugar intake, physically remove BF by oral care to inhibit QS.

口腔フローラのdysbiosisを調節する口腔バイオフィルム内シグナルシステムの解明

口腔のディスバイオーシス（dysbiosis，フローラの崩壊）は、腸内フローラと同様、免疫系に影響を及ぼし全身の健康にも影響を与える可能性がある。歯表面に形成される*Streptococcus mutans*によるバイオフィルム（BF）は、う蝕発症に関わる一方、日和見菌である*Staphylococcus aureus*を含む様々な菌を取り込む性質がある。このようなBFは、口腔のdysbiosisを起こし、免疫力に影響を与え、全身の健康を脅かす可能性がある。*S. mutans*は、グルカン産生に関わる分子（GtfB, GtfC）やクオラムセンシング（Quorum sensing, QS）に関わる分子を含む様々なシグナル機構を有している。そこで、本研究では、口腔フローラのdysbiosisに*S. mutans*の様々なシグナル機構が関与している仮説を立て検討を行った。その結果、*S. aureus*のBF形成は*S. mutans*によるグルカン形成に依存的ではなく、塩分濃度およびQSに制御された死菌形成に影響していた。菌の凝集が起こることで、QSが働き、菌を破壊、その結果、破壊された菌成分を利用して*S. aureus*が塩濃度依存的にBF形成することが明らかとなった。口腔フローラDysbiosisの阻止には、砂糖摂取よりむしろ塩分摂取量の制御およびQS阻止のための口腔ケアによる口腔BFの物理的除去が重要である。

研究発表内容の紹介

COVID-19パンデミックが起こり、口腔ケアによる口腔の健康維持が疎かになる傾向がみられるようになった。う蝕、歯周病および誤嚥性肺炎は口腔フローラに関係がある疾患であり、口腔フローラのdysbiosisを制御する意義を明確にし、国民に対して発信していくことが重要と考える。本研究は、口腔フローラのdysbiosisのメカニズムの一端を明らかにし、塩分摂取の制御、クオラムセンシング阻害と口腔ケアの重要性を明確にし、歯科医学の発展に寄与すると考える。（ファカルティー・アドバイザー：泉福 英信）

Elucidation of the signal system in the oral biofilm that regulates dysbiosis of the oral flora

(Problems)

The ratio of elderly people has increased, and in addition to dental caries, diseases associated with oral microorganisms such as periodontal disease and aspiration pneumonia have a great impact on health in Japan. In particular, oral dysbiosis (disintegration of flora) induced by biofilm formation due to accumulation of microorganisms and increase of opportunistic bacteria has occurred due to decreased oral function in oral frailty. Dysbiosis has been studied in the intestinal flora, which a healthy intestinal flora is disrupted and has a great effect on the systemic immune system. Therefore, oral dysbiosis may also affect the immune system and increase opportunistic bacteria in oral cavity. The oral biofilm is formed by aggregates of bacterial groups centered on oral streptococci attached to the acquired pellicle constructed by salivary protein on the tooth surface. These aggregates are largely supported by the polysaccharides produced by *Streptococcus mutans*, which is considered to be most associated with dental caries, synthesizes the polysaccharide (glucan) using sugar as a substrate. Furthermore, in order to survive in the oral environment, *S. mutans* has a signal production mechanism called quorum sensing, which activates bacteriocin production, extracellular gene uptake, acid resistance mechanism, and GtfB and GtfC production. *S. mutans* regulates oral biofilm formation by the signal systems of various molecules. The relationship between signal systems and dysbiosis of the oral flora has not been clarified in detail.

(Hypothesis)

Staphylococcus aureus, which is an intraoral opportunistic bacterium, is a salt-tolerant bacterium and also easily becomes a resistant bacterium to antibiotic medicine, and the detection rate increases in the oral cavity from the elderly. Aspiration pneumonia and heart disease are associated with the infection of *S. aureus* in the oral cavity. *S. aureus* has been shown to be present as an indicator of oral dysbiosis in oral biofilms. Therefore, it is investigated whether a biofilm of *S. aureus* is formed by the presence of a certain concentration of salt, and glucosyltransferases (GtfB, GtfC) that synthesize various polysaccharides of *S. mutans* and various molecules involved in quorum sensing (QS) in *S. mutans*. The study was conducted with the hypothesis that these signal systems affect the biofilm formation.

(Methods)

1) Biofilm formation assay : Biofilms were developed in 96-well polystyrene microtiter plates previously coated with human saliva. *S. aureus* cowan I, *S. mutans* UA159, and *S. mutans* UA159.*gtfBC*$^{-}$, and mix bacteria culture of *S. aureus* and *S. mutans* UA159 were inoculated in tryptic soy broth with 0.25% sucrose (TSBs) with and without various concentrations of sonic extracts from various bacteria, and with various concentration of NaCl. After incubation, the planktonic cells were removed by washing with distilled water (DW), and the adherent cells were stained with 0.25% safranin for 15 min. After washing with DW, safranin was extracted from biofilms with 70% (vol/vol) ethanol. Biofilm formation was quantified by measuring the absorbance of the extracted safranin at 492 nm.
2) Observation of live and dead bacteria in the biofilm : In order to observe dead and live bacteria, the biofilm was subjected to Live / Dead staining, and observed with a confocal laser scanning microscope.
3) Construction of mutants : Mutants to glucosyltransferase genes (*gtfB*, *gtfC*) and genes (*comD*, *comR*, *comX*, *comY*, *luxS*) associated with QS due to bacterial aggregation and mutants of various other genes (*pknB*, *gbpC*, *sacB*, *SMU574*, *SMU833*, *SMU1009*, *SMU1013*) were constructed. We investigated how sonic extracts from mutants affects the formation of *S. mutans gtfBC*$^{-}$ or *S. aureus* biofilms.

(Results)

1) Effect of NaCl on biofilm formation by mixing with *S. aureus* and *S. mutans*

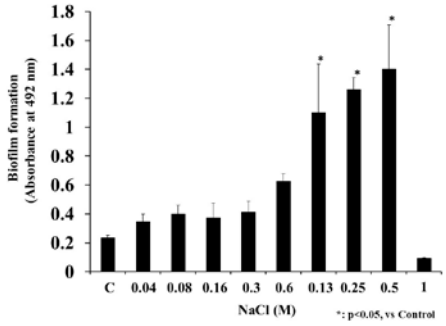


Figure 1 NaCl-dependent biofilm formation of *S. aureus*

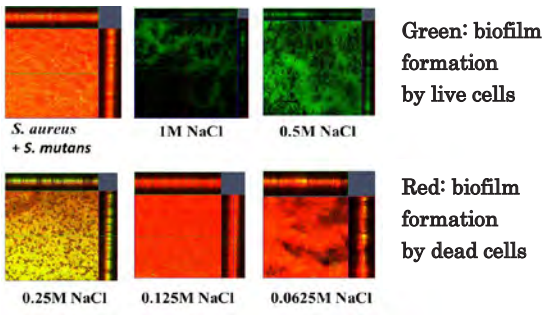


Figure 2 Effect of NaCl on the formation of mixed biofilms of *S. aureus* and *S. mutans*

S. aureus is not only salt-tolerant but also increases biofilm formation depending on salinity (0.125M, 0.25M, 0.5M) (Fig. 1). Furthermore, in the mixed biofilm formation of *S. aureus* and *S. mutans*, dead cell-dependent biofilm was remarkably formed at 0.125M NaCl (Fig. 2).

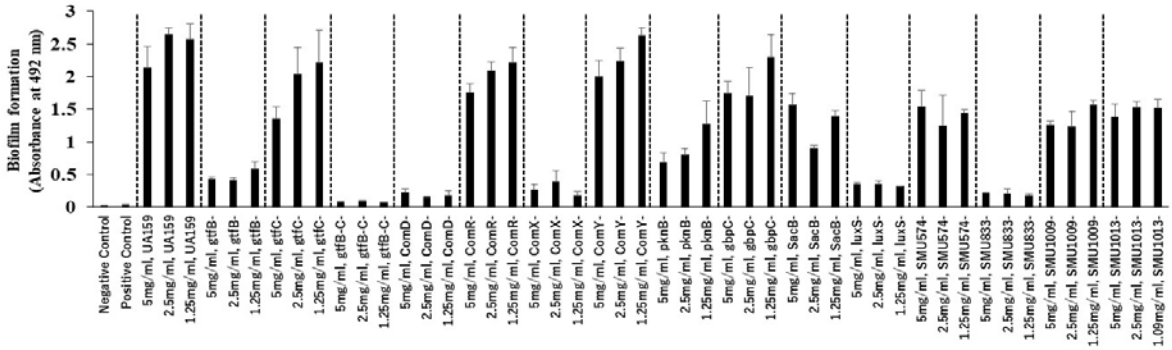


Fig. 3 Biofilm formation of *S. mutans gtfBC*$^-$ by cell sonic extract components of *S. mutans* mutant strains

The components from *gtfB*$^-$ and *gtfBC*$^-$ could not strongly induce biofilm formation in *S. mutans gtfBC*$^-$, which lacked biofilm-forming ability (Fig. 3). Ingredients from mutants of the QS-related genes *comD*, *comX*, *luxS* and *SMU833* involved in peptidoglycan synthesis also failed to induce biofilm formation.

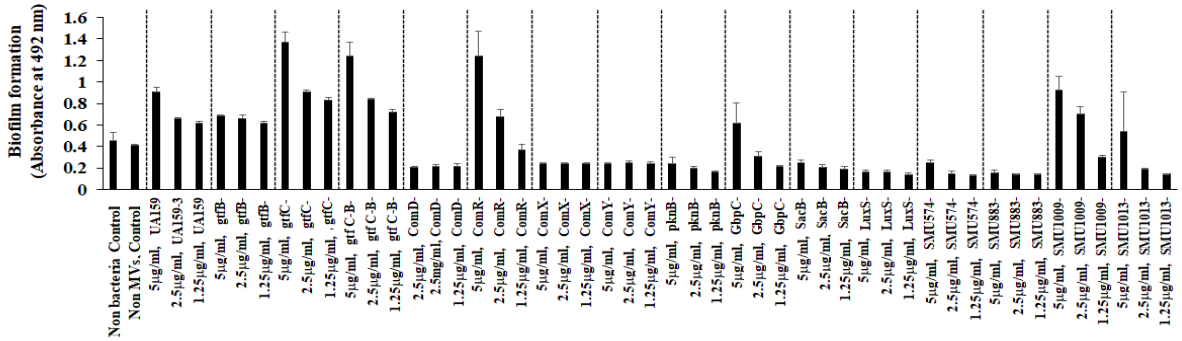


Fig. 4 Biofilm formation of *S. aureus* by cell sonic extract components of *S. mutans* mutant strains

The components of the glucan synthetic gene mutant induced salt concentration (0.125M) -dependent biofilm formation of *S. aureus* (Fig. 3). On the other hand, components of QS-related (comD, comX, comY, gbpC, luxS) and self-destroying autolysin-related genes (SMU574), fructan synthesis genes sacB and SMU833 mutants could not induce biofilm formation.

