SCRP

令和2年度

日本代表選抜大会

2020 JDA Student Clinician Research Program

研究発表抄録集

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

CONTENTS

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

令和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
日本代表選抜大会

歯科医師会館　（定礎：昭和63年）

ごあいさつ

公益社団法人 日本歯科医師会
会長 堀　憲郎

1959年に米国歯科医師会の主催によりスタートしたこのSCRP（スチューデント・クリニシャン・リサーチ・プログラム）は、現在世界各国を結ぶ歯学教育支援プログラムとして発展し続けています。

本年度は新型コロナウイルス感染症の世界的拡大による影響で、大学の教育もオンラインでの講義や受講学生の制限などを強いられ、様々な弊害が出ていることはご承知の通りです。

そのように大学での研究継続が困難であるにもかかわらず、本年度も18の歯科大学・歯学部から日本代表選抜大会にご参加いただきました。また、大会運営も、審査方法を書類およびビデオ発表による一次審査とオンラインでのプレゼンテーションによる二次審査という方式に変更するなど、これまでと異なる対応をお願いしたにもかかわらず、無事に日本代表を選抜できたことに対し、学生の皆様はもちろん、指導に当たられた皆様、関係者の皆様に感謝と敬意を表します。

日本歯科医師会は現在約65,000名の会員を有し、「医道の高揚、国民歯科医療の確立、公衆衛生・歯科保健の啓発、並びに歯科医学の進歩発達を図り、もって国民の健康と福祉を増進すること」という目的に沿い、多くの事業を展開しています。本会のそれらの取り組みの中で、国際戦略・国際貢献に関する分野については、歯科の国際組織であるFDI（世界歯科連盟）に約半世紀前の1969年より加盟し、毎年開催される世界大会に参加する中で、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。そしてそのような国際戦略のひとつとして、1995年からこのSCRPに参画しているところです。

この度の新型コロナウイルス感染症により、本年上海で開催予定であったFDI世界歯科大会は中止になりましたが、これまでの国際的友好関係を踏まえて、本会は米国歯科医師会との2カ国でのWEB会議、アジア太平洋地区の盟友であるオーストラリア、ニュージーランド歯科医師会等とのWEB会議等を開催し、組織運営や新型コロナウイルス感染症対策について意見交換をはかっています。

本会が、特にアジア地域をはじめとする国際貢献も重要な使命と位置づけ、国際感覚に優れた人材の育成を目指していることをお伝えし、今後とも皆様の変わらぬご理解とご支援により、このプログラムが日本の歯学教育と歯科の国際分野での発展に貢献することを祈念しご挨拶といたします。

公益社団法人 日本歯科医師会
常務理事　尾松 素樹

SCRP日本代表選抜大会は、昨年まではデンツプライシロナ社の後援により運営してきました。しかし今年度からデンツプライシロナ社が退き、日本歯科医師会の主催による単独開催となりました。これまでのデンツプライシロナ社のご支援に感謝申し上げます。

本会単独での開催については、運営面や予算面での課題がありましたが、歯科学生の研究を本会が支援できる貴重な事業ととらえ、実施に向けて準備を開始しました。

しかし、本年2月以降徐々に拡大した新型コロナウイルス感染症による影響は歯科医療現場のみではなく、全国の歯科大学・歯学部の教育現場にも影響を及ぼし、果たして大会が行えるだけの応募があるかの懸念が起こりました。さらに実施するにしても、従来のような各大学の代表が歯科医師会館に集合してのポスター発表による大会が、安全に開催できるかの問題も浮上しました。

そこで、本年度から運営を担当することになった国際渉外委員会で審査方法や日程について協議し、従来提出されている事前抄録に加えプレゼンテーションを収録したビデオ発表（一次審査）で上位入賞者を選び、次いで、オンラインでのプレゼンテーションと質疑応答（二次審査）で代表者を決める形式に変更しました。また、代表決定を8月下旬から二次審査の10月上旬へと変更しました。

このように従来の発表形式を大きく変更したにもかかわらず、18校の歯科大学・歯学部から応募があり、一次審査で選ばれた3名から、二次審査において日本代表1名を決定できました。

このような状況下で、本年度大会に参加されました18名の一人一人、そして共同研究者の皆さま、また、スチューデント・クリニシャンが安心して実験等発表準備に専念できるよう環境を整え研究指導に当たられたファカルティー・アドバイザーならびに研究指導協力者の先生方に敬意を表したいと思います。