(Conclusion)

Biofilm formation in *S. aureus* was not dependent on glucan formation by *S. mutans* and affected salinity and QS-controlled killing by *S. mutans*. Controlling salt intake rather than sugar inoculation, physical removal of oral biofilm by oral care and to block QS are important for blocking oral flora dysbiosis.

Regulation mechanisms of lipid metabolism in the proliferation and invasion of human oral cancer

明海大学歯学部　3年生　Meikai University School of Dentistry　Class of 2025

白石 琢弥　Takuya SHIRAISHI

ファカルティー・アドバイザー：生化学分野 講師　福田 正勝

Human oral squamous cell carcinoma（HOSCC）is the most common head and neck malignant neoplasm. It is an aggressive tumor that is difficult to treat with conventional therapies. Cancer cells can satisfy the energy requirement for their proliferation and invasion by glycolysis in glucose metabolism, called Warburg effect which had been described hundred-odd years ago. Although this effect is a less efficient pathway for producing ATP when compared to oxidative phosphorylation, it is quite efficient in cancer tissues which became in a hypoxic condition by abnormal proliferation. The metabolic reconstructing of cancer cells has been recently described for a wide range of other metabolic pathways, including lipid metabolism. Especially, E-FABP is expressed in tissues throughout the body and it is markedly up-regulated in esophageal carcinoma. It has been reported that SREBPs, are transcription factors, regulate the synthesis and uptake of fatty acids through activating their processing mechanism by SCAP in mammalian cells. However, it is unclear how lipid metabolism is regulated in oral cancer. This study examined the expression and regulation mechanism of SREBP1 and SCAP in proliferation and invasion of HOSCC.

口腔癌の増殖・浸潤における脂質代謝の調節メカニズムについて

ヒト口腔扁平上皮癌は、最も一般的な頭頸部領域の悪性腫瘍である。大変攻撃的な腫瘍で、従来の治療法では完治は難しい。ここで、癌細胞は100年余り以前に書かれたWarburg効果と呼ばれるグルコース代謝における解糖系によって増殖・浸潤のためのエネルギー要求を満たしている。この効果は酸化的リン酸化反応に比べATP産生の効率的な経路としては劣るけれども、異常な増殖によって低酸素状態になった癌組織においてはまったく効率的である。近年、癌細胞の代謝の再構築は脂質代謝を含め、他の代謝経路のとても広い範囲において描かれている。

とりわけ、上皮脂肪酸結合タンパク質（E-FABP）は、全身の至る組織に発現し食道癌において顕著に発現亢進している。哺乳細胞においてSREBP切断活性型タンパク質（SCAP）の活性化を通じて、転写因子のSRE結合蛋白（SREBP）が脂肪酸の合成と取り込みの調整を行なっていることが報告されているが、口腔癌において脂質代謝がどのようにして調節されているのか不明である。本研究は口腔扁平上皮癌の増殖・浸潤におけるSREBP1とSCAPの発現と調節メカニズムを検索した。

研究発表内容の紹介

本研究は、口腔癌のエネルギー代謝、特に近年注目され始めた脂質代謝の調節メカニズムについて詳細に解析したものです。すなわち、脂質代謝を抑制性にコントロールすることで口腔癌細胞の増殖阻止の可能性について検索したもので、もし口腔癌の脂質代謝を制御することができれば、口腔癌の増殖・浸潤を抑制し、細胞死の誘導に繋がるため、今後の歯科医学に寄与するところが非常に大きいと考えます。（ファカルティー・アドバイザー：福田 正勝）

Regulation mechanisms of lipid metabolism in the proliferation and invasion of human oral cancer

(Problem)

Human oral squamous cell carcinoma (HOSCC) is the most common head and neck malignant neoplasm. It is generally performed multidisciplinary approach that used chemotherapy, radiotherapy and surgery against patients with oral cancer, however, we cannot avoid the dysfunction due to the side effect or surgical defect and it significantly impacts the postoperative quality of life, unfortunately. Although the clinical outcome of HOSCC has gradually improved, the overall 5-year survival rate of patients is still disappointing, reflecting limited advances in our understanding of the pathogenesis of this disease and the molecular events that contributed to its development. Thus, a better understanding of the molecular mechanisms driving oral carcinogenesis may lead to new diagnostic and therapeutic approaches to this disease, and improve the prognosis of HOSCC patients.

(Hypothesis)

Energy metabolism, a phenomenon by which cancer cells can satisfy the fuel requirement for their proliferation and invasion in a harsh tumor microenvironment, has long been considered a pivotal feature of cancers. In addition to alterations in glucose metabolism, commonly called the Warburg effect, metabolic reconstructing of cancer cells has been recently described for a wide range of other metabolic pathways, including **lipid metabolism**. Fatty acid-binding proteins (FABPs) are capable of binding long-chain fatty acids, and comprise a family of cytosolic proteins consisting of over 10 isoforms. Among these, epidermal FABP (E-FABP) was first isolated from the epidermis, and is expressed in tissues throughout the body. Additionally, E-FABP is markedly up-regulated in human esophageal carcinoma. While most adult mammalian cells acquire fatty acid from the bloodstream, tumor cells are frequently associated with increased de novo biosynthesis of fatty acid, a process catalyzed by concerted actions of various enzymes, most of which are regulated by sterol regulatory element binding protein 1 (SREBP1), which are transcription factors involved in regulating the synthesis and uptake of fatty acids through activating their processing mechanism by SREBP cleavage-activating protein (SCAP) in mammalian cells. However, it is unclear how lipid metabolism is regulated in oral cancer. Then, we hypothesized that clarifying the regulation mechanism of lipid metabolism in oral cancer that might be a key for healing the disease. This study examined the expression and regulation mechanism of SREBP1 and SCAP in HOSCC.

(Methods)

At first, the expression levels of SCAP, SREBP1 mRNA and protein were analyzed by qRT-PCR and immunoblot analysis using 5 HOSCC cell lines, HSC-2, HSC-3, HSC-4, Ca9-22 and SAS. Monolayer SAS cells (1×10^4 cells/100µl/well) were incubated for 24 h on a 96-microwell plate and treated with 50 µM SCAP siRNA for 48 h and evaluated for decreasing SAS cell viability using the cell viability assay. The relative viable cell number was determined by measuring the absorbance of the dye solution at 450 nm. To investigate whether SCAP knockdown induced cell death in SAS cells, the levels of procaspase cleavage to active caspase-8, -9, and -3/7, markers of apoptotic activity, were examined. Cells were plated in white-welled 96-well tissue culture plates at a density of 5×10^3 cells/well in 100 µl of medium and incubated for 24 h. After treatment with SCAP siRNA for 48 h, Caspase-3/7, -8, and -9 activities were determined using the Caspase-Glo assay. Caspase-Glo reagents were added to each well in a 50 µl and incubated for 30 minutes before measuring luminescence as relative light units using a microplate luminometer. Then, to confirm whether SREBP1 has indeed been regulating E-FABP expression, we attempted to

Furthermore, it is well documented that resveratrol has an effective anti-oxidant activity. Then, it was examined that how SCAP, SREBP1 and E-FABP expressions were regulated in SAS cells upon treatment with 50 μM resveratrol for 24 h.

(Results)

The quantitative SCAP mRNA expression levels in HOSCC cells were the highest in SAS cells (n=3 experiments; means±SD) (Fig. 1). Detection of SCAP protein in HOSCC cells by Western blotting was detected at 130 kDa (Fig. 2). The levels of them were the highest in SAS cells, then we focused on SAS cells in this study. The knockdown of SCAP with SCAP siRNA in SAS cells obviously revealed to decrease the cell number (n=3 experiments; means±SD) (Fig. 3) and was greatly decreased SREBP1 expression (Fig. 4). We considered a possibility of apoptotic cell death, and examined to detect the activities of caspases. Then, activities of caspase-9 and -3/7 were increased in SCAP knockdowned SAS cells (n=3 experiments; means±SD) (Fig. 5). Furthermore, SREBP1 knockdown by SREBP1 siRNA was greatly decreased E-FABP expression in SAS cells, in comparison with the scrambled siRNA control or just control (Fig. 6). However, SCAP expression was upregulated by SREBP1 knockdown in SAS cells (Fig. 6). Additionally, SREBP1 and E-FABP were down-regulated by resveratrol treatment, however SCAP expression was up-regulated (Fig. 7).

(Conclusions)

These data suggested that SCAP might play a significant role with SREBP1 as a regulator of lipid metabolism in the proliferation and invasion of human oral cancer.

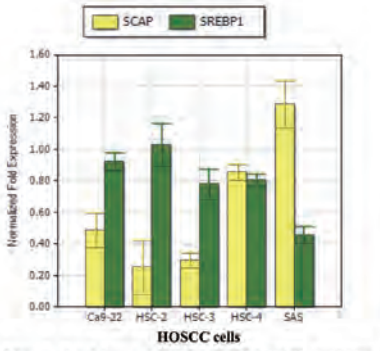


Fig. 1. Real-time quantitative RT-PCR analysis.

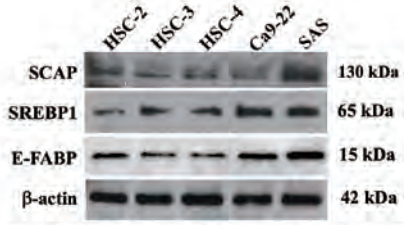


Fig. 2. Expression of SCAP, SREBP1 & E-FABP proteins in HOSCC cells was analyzed by Western blotting.

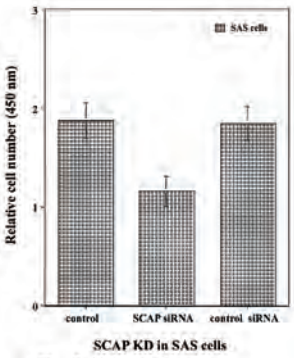


Fig. 3. Cell viability.

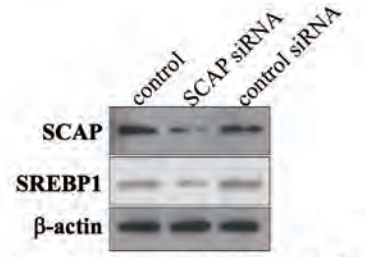


Fig. 4. Expression of SCAP & SREBP1 in SCAP-knockdowned SAS cells or control.

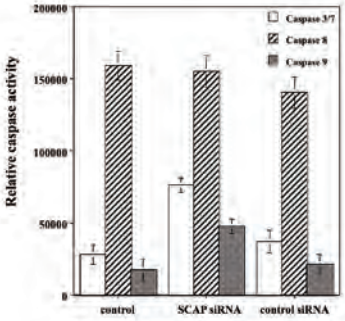


Fig. 5. Apoptosis detection assay.

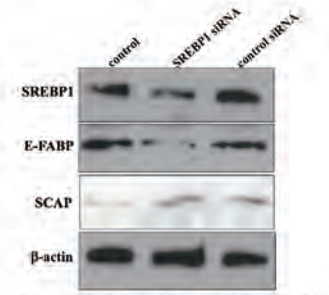


Fig. 6. SREBP1 regulates E-FABP expression and SCAP regulates SREBP1 expression in SAS cells.

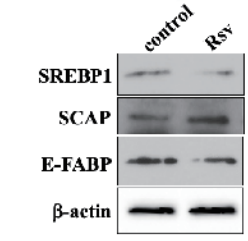


Fig. 7. Expression of SCAP, SREBP1 and E-FABP protein in SAS cells treated with resveratrol.

Effect of choline water or IFN-γ water on pneumococcal infected mice

大阪大学歯学部 4年生 Osaka University School of Dentistry Class of 2024

堀 新 Arata HORI

共同研究者：藤城 龍平（4年生）
ファカルティー・アドバイザー：バイオインフォマティクスユニット 准教授 山口 雅也
研究指導協力者：口腔細菌学教室 教授 川端 重忠

【Purpose】 *Streptococcus pneumoniae* is a major cause of pneumonia. The pneumococcal-related deaths are increasing because of aging society. In this study, we compared pathological condition of young and aged mice in pneumococcal pneumoniae model. We also investigated the effect of administration of choline chloride which prevents from autolysis of *S. pneumoniae* or IFN-γ which enhances bactericidal activation of neutrophils.
【Methods】 We infected *S. pneumoniae* nasally to young and aged mice. We evaluated bacterial burden and lung tissue after infection. In addition, we evaluated the survivability of infected young and aged mice by nasal administration of choline chloride, IFN-γ or PBS. We also observed the effect of drinking choline chloride for survivability of infected young mice.
【Results and Discussion】 Broncho-alveolar lavage fluid from aged mice showed a significantly higher bacterial burden as compared to that of young mice. Elastase markedly activated in lung of aged mice. Both young and aged mice showed no survival improvement with nasal administration of choline chloride, or IFN-γ. Survival of the group given choline chloride in drinking water was only slightly and not significantly greater than that of the control. In the near future, we intend to investigate effects of choline when given in combination with a variety of substances such as IFN-γ as an attempt to control pneumococcal infection.

肺炎球菌感染マウスに対するコリン、IFN-γの作用

【目的】 肺炎球菌は肺炎の主たる原因菌で、社会の高齢化に伴い死者数が増加することが懸念されている。本研究では、肺炎球菌性肺炎モデルにて、若齢マウスと高週齢マウスの病態を比較した。さらに、肺炎球菌の自己融解を阻害する塩化コリン、ならびに白血球の殺菌作用を強化するIFN-γの投与による病態制御の可能性について検討した。
【方法】 肺炎球菌を若齢、高週齢のマウスに経鼻感染し、感染後のマウスの菌数と肺の組織像を比較した。また、感染マウスに対して、それぞれ塩化コリン、IFN-γ、PBSを経鼻投与して宿主の生存率を比較した。また、感染若齢マウスに対して、塩化コリンを飲水投与し、宿主の生存率に及ぼす影響を観察した。
【結果と考察】 高週齢群のマウスでは、若齢群と比較して肺胞洗浄液中にて有意に高い菌数が検出された。また、高週齢群の肺では好中球の強い活性化が認められた。若齢、高週齢マウスともに、塩化コリンならびにIFN-γ投与による生存率改善は認められなかった。コリン飲水群の方が比較群に比べてわずかに生存率が高かったが、両群に統計学的な有意差は認められなかった。今後、コリンやIFN-γなどの組み合わせを検討し、肺炎球菌の病態制御法を探索していきたい。

研究発表内容の紹介

肺炎球菌は、mitis群の口腔レンサ球菌であり、世界の下気道感染症による死亡の約半数（120万人弱）の原因である。近年の研究で、肺炎球菌は類縁の口腔レンサ球菌の遺伝子を取り込み、抗菌薬やワクチンへの耐性を獲得することが明らかになってきた。
本研究は、特に感染リスクが高い高齢者を模した動物モデルにて、抗菌薬以外による感染制御の可能性を探索したものであり、新たな感染制御アプローチ確立の基盤形成につながるものである。（ファカルティー・アドバイザー：山口 雅也）

Effect of choline water and IFN-γ water on pneumococcal infected mice

Problem

Streptococcus pneumoniae is a Gram-positive facultative anaerobic bacterium and major cause of pneumonia. The phylogenetic relationship of bacterial 16S rRNA indicates that this pathogen belongs to the mitis group of oral streptococci. In 2020, pneumonia was the fifth leading cause of death in Japan, with more than 95% of fatal cases occurring at the age of 65 years or older. In developing countries, pneumonia also has a high mortality rate among children and accounts for 15% of all deaths of individuals under 5 years worldwide.

Although pneumococcal polysaccharide vaccines are now in practical use and show considerable benefits, non-vaccine serotype strains have increased worldwide. With drug-resistant pneumococcal strains also increasing, development of new preventive and therapeutic methods is urgently needed.

Hypothesis

The pneumococcal cell wall is composed of peptidoglycan, which consists of repeating units of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) as well as cell wall cross-linking peptides and teichoic acids. Enzyme are known to degrade the walls of those cells, with LytA, LytC, and CbpD choline-binding proteins that baind to phosphorylcholine associated with cell wall teichoic acids. The binding induces pneumococcal autolysis, resulting in release of pathogen-associated molecular patterns (PAMPs).

The present study was conducted to compare pathological conditions between young and aged pneumococcal pneumonia model mice. We hypothesized that administration of choline chloride would suppress host inflammatory response by competitively inhibiting pneumococcal autolysis and lung inflammation inhibition. Furthermore, it was speculated that IFN-γ administration could improve host survivability, as that cytokine is known to enhance the bactericidal ability of leukocytes.

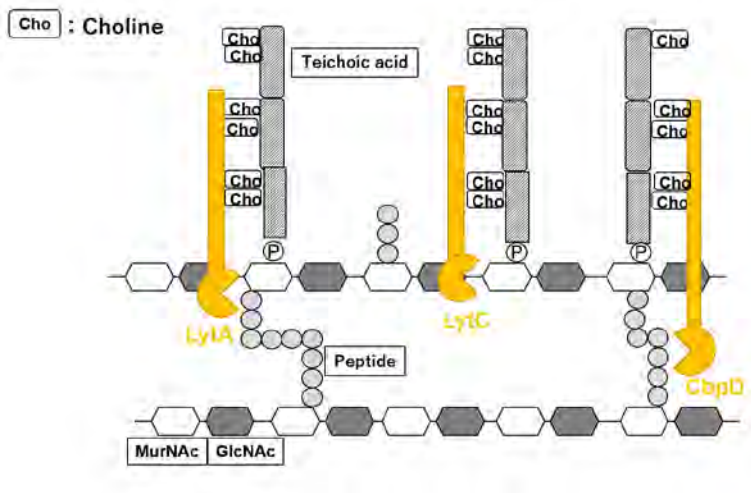


Figure 1. Structure of pneumococcal cell wall.
Teichoic acids on the cell wall are covalently attached to MurNAc by a phosphodiester bond. Cell wall teichoic acid has phosphorylcholine attachment. Choline-binding proteins (shown in yellow) bind to phosphorylcholine on the cell wall and degrade the peptidoglycans.

Methods

S. pneumoniae strain TIGR4 was cultured until mid-logarithmic growth phase. After suspending the bacterial cells with PBS, young (8 weeks old) and aged (73-78 weeks old) mice were nasally infected. At 24 hours after infection, bacterial burden was determined in nasal lavage fluid, broncho-alveolar lavage fluid, and blood. Additionally, histopathological analysis of infected lung tissues was performed by immunostaining.

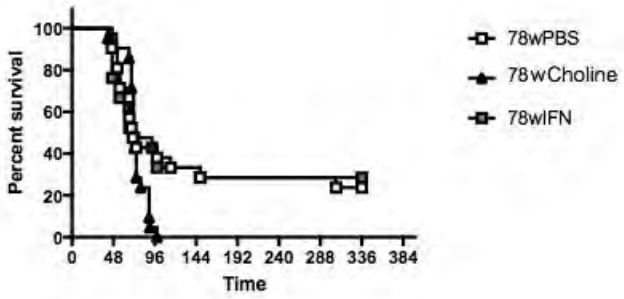
In another experiment, choline chloride (5 μg), IFN-γ (100 ng), or PBS (20 μL) was administered nasally to young and aged mice at one, two and three days after pneumococcal intranasal infection, then body weight changes and survival rates for two weeks after infection. In addition, young mice were given water with 5 mg/mL of aqueous choline chloride or sterile water starting from one day before infection, then body weight changes and survival rates were compared between the two groups.

Results

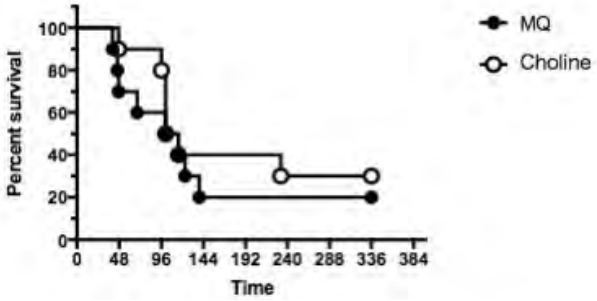
At 24 hours after infection, broncho-alveolar lavage fluid from aged mice showed a significantly higher bacterial burden as compared to that of young mice, whereas there were no significant differences regarding nasal lavage or blood. Histopathological analysis indicated no significant differences for the rate of neutrophils in lungs, while neutrophil elastase-positive cells were significantly increased in the aged mice. These results indicate that *S. pneumoniae* survives better in the lungs of aged as compared to young mice, though neutrophils showed greater activation in the former.

Regarding the effects of inhibition of autolysis by choline and enhancement of neutrophil bactericidal ability by IFN-ɤ- in the present mouse infection model, both young and aged mice showed no survival improvement with nasal administration of choline chloride or IFN-ɤ. On the other hand, nasal administration of choline induced aggressive coughing under anesthesia. Since choline chloride is used as a food additive, that was added to drinking water to examine whether it would improve host survivability. However, survival of the group given choline chloride in drinking water was only slightly and not significantly greater than that of the control.

Figure 2 A B



A. Survival curves of *S. pneumoniae*-infected aged mice following administration of choline chloride or IFN-γ. Differences between the infected mouse groups wwere compared using a log-rank test.



B. Survival curves of *S. pneumoniae*-infected young mice after drinking choline chloride in water or water only. Difference between the infected mouse groups were compared using a log-rank test.