日本代表として選ばれたスチューデント・クリニシャンは、2021年7月に米国マサチューセッツ州ボストン市にて開催予定の国際歯科研究学会米国部会（AADR）学術大会に招待され、全米の歯学系大学代表並びに各国代表と共に発表していただき、学術交流や親睦を深めていただきたいと思います。

なお、例年発行しています要旨を掲載した冊子を、研究発表抄録集として事前抄録も掲載し、研究記録として残すことにしました。本会ホームページでも閲覧できるようにし、大学での研究を志すこれからのスチューデント・クリニシャンの参考となるようにすることにしました。

次年度も多くの参加を期待いたします。

研究テーマ一覧

SC NO.	大学	氏名	研究テーマ（和文）	研究テーマ（英文）	ページ
1	九州大学歯学部	高本 侑立子	欠損補綴治療による起立運動機能への影響	Effect of prosthodontic treatment on standing movement function	5
2	九州歯科大学	高田 知佳	Evidence-Practice Gapの改善に関する研究	A study on the improvement of evidence-practice gap	8
3	奥羽大学歯学部	今井 千穂子	側貌シルエットに対する主観的評価に関する研究	Subjective evaluations of profile silhouettes	11
4	東京医科歯科大学歯学部	渡部 準也	Growth differentiation factor-5の発現が示すマラッセの上皮遺残の不均一性	Growth differentiation factor-5 expression indicates heterogeneity in epithelial rests of Malassez	14
5	東北大学歯学部	古内 聖弓	法医学的観点における歯冠色成形材料の熱による蛍光変化	Forensic considerations for fluorescence changes of tooth colored material due to heating	17
6	大阪大学歯学部	都原 志穂	*i*-GONAD法による遺伝子組み換えマウスの作製ー歯学部生による挑戦ー	Generation of genome-edited mice by *i*-GONAD - challenged by dental school students -	20
7	松本歯科大学	小野 亜美	象牙質形成過程と骨組織におけるDMP-1、FAM20C、FGF23局在の免疫組織化学的検討	Immunohistochemical localization of DMP-1, FAM20C, and FGF23 during dentinogenesis in comparison with bone	23
8	徳島大学歯学部	喜田 悠太	バイオフィルム細菌の生存戦略に関わる遺伝子の解析	An analysis of the genes related to the survival of biofilm bacteria	27
9	岡山大学歯学部	村田 志穂	ニューラルネットワークを使用した細胞の分化増殖能の評価	Evaluation of cell differentiation and proliferation potential using neural network	29
10	昭和大学歯学部	根岸 宗一郎	トレハロースは破骨細胞形成を抑制する	Trehalose suppresses osteoclast formation	32
11	福岡歯科大学	池本 梨央南	歯周病原細菌の増殖を抑制するヒト口腔内常在微生物の探索と同定	Screening and identification of human oral bacteria that inhibit growth of periodontal pathogenic bacteria	35
12	日本大学松戸歯学部	植草 信乃介	口腔内自然免疫応答の活性化に関する研究	A study for the induction of activating innate immune responses in the oral cavity	38
13	長崎大学歯学部	園木 美結	シェーグレン症候群による障害唾液腺の機能を修復する	Effectively conditioned peripheral blood mononuclear cells restore salivary gland function in mice with Sjögren-like disease	41
14	北海道大学歯学部	吉野 弘菜	アレンドロネート投与による骨特異的血管の組織学的変化	Histological alteration of bone-specific blood vessels by alendronate administration	44
15	鹿児島大学歯学部	佐藤 大幹	骨芽細胞の分化におけるHypoxiaの影響	The effect of hypoxia on osteoblast differentiation	47
16	日本大学歯学部	黒澤 佑介	歯周病原菌代謝産物酪酸の作用が歯肉３次元培養組織に及ぼす影響	The effects of butyrate-treatment on three-dimensional gingival tissue cultures	49
17	岩手医科大学歯学部	羽金 雅登	唾液腺腫瘍モデルマウスの確立とその性状解析	Establishment of a murine salivary gland tumor model and its characterization	52
18	大阪歯科大学	鈴田 真裕	力学的閾値同定を主軸とした破骨細胞分化（促進/抑制）制御法の確立	Control of promotion/suppression for osteoclast differentiation based on identification of mechano-threshold	56