Conclusion

The present results indicate that a pneumococcal infection causes excessive neutrophil activation in the lungs of aged mice, though that does not exhibit a sufficient bactericidal capacity. In addition, administration of either choline chloride or IFN-γ did not improve the survival of mice infected with *S. pneumoniae*. While IFN-ɤ increase the bactericidal activity of neutrophils, it might also have increased inflammatory activity, resulting in lung tissue destruction and survival of those mice was not improved. As for why administration of choline was not sufficient, it is speculated that the amount in drinking water was not sufficient for competitive inhibition. Additionally, it is possible that the choline did not reach the lungs, the site of infection, as it was orally ingested, and may have been metabolized and excreted, and thus not become concentrate in the lungs to a sufficient level. However, the dose was nearly the maximum that could be administered without causing harm to the mice and it would difficult to increase that.

In the near future, in addition to clarifying the biokinetics of choline by determining its concentration in blood, we intend to its effects when given in combination with a variety of substances such as IFN-γ as an attempt to control pneumococcal infection.

Effects of probiotic butyrate-producing bacteria on the immune responses of periodontitis in mice

福岡歯科大学　5年生　Fukuoka Dental College　Class of 2023

中村 麻衣　Mai NAKAMURA

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Periodontitis is an infectious disease caused by periodontal bacteria in the oral cavity. The pathogenesis of periodontitis is mediated by host immune response, especially IL-17A-producing Th17 cells. Th17 cells have been reported to exacerbate periodontitis by accumulating in the periodontal tissues and promoting bone loss. However, the regulatory mechanisms of Th17 cells in the development of periodontitis are not well understood. Recently, dysbiosis of gut homeostasis, including the gut microbiome, has been shown to cause various diseases. Furthermore, altering gut homeostasis by taking probiotic bacteria, such as butyrate-producing bacteria, is expected to improve some diseases by activating host's immune system. We hypothesized that administration of butyrate-producing bacteria, *Clostridium butyricum* in the gut would contribute to the inhibition of immune responses in periodontitis. In this study, we used a mouse model of *P. gingivalis*-induced periodontitis and investigated the effects of *C. butyricum* on the immune responses of periodontitis. Mice were treated with antibiotics to partially deplete the intestinal microbiome. *C. butyricum* was then administered into the intestinal tract of the mice. After infecting with *P. gingivalis*, Th17 cells and neutrophils in the periodontal tissue were analyzed with flow cytometry. As a result, intestinal colonization of *C. butyricum* in the antibiotic-treated gut with disturbed microbiome could inhibit the accumulation of Th17 cells and neutrophils in the periodontal tissues.

酪酸菌プロバイオティクスが歯周病の免疫応答に及ぼす効果

歯周病は口腔内に常在する歯周病原細菌による感染症である。歯周病原細菌に対する宿主の免疫応答、その中でサイトカインIL-17Aを産生するTh17細胞が歯周組織に集積することで歯周病の病態形成に関わることが知られている。しかし、その免疫制御機構は不明な点が多い。近年、腸内細菌叢を含む腸内環境の変化が、全身の免疫機能を制御することが明らかになってきた。特に、酪酸菌などのプロバイオティクスが宿主の免疫を活性化し、種々の疾患の発症を抑制することが報告されている。そこで、酪酸菌を腸管に定着させることで歯周病の病態形成を制御できるのではないかと仮説を立てた。本研究では歯周病原細菌*Porphyromonas gingivalis*を感染させる歯周病マウスモデルを用いて、酪酸菌の腸管への定着が歯周病の免疫応答に及ぼす影響を解析することを目的とする。抗生物質投与により腸内細菌叢を一部破壊したマウスに酪酸菌を腸管へ定着させた。さらに、*P. gingivalis*を感染後、マウスの歯周組織に集積するTh17細胞と好中球をフローサイトメトリーにより解析した。その結果、酪酸菌が定着したマウスでは歯周組織におけるTh17細胞や好中球の集積が抑制され、歯周病の病態形成を抑制する可能性が示唆された。

研究発表内容の紹介

本研究の成果は、プロバイオティクスである酪酸菌の腸管への定着が、歯周病の病態形成に関わるTh17細胞や好中球の歯周組織への集積を抑制することで、歯周病の発症を抑制する可能性を示唆している。本研究は口腔と腸管の連関を示したものであり、腸内細菌叢の制御による新しい歯周病の治療・予防法の開発に寄与する可能性を秘めている。（ファカルティー・アドバイザー：田中 芳彦）

Effects of probiotic butyrate-producing bacteria on the immune responses of periodontitis in mice

(Problem)

Periodontitis is an infectious disease caused by periodontal bacteria in the oral cavity and is the most common cause of tooth loss. *Porphyromonas gingivalis* (*P. gingivalis*), a bacterium of the red complex, is especially known to be highly correlated with the development of periodontitis. The pathogenesis of periodontitis is mediated by host immune response, especially IL-17A-producing Th17 cells. Th17 cells have been reported to exacerbate periodontitis by accumulating in the periodontal tissues and promoting bone loss. However, the regulatory mechanisms of Th17 cells in the development of periodontitis are not well understood. Understanding how Th17 cells regulate periodontal bacteria, as well as how to suppress Th17 cells, would lead to progressing new preventive and therapeutic strategies for periodontitis.

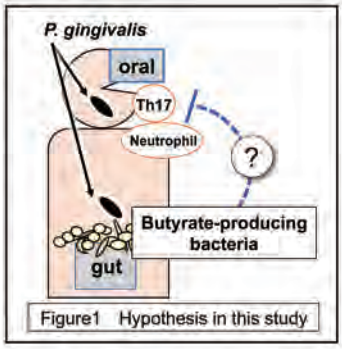

Figure1 Hypothesis in this study

(Hypothesis)

Recently, dysbiosis of gut homeostasis, including the gut microbiome, has been shown to cause various diseases such as rheumatoid arthritis, and regulates systemic immune responses. Furthermore, altering gut homeostasis by taking probiotic bacteria, such as lactic acid bacteria and butyrate-producing bacteria, is expected to improve some diseases by activating host's immune system and to attract attention as a new treatment method. In this study, we hypothesized that administration of butyrate-producing bacteria, *Clostridium butyricum* in the intestine would contribute to the inhibition of immune responses in periodontitis (Fig. 1).

(Methods)

We used a mouse model of *P. gingivalis*-induced periodontitis and investigated the effects of *C. butyricum* on the immune responses of periodontitis. The schedule of experiments is shown in Fig. 2. IL-17A-GFP reporter mice were used to monitor the accumulation of Th17 cells in the periodontal tissues.

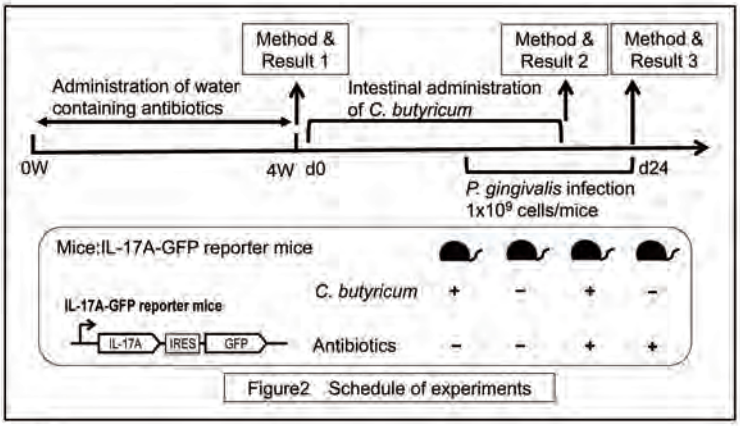

Figure2 Schedule of experiments

Method 1: Analysis of microbiota after antibiotics treatment.

Mice were treated with antibiotics for 4 weeks to partially deplete the intestinal microbiome. Feces from mice were homogenized and spread onto blood-agar plate and incubated for 5 days under anaerobic conditions at 37°C.

Method 2: Analysis of *C. butyricum* colonization in the gut.

C. butyricum was administered into the intestinal tract for 19 days. Bacterial DNA was collected from the feces, and then total bacterial DNA and *C. butyricum* specific DNA were analyzed with qPCR.

Method 3: Analysis of immune cells in the periodontal tissues after *P. gingivalis* infection.

After infecting with *P. gingivalis* for 9 days, Th17 cells and neutrophils around the maxilla were analyzed with flow cytometry.

(Result)

Result 1: Analysis of microbiota after antibiotics treatment

The CFU/g-feces from the group with antibiotics was comparable with the group without antibiotics. The antibiotic-treated mice group showed a greater change in the type of colonies on agar media compared to the non-treated group, suggesting a change in the intestinal microbiome, especially the type of anaerobic bacteria (Fig. 3).

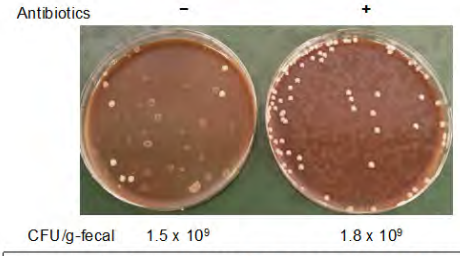

Figure3 Analysis of microbiota after antibiotics treatment

Result 2: Analysis of *C. butyricum* colonization in the gut

First, we investigated changes of total bacteria in feces by using qPCR. As a result, total bacteria in feces from the group with antibiotics decreased compared to the group without antibiotics. However, *C. butiricum* administration did not change the amount of total bacteria. Next, we analyzed whether *C. butiricum* was colonized in the gut by using qPCR with a *C. butyricum* specific primer. *C. butyricum* was detected in the feces from the group with *C. butyricum* administration but not without the administration (Fig. 4). These data suggested that *C. butyricum* colonized in the gut by intestinal administration.

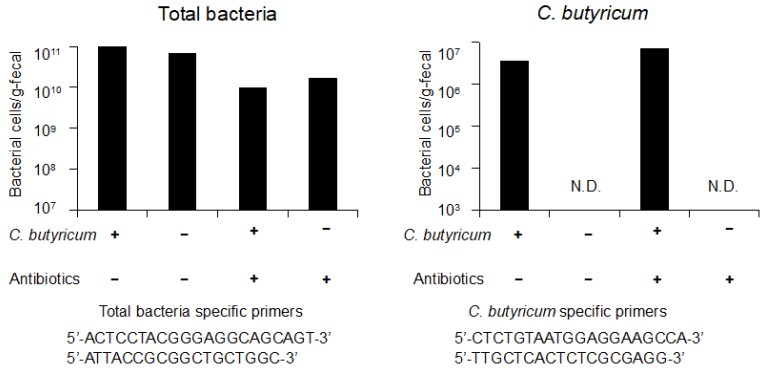

Figure4 Analysis of *C. butyricum* colonization in the gut

Result 3: Analysis of immune cells in the periodontal tissue after *P. gingivalis* infection

Neither absolute number of Th17 cells nor neutrophils were changed in antibiotics-untreated groups, regardless of *C. butyricum* administration. On the other hand, the number of Th17 cells and neutrophils were increased by antibiotic administration. However, this increase of the cells was inhibited by *C. butyricum* administration (Fig. 5). These data suggested that intestinal colonization of *C. butyricum* in the antibiotic-treated gut with disturbed microbiome could inhibit the accumulation of Th17 cells and neutrophils in the periodontal tissues.