Effect of prosthodontic treatment on standing movement function

九州大学歯学部　5年生　Kyushu University School of Dentistry　Class of 2021

高本 侑立子　Yuriko TAKAMOTO

共同研究者：小野 涼平（5年生）　遠藤 真緒（5年生）
ファカルティー・アドバイザー：大木 郷資　クラウンブリッジ補綴学分野 助教
古谷野 潔　インプラント・義歯補綴学分野 教授

Problem / hypothesis: Recently, Japan is experiencing "super-aging" society that is unprecedented in the world. It was reported elderly people with frailty increased risk of falls. However, the appropriate intervention can improve "frail". To reveal the effect of prosthodontic treatment on standing movement function shows importance of occlusion.
Methods: The subjects were 48 people and more than 65 years-old who received denture treatment. Experimental protocols were approved by University Institutional Review Board for Clinical Research (approval no.: 2019-167). After obtaining the consent, the oral function and the ability of standing movement before and after a prosthodontic treatment were evaluated.
1) The occlusal force and the masticatory performance were compared with and without dentures.
2) All subjects were categorized by 2 groups which are with and without occlusal support in molar region and evaluated. Statistical analyses were performed using a Wilcoxon signed rank test.
Results: 1) The result (the occlusal force, masticatory performance and the evaluation item of standing movement) with dentures were significantly improved compared without dentures.
2)The occlusal force and the masticatory performance with dentures were significantly improved compared without dentures in both groups. In the groups of without occlusal support, a part of masticatory performance was improved with dentures compared without dentures.
Conclusion: It was suggested that the prosthodontic treatments for the patients without occlusal support in the molars affect the standing ability, which is one of the systemic functions.

欠損補綴治療による起立運動機能への影響

問題点・仮説：日本は世界に類を見ない超高齢社会である。「フレイル」状態の高齢者は転倒リスクが高いが、適切な介入で改善できる。欠損補綴治療における起立運動機能への影響を解明することで、咬合支持の確立の重要性を示し、咀嚼だけではない高齢者の補綴治療の意義を明らかとできる。
方法：可撤性部分床義歯治療を行い研究への同意を得た65歳以上の48名を被検者とした。臨床研究倫理審査委員会の承認を得た（許可番号2019-167）。咬合力、咀嚼能力、起立動作能力の各項目に関して、1）被験者全体の義歯装着の有無での比較、2）咬合支持の有無によって被験者を2群に分け、各群の義歯装着の有無における比較を行った。群間比較には、ウィルコクソンの符号付順位検定を用いた。
結果：1）咬合力、咀嚼能力、一部の運動機能項目に関して、義歯装着なしの状態と比較し、義歯装着ありの状態の方が有意に向上した。2）両群とも咬合力、咀嚼能力に有意な差を認めた。咬合支持がない群で一部の運動機能項目において有意差を認めた。
結論：咬合支持を失った者に対する可撤性部分床義歯治療は、全身機能の一つである起立動作能力に影響を与えることが示唆された。

Effect of prosthodontic treatment on standing movement function

（Problem）

Recently, Japan is experiencing “super-aging” society that is unprecedented in the world. The main cause of the accident of the elderly’s daily life is "falls", and there are many cases that it leads the need for long term nursing care. It was reported that many elderly people are frail and these people have significantly increased risk of falls. The appropriate intervention can improve “frail”. As a dental intervention, the usefulness of dentures has been indicated. However, the effects on physical function regarding “frail” before and after prosthodontic treatment for missing teeth is still incompletely understood. The objective of this study was to reveal the effects on physical function of patients who have prosthodontic treatment for missing teeth focusing on standing motion measurement, because this way is easily and isn’t invasive.

（Hypothesis）

We hypothesized that the prosthodontic treatment for missing teeth had a positive impact on the physical function such as sit-to-stand motion of patient and it shows the importance of occlusion and the meaning of prosthodontic treatment for eating. Moreover, it also leads to show the role and the meaning of dentistry in super-aging society.