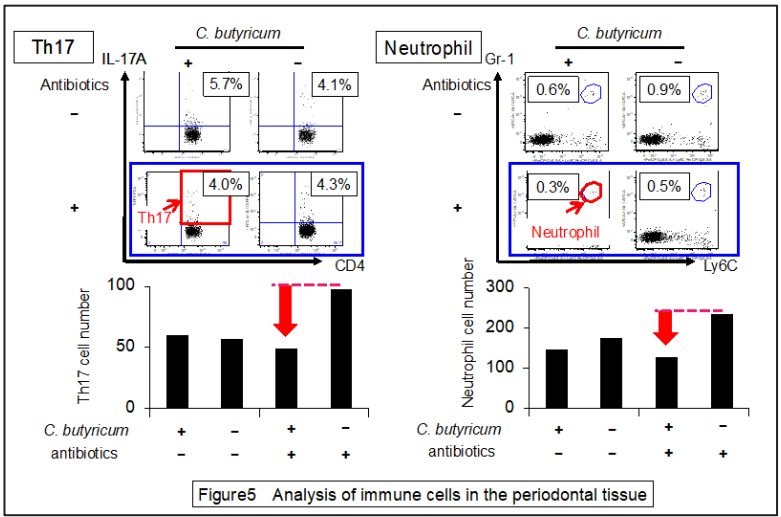

Figure5 Analysis of immune cells in the periodontal tissue

(Conclusion)

Collectively, intestinal colonization of the probiotic butyrate-producing bacterium *C. butyricum* may suppress periodontitis by inhibiting the accumulation of Th17 cells and neutrophils. This study may provide possible prevention and treatment of periodontitis by controlling the intestinal microbiota with probiotics.

In the future, we would like to study the effects of *C. butyricum* administration on the intestinal microflora and immune response, as well as on the development of periodontitis.

Development of a chairside-type pressure variable sandblaster

朝日大学歯学部 2年生 Asahi University School of Dentistry Class of 2026

デュアー ヒュー Hugh DEWAR

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Objectives: Development of CAD/CAM system enabled the production of dental-restoration products through numerically controlled machining, thereby opening up avenues for a wider range of materials such as CAD/CAM resin blocks and zirconia ceramics. However, those materials require alumina-blasting immediate before cementing. Thus, a major concern hindering the use of CAD/CAM materials is to control alumina-blasting pressure at chairside. Despite the usefulness of chairside micro blaster, alumina blasting pressure depends on the supply level. The aim of this study was to develop and validate a new pressure variable chairside sandblaster.
Methods: A pressure variable chairside sandblaster（CSB1, Morita）was newly developed. A micro sandblaster（Miniblaster, Deldent）was used as a control with an air-pressure regulator. Validation tests were performed with different air-pressure onto 1）surface roughness of PMMA plate, 2）area of blasting at 1 cm distance, 3）amout of Al_2O_3 powder, 4）efficiency of roughness and 5）micro-tensile bond strength test with a CAD/CAM resin block and a resin cement.
Results: CSB1 showed superior surface roughness and smaller area of blasting comparing with Miniblaster with regulator. Amount of Al_2O_3 powder were not different each other, therefore CSB1 showed higher efficiency of roughness. There was no difference in micro-tensile bond strength with 0.2 MPa pressure.
Conclusion: A new pressure variable chairside sandblaster will be a useful device for preparation of adherend surface at chairside.

チェアサイドで使用可能な圧力可変式サンドブラスターの開発

デジタルデンティストリーの発展とともに、CAD/CAMを用いた精密な歯冠修復材料の加工が可能になってきている。従来では使用できなかったジルコニアやCAD/CAM用レジンが使用可能になってきてはいるものの、接着前処理としてのアルミナを用いたサンドブラスターは材料ごとに必要な圧力が異なる。装着直前にサンドブラストすることが理想的ではあるが、現在チェアサイドで使用可能な小型のサンドブラスターでは調圧を行うことができない。そこで本研究では、調圧弁を内蔵した新型の小型ブラスターを試作し、その性能評価を行うことを目的とした。歯科メーカーの協力のもと試作チェアサイド用ブラスター（CSB1）を作製し、従来品に外部調圧装置を接続した製品との比較試験を多面的に実施した。その結果、CSB1は内蔵した調圧機構によって、従来製品と同等以上の性能を発揮し、臨床に即した微小引張り接着試験においても十分な性能を示した。現在この試作品は実用製品化に向けて動いており、世界初のチェアサイド型サンドブラスターとして、今後のデジタルデンティストリーにおける修復物の装着の質の向上に大きく寄与するものと考えられる。

研究発表内容の紹介

デジタルデンティストリーの発展とともに日進月歩で発達していく歯冠修復材料の接着前処置において、アルミナを用いたサンドブラスターは必要不可欠となっている。しかしながら、チェアサイドにおいて各材料ごとに確実に調圧してサンドブラストを行う装置は現状存在していない。本研究は歯科メーカーとの産学連携の中で、チェアサイドで取り扱うことが可能なサイズで、適切な圧力で出力できるサンドブラスターを新規試作し、その性能を検証した。試作機は現在製品化が予定されており、研究成果は明確に歯科臨床における接着前処理の質を向上させていくことに寄与するものと考える。（ファカルティー・アドバイザー：髙垣 智博）

Development of a chairside-type pressure variable sandblaster

(Problem)

With all the technological advancements in recent years, more and more dentists are using digital means of dental treatments. Development of CAD/CAM system enabled the production of dental-restoration products through numerically controlled machining, thereby opening up avenues for a wider range of materials such as CAD/CAM resin blocks and zirconia ceramics. In order to obtain a durable bonding performance, mechanical retention between the crown material and cement is critical. However, unlike lithium-disilicate glass-ceramics. CAD/CAM resin cannot be etched via chemical treatments such as acids, due to its material properties. Thus, alumina blasting is essential to obtain stable bonding performance onto CAD/CAM materials. Different materials have different stress tolerances. If the blasting pressure is too weak, Alumina will not be able to roughen the surface enough, leading to less mechanical retention. On the other hand, if the blasting pressure is too high, the material will start to crack due to stress leading to premature failure (Fig. 1). Latest evidence proved blasting immediately before cementing is ideal to remove contaminations and prepare a sound surface for chemical treatment. Thus, a major concern hindering the use of CAD/CAM materials is to control alumina-blasting pressure at chairside.

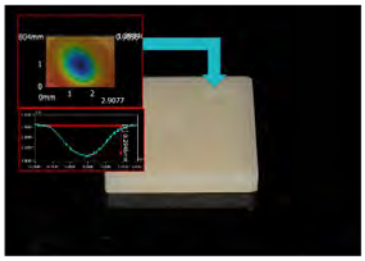


Fig.1 A hole and cracks by exceeded pressure of sandblasting

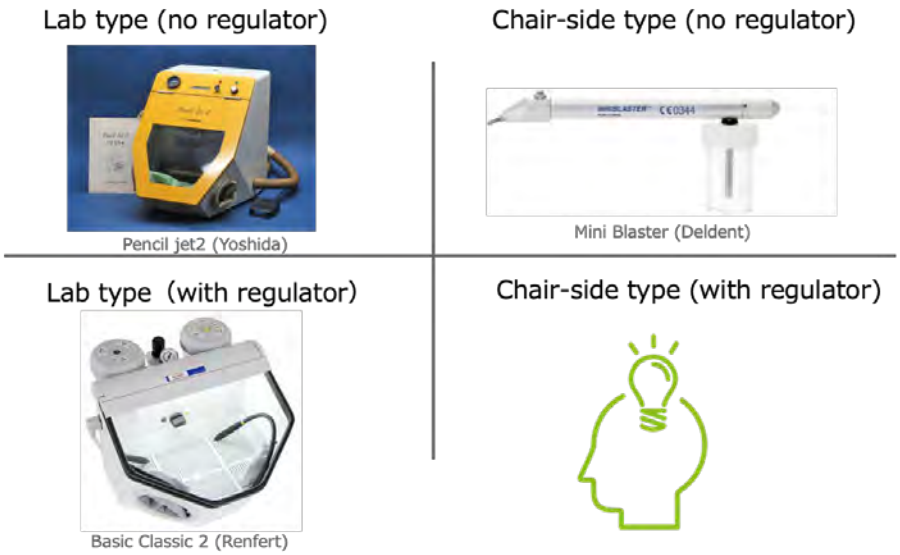


Fig.2 Types of sandblasting devices available in the marlet

(Hypothesis)

Despite the usefulness of chairside micro blaster, alumina blasting pressure depends on the supply level (0.4-0.5 MPa). There are no sand blasters on the market as of now that fits both of these two criteria (Fig.2):

1. Blasting pressure control
2. Small enough to be used chair-side

Therefore, the hypothesis of this study was to develop a new pressure variable chairside sandblaster (CSB1) and perform validation tests to evaluate its performance.

Fig.3 Pressure variable chairside sandblaster CSB1 and Miniblaster with an external regulator

(Methods)

To compare with CSB1, Miniblaster (Deldent) was pared with an external regulator as control (Fig 3). A PMMA plate was sandblasted with 50 micro meter alumina with different pressure, from a distance of 10mm for 10 seconds. Four different factors were recorded by using a laser scanning microscope (VK-X150/160, Keyense), 1) surface roughness after blasting, 2) area of blasting by measuring the diameter of blast area, 3) Amount of alumina used per second 4) Efficiency of roughness, obtained surface roughness divided by the amount of blasting (Fig. 4).

To evaluate clinical performance of CSB1, micro-tensile bond strength test with a CAD/CAM resin block were performed (Fig. 5). A CAD/CAM resin block (KATANA Avencia Block, Kuraray Noritake Dental) was sectioned into 2mm thick slabs, and polished with 600 grit silicon carbide paper to standardize the adherend surface. Sandblasting was performed using 0.2 and 0.4 MPa on each device, then treated with a silane coupling agent (Clearfil Ceramic Primer Plus, Kuraray Noritake Dental). Two slabs of resin blocks were cemented with an adhesive resin cement (Panavia V5, Kuraray Noritake Dental) and light cured for 60 s on each side. After storing in distilled water at 37℃ for 24 hours, these specimen were sectioned into 1mm^2 sticks. Micro-tensile test were performed with a universal testing machine under 1mm/min crosshead speed (n=30).