（Methods）

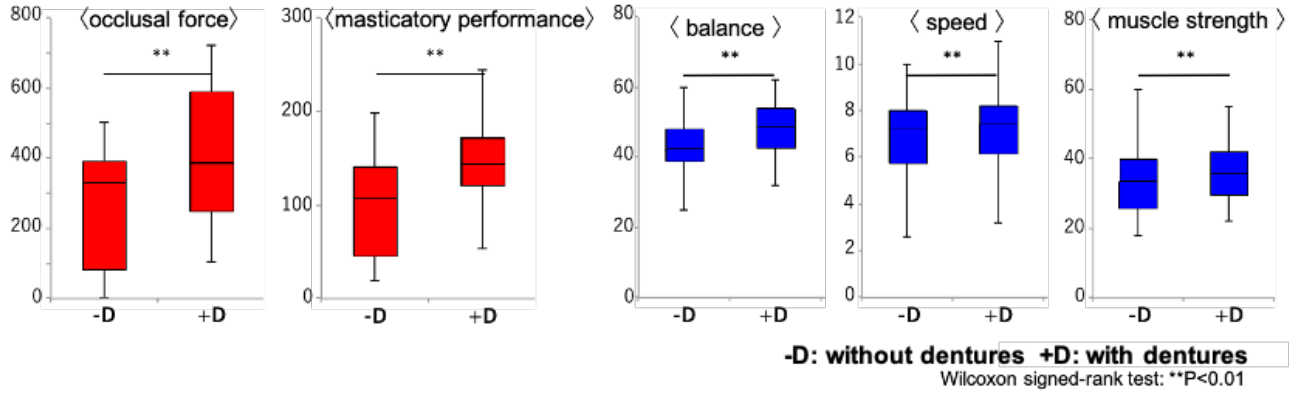
Types of Research designs: Before-after study

1) The subjects; The subjects were more than 65 years-old who received denture treatment in University Hospital (Section of Fixed Prosthodontics and Removable Prosthodontics). The subjects were total 48 people and grouped two. One is included 24 subjects who have occlusal support in molar region（Eichner B1～B3), and the other is included 24 subjects who don’t have it（Eichner B4～C3)) and agree with this research. After approving by research ethics committee (REC) of University Hospital, this research was conducted. (approving number: 2019-167)
2) List of measurement; The occlusion with and without prosthodontic treatment (the occlusal number of teeth, occlusal area of contact, occlusal force and masticatory performance), the ability of standing movement (①power, ②speed, ③muscle strength, ④balance) .

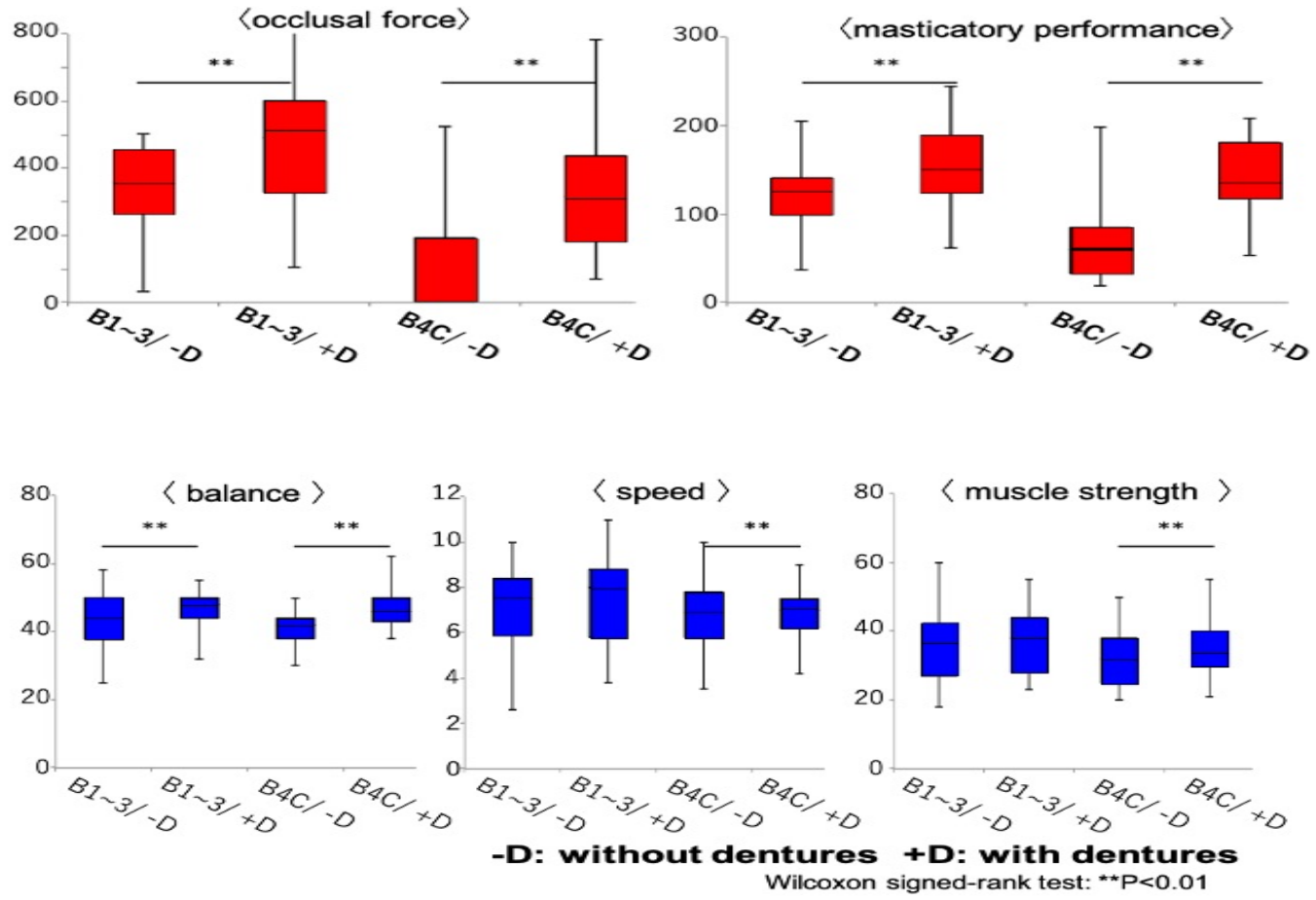
   Measurement method: First, the subjects were advised to bite Dental Prescale (GC, Tokyo) for 3 seconds. After the researcher removing it, they scanned using the Bite Force Analyzer (GC, Tokyo) and analyzed bite force. Masticatory performance was analyzed by using GLUCO SENSOR GS-II (GC, Tokyo) after chewing gummy jelly. Standing movement was measured with zaRitz BM-220 (TANITA, Tokyo). The items measured were: “power” “speed” “balance” and “muscle strength”. All measurements were compared between with and without dentures in another day.
3) Statistical analysis: First, all subjects were compared with dentures and without. All subjects were categorized by 2 groups which are with and occlusal support in molar region, and each lists of measurement are compared and examined. They were compared with and without dentures in each category. Statistical analyses were performed using a Wilcoxon signed rank test.