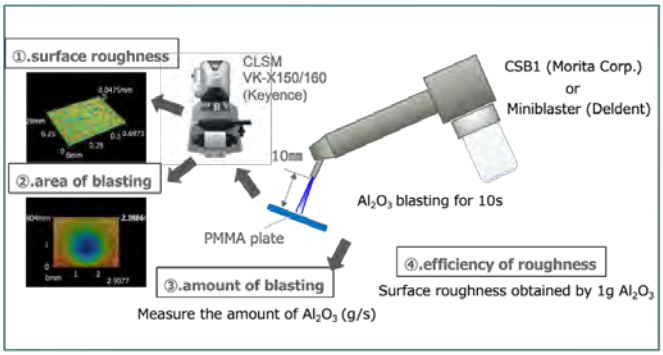


Fig.4 Validation test with laser microscope

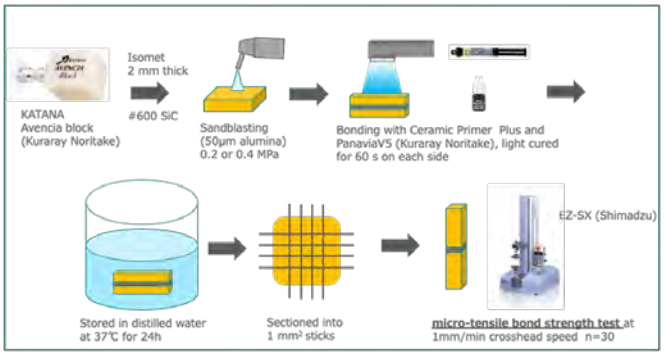


Fig.5 Micro-tensile bond strength test with CRB

(Results)

Results of validation test were shown in Fig. 6. Surface roughness of PMMA plates increased according with pressure in both devices, CSB1 tended to show higher Ra value at any pressure. Area of blasting also tend to increase as the pressure increased, however CSB1 showed smaller blasting area at any pressure. Amount of blasted powder did not differ at any condition, therefore efficiency of roughness were superior in CSB1. Results of micro-tensile bond strength test were shown in Fig. 7. Bond strength tend to be higher at 0.4 MPa in both device. However clinically 0.4 Mpa does too much damage on CAD/CAM resin blocks. At 0.2 MPa, CSB1 showed higher bond strength comparing with Miniblaster. However, there were no significant difference between them.

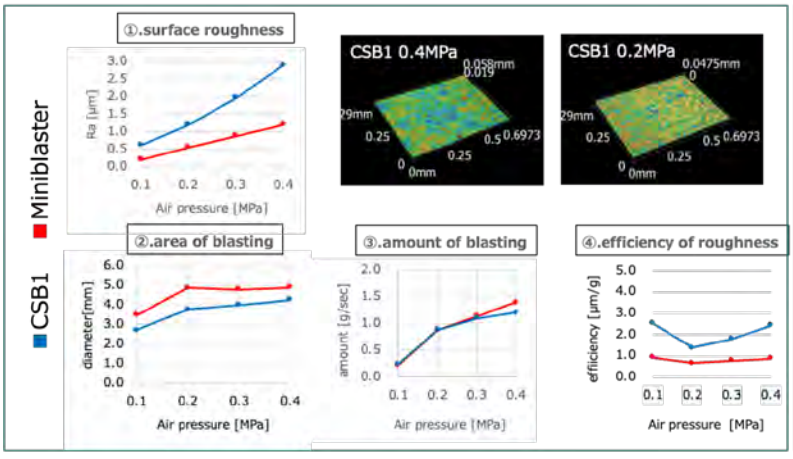
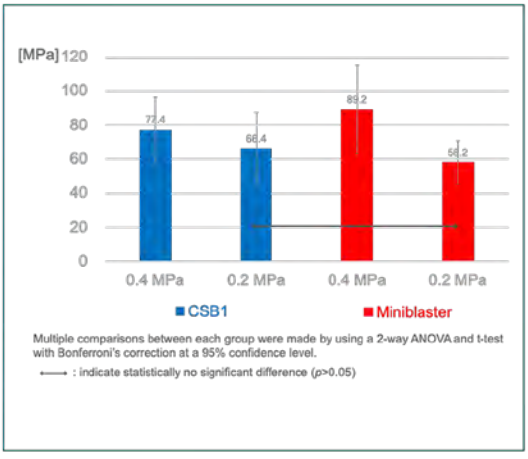


Fig.6 Results of validation tests



Fig, 7 Micro-tensile bond strength

(Conclusion)

A new pressure variable chairside sandblaster will be a unique and useful device for preparation of adherend surface with appropriate pressure for each CAD/CAM material at chairside. This device will solve the problem of surface pretreatment of CAD/CAM materials before cementation, ensure the clinical success of digital dentistry.

審査日程および実施方法

一次審査：2022年7月11日（月）～7月21日（木）
書類（研究発表抄録等）＋発表ビデオ

二次審査：2022年8月26日（金）
オンライン発表＋質疑応答

上位入賞結果

● 優勝

朝日大学歯学部 2年生　デュアー ヒュー　Hugh DEWAR

チェアサイドで使用可能な圧力可変式サンドブラスターの開発
Development of a chairside-type pressure variable sandblaster

● 準優勝

大阪歯科大学 5年生　木畑 佑基　Yuki KIBATA

分子動力学法を利用して脂質二重膜上で成長するハイドロキシアパタイトナノ結晶を制御する
Controlling the growth of hydroxyapatite nanocrystals on lipid bilayers with the guide of molecular dynamics simulation

● 第3位

北海道大学歯学部 4年生　水野 天音　Amane MIZUNO

SARS-CoV-2感染マウスモデルを用いたCOVID-19重症化における血管内皮細胞の役割の検討
Investigation of the role of vascular endothelial cells in severe COVID-19 using a SARS-CoV-2 infected mouse model

● 優秀賞

東京歯科大学 4年生　冨秋 智博　Chihiro TOMIAKI

ゾレドロン酸はT細胞依存的な免疫応答を負に制御する
Zoledronate negatively regulates T cell-mediated immune responses

東北大学歯学部 6年生　遠山 学　Manabu TOYAMA

歯周炎による炎症増悪機構: *Porphyromonas gingivalis*線毛はヒト単球におけるインターロイキン-6産生を相乗的に誘導する
Exacerbation of inflammation in periodontitis: *Porphyromonas gingivalis* fimbriae induce synergistic interleukin-6 production in human monocytes

岡山大学歯学部 4年生　山本 結　Yui YAMAMOTO

癌チップの独自開発と口腔癌の免疫抵抗性の発見
Original development of cancer chip and discovery of immunoresistance of oral cancer

優勝　朝日大学歯学部　デュアー ヒュー


二次審査風景　2022年8月26日　於歯科医師会館

審査講評

2022年になっても新型コロナウイルスが終息することはなく、3年間オンライン開催となりましたことは残念でなりません。このような中でも本年度は21校の参加をいただきました。スチューデント・クリニシャンおよび指導された先生方には敬意を表します。

一次審査では、研究発表抄録・発表スライド・発表ビデオをお送りいただきまして、審査員全員で主題・内容・発表等について採点し、上位6名を選出させていただきました。8月26日（金）の二次審査では、6名のスチューデント・クリニシャンにより、オンライン上で発表していただき、発表後には、審査員による質疑応答が行われ、英語での説明力、研究内容の理解度と質問に対する回答力など、英語力を中心に審査いたしました。審査員長からは、研究で最も難しかった点、副審査員長である私からはその研究がノーベル賞を取るに値するかどうか、という同じ質問を全員に投げかけさせていただきました。

一次審査・二次審査共に審査員の最高点と最低点を削除し、残りの審査員の平均点を算出して順位をつけさせていただきました。なお、スチューデント・クリニシャンの大学出身者が審査員となっている場合には、当該審査員の点数は削除して計算いたしました。

今回も21大学のスチューデント・クリニシャン全員の研究は大変すばらしく、今後の歯科界に役立つものと信じております。世界的な新型コロナウイルスのパンデミックや地政学的問題、さらに経済財政状況などが時々刻々と変わる中で、歯科界もその変化に追随していく必要があると思っています。参加いただいたスチューデント・クリニシャンの皆様には、今後の歯科界のために活躍されることを祈念して、講評とさせていただきます。

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

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東京歯科大学　名誉教授

審査員

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解剖学第1講座　教授

審査員

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東京医科歯科大学大学院医歯学総合研究科
東京医科歯科大学統合教育機構　准教授

*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

平成10年度（1998年）日本代表選抜大会

日本歯科医師会雑誌2022年5月号より転載

2021 年 AADR※/SCRP 大会参加報告

北海道大学病院　臨床研修医（歯科）／北海道大学大学院歯学院硬組織発生生物学教室　**吉野 弘菜**
（日本代表選抜大会参加時：6 年）

私は，令和 2 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（SCRP）日本代表選抜大会にて，総合優勝という栄誉にあずかりました。そして，2021 年 7 月米国にて開催された AADR/SCADA 大会において，日本代表として研究発表をさせていただきました。この度は，AADR/SCRP 大会での貴重な経験をご報告させていただきます。

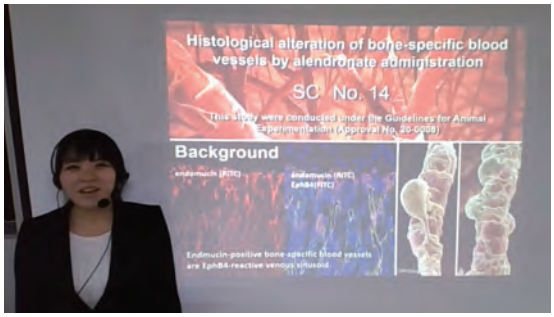
2021AADR/SCADA 大会は，2021 年 7 月 21 日～2021 年 7 月 24 日（EDT）の 4 日間にわたって執り行われました。当初は米国マサチューセッツ州ボストンにて開催される予定でしたが，新型コロナウイルスの蔓延により，Zoom を用いたオンライン形式での開催を余儀なくされました。SCRP 大会時から渡米の機会を心待ちにしていたので，ボストンへ赴くことができなかったのはとても残念でした。しかし，学会のホームページは細やかな工夫が凝らされており，閲覧しているだけでも学会の雰囲気を楽しむことができました。中でも，各セッションの演者名簿とは別に“Abstract Gallery”というものが設置されており，そこに演者のポスターや PowerPoint のファイルと，音声ファイル（MP 3）あるいは発表動画の 2 つが，セットで投稿される形となっていました。そのため，実際に演者の発表を聞く以前に，発表の趣旨や大まかな要点を知ることができました。また，事前に演者へ質問を送ったりコンタクトをとったりできるようなフィードも設置されていました。

私も演者として，学会が開催される前に音声ファイルおよび発表用ポスターを提出することとなりました。しかし，これは想像以上に骨の折れる作業でした。というのは，一昨年行われた SCRP 大会の発表は，オンライン開催に伴い，例年とは異なりスライド形式の発表だったからです。厳しい枚数制限の中で一度まとめ上げたスライドを，ポスターの形に変換する作業は大変手間が掛かりました。