（Results）

1) The occlusal force and the masticatory performance were compared with and without dentures, and the results with dentures were significantly improved compared without dentures ($p<0.05$). Among the evaluation items of standing



2) When grouped and evaluated by the presence or absence of occlusal support in the molar region, the occlusal force and the masticatory performance with dentures were significantly improved compared without dentures ($p<0.05$) in all groups. Especially, in the groups of absence of occlusal support (Eichner B4C), “balance”, ”speed” “muscle strength” was more improved with dentures compared without dentures ($P<0.05$).



（Conclusion）

It was suggested that the prosthodontic treatments with removable partial dentures for patients without occlusal support in the molars affect not only the masticatory function but also the standing ability, which is one of the systemic functions.

A study on the improvement of evidence-practice gap

九州歯科大学 3年生 Kyushu Dental University Class of 2023

高田 知佳 Tomoka TAKATA

ファカルティー・アドバイザー：角舘 直樹 臨床疫学分野 教授

An Evidence-Practice Gap (EPG) is defined as a difference between high-quality evidence in clinical research and actual clinical practice. We conducted a before-after intervention study using web-based questionnaire surveys among Japanese dentists to clarify the following: 1) whether an EPG can be improved by updating dentists with the best research evidence for the caries treatment; 2) dentist-specific factors that are associated with improvement of this EPG; and 3) situations in which evidence-based caries treatment is difficult. We measured the EPG using 6 questions about diagnoses of primary caries, deep caries, and restorative treatments. The educational intervention, providing the dentists with the updated evidence, improved mean overall concordance between the published evidence and actual clinical practice from 66% (pre-intervention) to 89% (post-intervention, $p<0.001$). The higher improvement of EPG was associated with two dentist specific factors: "female dentist" and "employed dentist." A qualitative content analysis found that difficult situations in the evidence-based caries treatment could be classified into 5 categories: "cases where decision making is difficult on treatment and prognosis", "inadequate practice resources", "limitations on patient visit and treatment period", "discrepancy between the patient's values and dentist's values", and "limitations on health insurance and social understanding." These results suggest that improving EPG in Japan requires not only that dentists be provided with updated evidence, but will also require improvements in the 5 categories above.

Evidence-Practice Gapの改善に関する研究

臨床研究で良質のエビデンスが得られても診療現場で実施されず、研究と診療の間にギャップが存在することを、Evidence-Practice Gap（以下、EPG）と呼ぶ。本研究では日本の歯科医師を対象に、1）う蝕治療に関するエビデンスのアップデートによるEPGの改善効果を評価すること、2）EPGの改善に関連する要因を明らかにすること、3）エビデンスに基づくう蝕治療の実施が難しい場面を明らかにすること、を目的としてウェブを用いた前後比較研究を行った。EPGは「初期う蝕」、「深在性う蝕」および「修復処置」の診断と治療に関する6設問で測定した。参加者にエビデンスをアップデートした結果、エビデンスと実際の診療との平均一致率は66%から89%へと改善した（$p<0.001$）。また、「女性歯科医」および「勤務医」が高いEPG改善率と有意に関連していた。エビデンスに基づくう蝕治療の実施が難しい場面に関する内容分析の結果、①治療法や予後の判断が難しい、②診療環境が整っていない、③通院・治療期間に制約がある、④患者の価値観と異なる、⑤制度・社会的な制約がある、の5カテゴリーが抽出された。我が国のEPG改善にはエビデンスのアップデートに加え、上記5つの場面を改善することが有効であろう。

A Study on the Improvement of Evidence-Practice Gap

[Problem]
High-quality evidence obtained from clinical research may not be appropriately reflected in clinical practice. This is defined as the evidence-practice gap (EPG). A study of EPG in medicine outside Japan reported that more than 40% of patients were not given established effective treatment. In dentistry, EPG has been shown to exist in about 40% of cases of caries treatment both in Japan and the United States, and improving the EPG issue is an international priority. To date, however, no study has investigated educational interventions to achieve specific improvements in EPG, nor has any study reported on difficulties in evidence-based dental practice in Japan.

[Hypothesis]
1) Dentists can improve EPG by updating the evidence for caries treatment.
2) Dentist-specific factors are associated with EPG improvements.
3) Situations exist in which the provision of evidence-based treatment is difficult, and these situations can be classified into categories.

[Methods]
1) Study design: Before-after intervention study and cross-sectional study (two web-based online questionnaire surveys)
2) Study period: January 2017 to September 2018
3) Participants: 197 Japanese dentists working in outpatient dental practices
4) First survey: A questionnaire used by the US Dental Practice-Based Research Network (Dental PBRN) was translated into Japanese, and a web-based questionnaire survey was conducted. EPG was measured in terms of minimal intervention dentistry (MID). The questionnaire had 6 EPG-related questions, covering 3 clinical areas: “Primary caries diagnosis and treatment”, “Deep caries diagnosis and treatment”, and “Restoration diagnosis and treatment” (Table 1). To answer these questions, the respondents reported their diagnosis and treatment behaviors in daily practice.
5) Feedback material: Feedback about EPG was electronically prepared, including results of the first survey, international comparisons with a previous study from the US, and a summary of recent evidence on MID (a total of 13 pages in A4 format).
6) Second survey: The EPG was re-measured after participants read the feedback material. To verify educational effects, EPG measurements from the first (baseline) and second surveys (after feedback) were compared. We also performed qualitative content analysis using free-text responses to a question about “difficult situations when conducting MID based on evidence and guideline recommendations in actual caries treatment”.
7) Primary outcome: The mean overall concordance between published evidence and clinical practice was used as a primary outcome. To examine overall concordance for the six questions, we coded responses to each of the six clinical procedures as consistent or inconsistent with published evidence. An overall concordance was calculated as the percent of responses that were consistent with the evidence, with a higher percentage indicating higher concordance between published evidence and clinical practice. The criteria for assessment of concordance between evidence and clinical practice was used according to the categorization in the prior US study (Table 1).
8) Statistical analyses: Differences in concordance between before and after interventions were tested using the chi-square test and paired t-test. Multiple logistic regression analysis was then conducted to examine the associations between dentist-specific factors (independent variables) and the improvement of the overall concordance to 100% for all six questions (dependent variable). The statistical significance level was set at 5%.