私が発表を行ったセッションは SCADA: Basic & Translational Science Research I で，これは 7 月 24 日の午前 4：45 ～午前 5：45（JST）に行われました。私は自宅の私用 PC から，一人で参加しました。時差により日本での開始時刻が早朝だったため，寝過ごしてしまわないか心配でしたが，当日は無事に参加することができました。私以外に，各国から選ばれた 10 名の学生が 5 分間のポスター発表を行いました。今回はオンラインだったため，各自が自分のポスター（PDF）を Zoom で画面共有して発表するというスタイルでした。

私は，アレンドロネート投与による骨特異的血管の組織科学的変化について発表しました。発表後に 1 名の学生が骨吸収抑制薬関連顎骨壊死（ARONJ）と絡めた臨床的な質問をしてくださったので，日本でも ARONJ が深刻な問題になっていることや，その発症機序に関する話をしました。今回，授賞式および受賞者交流の場が設けられなかったことは大変残念でしたが，このように少しでも他国の学生と会話をする機会があって嬉しかったです。

国際学会に初めて参加した感想としては，私がこれまで参加した国内の学会と比較して，大変フランクな印象を受けました。学会期間中，私は他のセッションも多数拝聴しましたが，演者やホストの大半がプライベートな自室から学会に参加しており驚きました。発表中に，自分の子どもの声や飼い犬の鳴き声が入ったりしても，あまり気に留めていない様子でした。ゲーミングチェアに座ってポップコーンを食べながら発表を聞いている方もおり，とてもアメリカンだなと思いました。また，スーツを着用していた演者は私のみで，他の方はラフな私服で参加していました。日本国内の学会の多くは，ランチョンセミナーを除いては大学の講義の延長といった雰



日本代表選抜大会二次審査プレゼンテーション

YouTube 配信による表彰式の様子

※ AADRはAADOCR（American Association for Dental, Oral, and Craniofacial Research）に改称されました。

囲気を感じます。それに対して国際学会は，一方通行なレクチャーではなく，双方向で自由に意見を交わす場としての在り方を重視しているようでした。

オンライン学会に参加した感想としては，人的移動の負担や費用を削減できるというメリットがある一方で，デメリットも多くあると感じました。例を挙げると，対面形式と同じようにポスター発表を行うことには限界があると思いました。これは私が参加したセッションで起こった話ですが，画面共有の際に，拡大率の問題で所見が小さ過ぎて見えないといったことがありました。また，初めて出会った研究者と交流する機会が持てなかったり，どのような発表に人気が集まっているのかを目視できなかったりする欠点も感じました。学会開催地を散策する楽しみがなくなってしまったことも心さみしく感じます。コロナ禍が終焉し，これまでのような活発な討論が再開することを願います。

最後になりますが，お世話になった北海道大学硬組織発生生物学教室の先生方に心から感謝申し上げます。そして，関係者の皆様に，この場を借りて厚く御礼申し上げます。ありがとうございました。

令和４年度日本代表選抜大会募集案内

令和２年度 SCRP 日本代表選抜大会大会発表内容要旨

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

【目的・方法】近年，CD31/endomucin/EphB4 陽性骨特異的血管が発見され，骨芽細胞活性に寄与する可能性が報告されている。本研究は，アレンドロネート（ALN）が骨特異的血管や骨芽細胞に与える影響を組織化学的に解析した。
【結果】ALN 投与マウスの大腿骨では，endomucin 陽性血管が狭小化するとともに，血管壁に小突起や小胞が形成されていた。一方，血管壁の管腔維持に関わる *Gata2* や *Endomucin*，血管新生抑制因子である *Vash1* の遺伝子発現が上昇していた。また，ALN 投与マウスで TRAP 陽性破骨細胞が認められたにも関わらず，骨梁表面の ALP 陽性骨芽細胞が減少しており，狭小化した endomucin 陽性血管が骨表面から離れて局在した。
【考察】ALN 投与マウスの骨組織では，血管の異常が生じる一方，異常を修復するフィードバック機構が働いている可能性が推測された。また，ALN によって障害を受けた血管は，骨芽細胞にも影響を及ぼすと考えられた。
【結論】アレンドロネートは，破骨細胞のみならず骨特異的血管に影響を及ぼすとともに，骨芽細胞にも影響を与える可能性が示唆された。

Histological alteration of bone-specific blood vessels by alendronate administration

IIntroduction: Recently, the presence of CD31-/endomucin-/EphB4-positive blood vessels specific to bone has been reported to be involved in the osteoblastic activities. In this study, I have histochemically examined the biological effects of alendronate on bone-specific blood vessels and osteoblasts in bone.
Results: Alendronate-treated endomucin-positive blood vessels were markedly reduced in size, in that they appeared to have shrunk in femoral metaphysis. Under TEM, the alendronate-treated vascular endothelial cells showed several small processes and released many small vesicles from the luminal surfaces. In contrast, *Endomucin*, *Gata2* and *Vash1* genes related to angiogenesis or maintaining vasculature had increased after alendronate administration. Although the distribution of TRAP-positive osteoclasts was not as obviously affected by the regimen of alendronate administration, alkaline phosphatase-positive osteoblasts were not detectable in the secondary trabeculae, where only the shrunken and small endomucin-reactive blood vessels were seen.
Discussion: Alendronate administration resulted in small and shrunken endomucin-positive blood vessels, which showed irregular shapes and small protrusions from the vascular endothelial cells. Enhanced expression of *Gata2*, *Endomucin*, and *Vash1* may indicate the presence of a feedback mechanism to maintain the vascularity of bone-specific blood vessels. Interestingly, after alendronate administration, the inhibition of the osteoblasts appeared to be consistent with the decreased size of bone-specific blood vessels, even without the markedly reduced activities of osteoclasts.
Conclusion: Alendronate may not only affect osteoclasts but also bone-specific blood vessels, which subsequently influence osteoblasts.

ファカルティー・アドバイザー：網塚　憲生　硬組織発生生物学教室　教授
長谷川智香　硬組織発生生物学教室　助教

「令和２年度 SCRP 日本代表選抜大会研究発表抄録集」より転載

日本歯科医師会雑誌2022年6月号より転載

2022 年 AADOCR /SCADA 大会参加報告

岡山大学歯学部５年　棚井 あいり
（SCRP 日本代表選抜大会参加時：４ 年）

AADOCR での発表の経験を通して

この度 SCRP 日本代表として，AADOCR[*1] での発表の経験を報告する機会をいただきありがとうございます。今回は，令和３年度の SCRP[*2] 日本代表選抜大会後に，どのように AADOCR の参加・発表に至ったかについてお話ししたいと思います。正直なところ，SCRP 日本代表選抜大会で優勝することこそ，私の目標でした。そのため，AADOCR に出場することは，さらなる栄誉をいただいたようで大変うれしかったです。

さて，AADOCR の出場には年末から年始にかけて様々な準備がありました。事務局からはオンサイトで行う旨を伝えるメールをいただきました。新型コロナウイルス感染症のために，大学の方針として海外への渡航は許可されない状況でした。しかしながら，歯学部長，大学の事務の方々のご助力により，例外的に渡航を認めていただけることになりました。結果的には，その後の AADOCR 事務局の判断により，オンラインでの開催となりましたが，数多くの方々の支援をいただけたことに感謝申し上げます。

AADOCR はいくつかのセッションに分かれていました。まずは，SCADA の参加者が交流できるオープニングセレモニーがありました。私はそこに参加し，各国代表の学生たちと研究について話ができると楽しみにしていました。残念ながら，発表準備や時差などの関係もあってか，大半の学生は，大会の参加方式の説明を聞くだけで，すぐに退席してしまいました。そのような状況でしたが，米国参加者の中に母親が日本人の方がおり，話をすることができました。彼女の話は多岐にわたり，米国歯学部の学生の勉強や研究について知ることができました。

米国のコンペティションの様子も観ることができました。SCRP 日本代表選抜大会は，厳正な審査を行うため，それぞれの出身大学や個人名を公表せずに行われました。それとは異なり，米国大会では大学名や指導者・発表者名が伏せられていないほか，審査委員も知ることもできました。そのため，発表者は，事前に審査委員の研究分野なども知ることができましたが，評価の公平性の観点からは，日本のシステムが良いと感じました。

実際の発表では，私は最終日の “Periodontal Disease, Microbiome, Inflammation & Immune- modulation III” のセッションに入りました。発表の内容や原稿を確認し，午前３時になってオンライン接続をしました。セッションのファシリテーターの司会進行で発表が始まりました。オンラインでしたので，発表者のほとんどは研究室や自宅から参加していました。発表のほとんどは疫学的研究や，その後の in silico のデータ解析が多かった印象です。ここでの発表は Journal of Dental Research 誌にまとめられることから，後に論文として公表したい発表者や指導者は主なデータを発表できないといった感がありました。

私は，プロテオミクス解析に加え，動物実験，組織学的実験，細胞を使った実験を発表しました。他の発表者とは異なり，様々な実験手法を用いた発表でしたので，多くのセッション参加者に興味を持っていただくことができました。中には「実験に使用したマクロファージや小胞サイズは」といった質問がありました。発表は録画

発表は録画され，AADOCR のホームページにてオンデマンド配信も行われた

[*1] AADOCR は，American Association for Dental, Oral, and Craniofacial Research の略称です。
[*2] SCRP は，スチューデント・クリニシャン・リサーチ・プログラムの略称です。

して，ホームページ上でオンデマンド配信されました。

発表を通して印象深かったことは，たくさんの女性研究者が生き生きと発表や質問をされていることでした。日本でも，以前と比べて女性の研究者・指導者が増加していますが，海外の研究機関の環境とは異なる点が多いと思います。私は，このような今回の経験を積極的に岡山大学の学生，特に女子学生に伝えていきたいと思います。私自身も後輩にとって魅力的な研究者となれるよう，今後も頑張っていきたいと感じました。

今回 SCRP 日本代表として AADOCR での発表を経験し，日本の研究のレベルは世界にも引けを取らないこと，様々な人々が私も含めた学生のために尽力していただいていることを知りました。この経験は一生の宝物です。最後になりましたが，このような機会をいただき，日本歯科医師会および関係者の方々に心よりお礼申し上げます。ありがとうございました。

令和３年度 SCRP 日本代表選抜大会大会発表内容要旨

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 M φ 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 M φ 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 M φ 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 M φ 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。

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

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 M φ EVs (***Pg***-inf M φ 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 M φ 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 M φEVs-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 M φ 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 M φ EVs and inhibits placental angiogenesis through the downregulation of VEGFR1, which results in fetal abnormalities.