Table 1. Categorization of concordance between clinical practice and published evidence

Clinical Area	Clinical Question or Scenario	Response option(s) classified as consistent with evidence	Response option(s) classified as inconsistent with evidence
Primary Caries diagnosis and treatment	Q1. Assessment of caries risk for individual patients in any way	Yes	No
	Q2. Treatment of unrestored occlusal surface of a mandibular left first molar that has brown discoloration in some of the fissures in the occlusal surface and no cavitation	Any non-invasive restoration procedure	Composite restoration or indirect restoration or amalgam restoration
	Q3. Treatment of unrestored occlusal surface of a mandibular left first molar that has brown discoloration in most of the fissures in the occlusal surface and no cavitation	Any non-invasive restoration procedure	Composite restoration or indirect restoration or amalgam restoration
Deep caries diagnosis and treatment	Q4. Treatment options for excavation of caries deeper than anticipated for a patient with deep occlusal caries in the mandibular right first molar and perhaps involving the mesio-buccal pulp horn	Stop removing decay near the pulp horn and remove it elsewhere	Continue and remove all the decay; Temporize and treat or refer the tooth for endodontics
Restoration diagnosis and treatment	Q5. Defective composite restoration with enamel margins	Response included (but not limited to) polish, re-surface, or repair restoration but not replace	Response included (but not limited to) replace entire restoration
	Q6. Lesion depth for permanent restoration instead of only preventive or non-surgical therapy (proximal caries)	Radiographs #3, 4, or 5 (lesion into dentin)	Radiograph #1 or 2 (lesion in enamel only)

[Results]
1) Demographic characteristics of participants (Table 2)
Web-based questionnaires were distributed by e-mail to 197 dentists, from whom 105 (53%) responses were collected.

2) Before and after comparison of concordance between first (baseline) and second surveys (after feedback) (Table 3)

Regarding comparisons of concordance between pre- and post-intervention, concordance for five questions improved significantly ($p<0.05$), including "Q1: Caries risk assessment", "Q3: Occlusal surface primary caries treatment", "Q4: Deep caries treatment", "Q5: Defective composite restoration", and "Q6: Proximal caries diagnosis".

3) Before and after comparison of mean overall concordance for all six questions (Table 4) and proportion of respondents who had the right answers to all six questions (perfect concordance) (Table 5)

Mean overall concordance for all six questions improved significantly, from 66% to 89% ($p<0.001$). Furthermore, the proportion of respondents who had the right answers to all six questions increased from 12% to 57% ($p<0.001$).

4) Dentist-specific factors associated with the improvement of concordance (Table 6)

A multiple logistic regression analysis identified two dentist-specific factors significantly associated with the improvement of the overall concordance to 100% for all six questions: "female dentist" and "employed dentist".

5) Qualitative content analysis on difficult situations when conducting MID (Table 7)

Qualitative Content analysis concerning "difficult situations when conducting MID based on evidence and guideline recommendations in caries treatment" identified five categories: "cases where decision making is difficult on treatment and prognosis", "inadequate practice resources", "limitations on patient visit and treatment period", "discrepancy between the patient's values and dentist's values", and "limitations on health insurance and social understanding".

[Conclusion]

1) The concordance improved significantly by updating dentists with the latest evidence. These results suggest that it is possible to reduce the EPG by using a web-based educational intervention.
2) Although the educational effects of this intervention were demonstrated, perfect concordance was not achieved by all participants. This is possibly due to the five identified situations that Japanese dentists face when conducting MID. Creating an environment to improve these situations would facilitate reducing the EPG in Japan.

Table 2. Participants characteristics (n=105)

	N (%) or Mean±SD
Years since graduation from dental school	19.6±12.7
Gender	
Male	81 (77%)
Female	24 (23%)
Type of practice	
Employed by another dentist	39 (37%)
Self-employed without partners and without sharing of income, costs, or office space	66 (63%)
City population	
Government-ordinance-designated city	52 (50%)
Non-government-ordinance-designated city	53 (50%)
Practice busyness	
Busy	51 (49%)
Not busy	54 (51%)

Table 3. Before-after comparison of concordance between first and second surveys (n=105)

		Concordance (1st survey)	Concordance (2nd survey)	p-value*
Q1	**Caries risk assessment**	53% (56/105)	90% (95/105)	P<0.001
Q2	**Occlusal surface discoloration**	98% (103/105)	100% (105/105)	0.477
Q3	**Occlusal surface primary caries treatment**	82% (86/105)	92% (96/104)	0.037
Q4	**Deep caries treatment**	53% (56/105)	86% (90/105)	P<0.001
Q5	**Defective composite restoration**	60% (61/102)	86% (90/105)	P<0.001
Q6	**Proximal caries diagnosis**	50% (53/105)	81% (85/105)	P<0.001