ファカルティー・アドバイザー：岡村　裕彦　口腔形態学分野　教授
研究指導協力者：池亀　美華　口腔形態学分野　准教授
福原　瑶子　口腔形態学分野　助教
江口　傑徳　歯科薬理学分野　助教

「令和３年度 SCRP 日本代表選抜大会研究発表抄録集」より転載

先輩SCからのメッセージ

産業医科大学産業保健学部人間情報科学 准教授　中富 満城　Mitsushiro NAKATOMI

今年度も日本歯科医師会はじめ関係される皆様方のご尽力によりSCRP日本代表選抜大会が無事開催される運びとなりました事を、OBの1人として大変喜ばしく思います。心より御礼申し上げます。

私とSCRPとの縁は九州大学歯学部4年生だった1999年に遡ります。当時の学部長より「東京で歯学部生の研究発表大会があるらしいから、見学に行ってきて欲しい」とのご依頼があり、その夏に開催された第5回大会に見学者として派遣されました。その時点では私は全く研究活動などしておらず、SCRPについて何の知識も無い状態でした。SCRP自体もまだ黎明期で、それまでに九州大学からの出場者は1人もおらず、学内での知名度は皆無という状況でした。

そして初めて足を踏み入れた歯科医師会館で目の前に展開される光景に、私は圧倒されるばかりでした。同年代の学生達がパネルの前で堂々と研究内容のプレゼンテーションを行い、表彰式と懇親会は華やかで、それまでに全く知らなかった世界が広がっていました。私は衝撃を受けると同時に、翌年は必ず出場者としてこの場に戻ってきたいと強く思いました。

その後歯科放射線科で研究活動をさせて頂ける事となり、2000年の第6回大会に九州大学から初出場を果たす事ができました。同期の出場者は会場で初めて会う人ばかりでしたが、皆それぞれに苦労して参加を果たした様子が窺え、初対面でありながら既に同志としての連帯感がありました。大会後も折に触れて再会する機会があり、20年来の仲間として現在に至っています。

私は祖父も父も開業歯科医なので元々家業を継ぐつもりで歯学部に入りましたが、在学中にこのSCRP出場をはじめとして様々な契機に触れた結果、次第に研究者を志望するようになりました。中でも再生医療につながる基礎研究としての発生学に興味を惹かれ、卒業後は東京医科歯科大学大学院博士課程に進学しました。その際にも既に同大学院に進学されていた複数のSCRPのOBの方から様々な助言を頂戴する事ができ、大変参考になりました。大学院在学中はお世話になったSCRPへの恩返しがしたいという思いから、OB会であるSCADA-Japanの役員・代表も務めさせて頂きました。

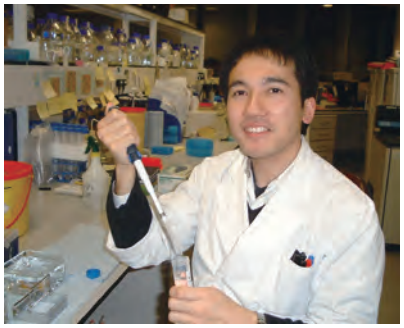
学位取得後は英国ニューキャッスル大学にて3年間ポスドクとして発生学研究に従事し、2009年に帰国して新潟大学歯学部の助教となりました。2010年と2011年にはSCRP出場学生のファカルティー アドバイザー（FA）を務めさせて頂き、英語指導や予演会の運営等を行いました。今度は教員としてこのSCRPの場に戻ってくる事ができ、大変感慨深かったです。現在ではSCRP出場経験者が教員となってFAを務める例が珍しくありません。将来はOBが審査員を務める日も来るのではないかと思います。その後2014年より九州歯科大学講師、2021年より産業医科大学准教授となり現在に至ります。

振り返るとSCRPのお蔭で学生時代に研究活動の醍醐味に触れる事ができ、研究者になるという人生の選択に大きな影響を与えました。勿論将来臨床歯科医を目指す学生にとっても在学中に研究を経験する事はリサーチマインドを涵養する上で非常に大きな財産になると思います。今後とも全国の歯学部生に対する貴重な機会としてのSCRPが末永く継続して開催される事を念願しつつ、メッセージとさせて頂きます。

プロフィール

2002年	九州大学歯学部卒業
2006年	東京医科歯科大学大学院修了
2006~2009年	英国ニューキャッスル大学研究員
2009~2014年	新潟大学歯学部助教
2014~2021年	九州歯科大学講師
2021年~現在	産業医科大学産業保健学部准教授

平成12年度日本代表選抜大会（2000年）



英国留学中の研究室にて

先輩SCからのメッセージ

北海道大学大学院歯学研究院硬組織発生生物学教室 助教　本郷 裕美　Hiromi HONGO

私は2010年、第16回SCRP大会に北海道大学歯学部の代表として出場した本郷裕美と申します。SCRP同窓会であるSCADA-Japanの役員も務めております。

私がSCRP大会に参加した当時、北海道大学には5〜6年生にかけて「研究実習」という授業があり、研究成果をSCRP大会で発表するものでした。しかし、発表時が6年生で、毎年代表のなり手がいない中、部活の先輩でもあり、当時SCADA-Japan代表だった鷲巣太郎先生（現SCADA-Japan副代表）に指名され、大会に参加することになりました。幸いファカルティー アドバイザー（現在の上司である網塚憲生教授）に恵まれ、研究はとても楽しく、自由に取り組むことができました。ところが会場に着くと、氏名・大学名は非公表で、各校の代表者の緊張感が伝わってきて、あまり深く考えずSCRP大会に参加した私は、これだけ多くの学生が『研究』に真剣に取り組んでいたことに驚き、大きな影響を受けました。

この時の研究が大変楽しく、もう少し続けてみたいという思いもありましたが、私の実家は開業医で、継ぐなら私しかおらず、当時は実家を継ぐことも考えていました。しかし、院長である母が「歯科医師は一生できるけど、研究は誰もができることではない」と背中を押してくれ、基礎の大学院に進学することを決めました。

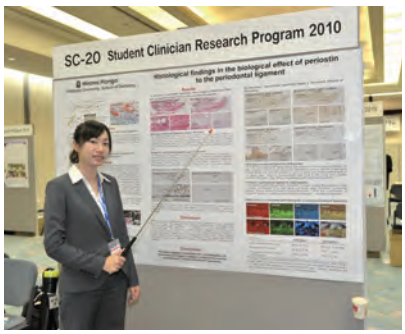
大学院時代は学術振興会特別研究員（DC1）になることができ、朝から晩まで研究ずくめ・・・というわけでもなく、基礎の大学院生ではありましたが、大学病院の外来で診療も行っておりました。そのおかげで、診療するにあたってはevidenceに基づいた治療を行う重要性を理解し、研究を行うにあたっては、臨床に活かせるevidenceをつくる根幹であることを意識して取り組むことができました。その間に国内外を問わず、たくさんの学会に参加させていただきましたが、学会先でSCADA-Japanの先生方にお声がけいただいたり、SCADA-Japan飲み会に参加したり、大会後も楽しい時間を共有することができました。

そして、今となっては自分がSCRP大会に参加する学生の指導に携わり、研究という道を提示できることを嬉しく思っています。また、SCRP大会に参加するということは、やはりresearch mindを持った先生方が多く、SCADA-Japan会員として多様性に富んだ先生方と知り合えたことに感謝しています。現在はコロナ禍のためオンラインを活用し、ベテランから若手まで気軽に参加できる症例検討会や勉強会を開催していますので、ぜひ、ご参加ください。

近年のSCRP大会は発表内容も非常にレベルが高く、質問にもしっかりと答えることができ、英語も堪能で優秀な学生が多く、それは歯科全体にとって喜ばしいことと思っています。臨床・研究、何をするにしても一生懸命取り組み、努力し、最善を尽くすことが、自分自身のためになります。みなさんの未来が、ますます素晴らしいものになることを祈念しています。

プロフィール

2011年	北海道大学歯学部卒業
2016年	北海道大学大学院歯学研究院 博士課程修了
2020年	北海道大学大学院歯学研究院 硬組織発生生物学教室　特任助教
2021年〜現在	北海道大学大学院歯学研究院 硬組織発生生物学教室　助教



平成22年度日本代表選抜大会（2010年）


2022年　研究室にて染色中

歴代優勝者/日本代表

第 1 回（H.7/1995 年）■東京歯科大学　黒田 俊太郎
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
新規歯垢染色液の開発
Ayano KANEOYA - Nihon University School of Dentistry at Matsudo
“Development of novel disclosing agents”

第 8 回（H.14/2002 年）■神奈川歯科大学　川越 俊美
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
音声音響分析による開咬を有する小児の構音評価
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”

第 12 回（H.18/2006 年）■北海道医療大学歯学部　大迫 利光
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
視認性に優れたオリジナル shade guide の製作
Yuko AKIYAMA - Nihon University School of Dentistry
“Shade determination using visibly optimal custom shade guide”

第 14 回（H.20/2008 年）■日本大学松戸歯学部　會田 悦子
携帯電話とパソコンを利用したブラッシング効果の検討
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”

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SCADA-Japanへようこそ

SCADA Associates in Japan
代表　井田 有亮　Yusuke IDA

今年度もSCRP日本代表選抜大会が開催されますことをSCADA Associates in JAPANを代表してお慶び申し上げます。この大会出場を経験した皆さんを同窓会であるSCADA会員としてお迎えできることを大変嬉しく思います。

各校代表として研究発表に臨まれたStudent Clinician・Co-Clinicianの皆さんは、大変多忙な学生生活の中にもかかわらず研究活動を継続され、大学代表の栄誉を背負って発表する中で他では得難い貴重な経験を蓄積されたことと拝察いたします。本年は世界規模の感染症流行が継続する中で、例年に比べて一段と活動に制約が加わる状況でも研究活動を継続されたことに、心から敬意を表します。

さて、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の機会を得ました。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。その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。今年度も集合しない形式での開催となりましたが、大会における交流をSCADAがサポートさせていただきたくことになりました。

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、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
▶現：東京大学大学院医学系研究科 特任講師　▶2007年SCRP大会出場第2位　▶2009年北海道医療大学歯学部卒
▶博士（歯学）・公衆衛生学修士（専門職）　▶大学院時代は金属系生体材料、現在は医療管理学・医療情報システム学を専門とする。
▶2018年よりSCADA Associates in Japan代表

SCADAについて

SCADAは世界のSCRP参加経験者で構成されるアメリカを本拠とする国際的な同窓会組織であり、世界各地で学術交流の場を広げています。1999年には日本人会員により、日本におけるSCRP大会同窓会組織としてSCADA Associates in Japan（SCADA-Japan）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大会参加者は、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。

あとがき

コロナ禍3年目を迎え、今年も一次審査からスチューデント・クリニシャンの熱い思いに圧倒される思いで審査に臨ませていただきました。止むを得ずオンライン開催となりましたが、予断は許さないものの、今後ウィズコロナにシフトしていく過程で、次年度以降は対面でスチューデント・クリニシャンの情熱を直接感じることができればと考えております。

年々、研究内容が高度化し、発表のスキルも上達していく中、思いを果たせた人もおられれば、熱意が届かず歯がゆい思いをされた方もおられたのではないかと思います。しかし、結果はどうあれ、皆さんの素晴らしい先輩の活躍が示されているように、この経験は必ずSCRPを経験された皆さんならではのステップアップに役立つと考えています。

今後、国際舞台で活躍される皆様の勇姿を拝見することを期待して、あとがきに代えさせていただきます。

審査員長　平野　裕之

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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。

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