* Chi-square test

Table 4. Before-after comparison of mean overall concordance for all six questions (n=105)

	1st survey (Baseline)	2nd survey (After feedback)	p-value*
Mean overall concordance for all six questions (%)	66.3±20.8	89.2±15.0	P<0.001

* Paired t-test

Table 5. Proportion of respondents who had the right answers to all six questions (perfect concordance) (n=105)

	1st survey (Baseline)	2nd survey (After feedback)	p-value*
Proportion of respondents with perfect concordance	12% (13/105)	57% (60/105)	P<0.001

* Chi-square test

Table 6. Dentist-specific factors associated with the improvement of concordance (n=105)

Independent variables	Odds Ratio (95%CI)	p-value*
Years since graduation from dental school**	1.03 (0.98-1.08)	0.260
Gender (reference: male)	3.63 (1.09-12.13)	0.036
Type of practice (reference: self-employed)	4.71 (1.29-17.11)	0.019
City Population (reference: government-ordinance-designated city)	2.15 (0.76-6.13)	0.151
Practice busyness (reference: busy)	1.27 (0.52-3.10)	0.607

* Logistic regression analysis ** Continuous variable
Dependent variable: improvement of the overall concordance to 100% for all six questions

Table 7. Qualitative content analysis on difficult situations when conducting MID based on evidence and guideline recommendations (n=62)

Main category	Sub-category	Representative participant quotes
1. Cases where decision making is difficult on treatment and prognosis (n=8)	1) Necessity to consider both the oral and systemic situations (n=4)	When the patient cannot open his/her mouth adequately for reasons such as temporomandibular joint disorders.
	2) Unusual cases (n=2)	There are non-textbook cases.
	3) Lack of confidence about prognosis (n=1)	When I am not confident about the prognosis for fear of pain or spreading of caries.
	4) Repair of sites treated at other clinics (n=1)	I cannot repair the parts that have been treated at other clinics. I don't have trust in other clinics because there are too many dental clinics providing insufficient treatment.
2. Inadequate practice resources (n=18)	1) Lack of time to explain or treat (n=7)	Depending on the clinic's situation, treatment time may cause the possibility of "drilling teeth immediately".
	2) Variation of perceptions of MID among local dental practitioners (n=6)	In some cases, a patient at my clinic is told that he/she will be followed up without treatment but has been told at another clinic that he/she needs treatment, which makes it difficult for the patient to understand which is right, and makes the patient anxious.
	3) Problems with skills and equipment (n=5)	Increase in the amount of excavation is inevitable when the dentist has insufficient skills or when we don't have the appropriate tools for minimal intervention.
3. Limitations on patient visit and treatment period (n=10)	1) Irregular patient visits (n=6)	Although I have given patients an explanation on the necessity of follow-up, they stop visiting me. They eventually come to see me again when pain comes back.
	2) Limitation on treatment period (n=4)	When the patient is supposed to stay in an area without dental institutions for a long time (e.g., overseas assignment).
4. Discrepancy between the patient's values and dentist's values (n=17)	1) Difference between MID and patient wishes (n=12)	Many patients actually want us to remove all the areas suspicious for dental caries.
	2) Patient's unacceptance of MID-based treatment (n=5)	There are individual differences in patients' sensitivities and characters. For example, I have to select a treatment option that can definitely resolve the symptoms in patients who complain of persisting symptoms after one-time treatment even though it represents overtreatment.
5. Limitations on health insurance and social understanding (n=9)	1) Difficulty in implementation of MID due to health insurance system and dental fees (n=8)	Regarding the treatments not covered by the National Health Insurance (e.g., caries risk assessment), I have some difficulties in performing them (e.g., when I do not have enough time) even though I understand their necessities.
	2) Insufficient social understanding regarding MID (n=1)	It seems necessary to promote understanding of MID more broadly in society.

Subjective evaluations of profile silhouettes

奥羽大学歯学部　4年生　Ohu University School of Dentistry　Class of 2022

今井 千穂子　Chihoko IMAI

ファカルティー・アドバイザー：福井 和徳　成長発育歯学講座 教授

Introduction: Improving facial harmony is one of the important objectives of orthodontic treatment and proper facial evaluation is important for treatment planning. We conducted research of subjective evaluations of side profile silhouettes performed by undergraduate students and dental practitioners.
Material and methods: A standard silhouette model for profile evaluation was prepared using a male patient with an ANB of 3.7° and good E-line, who was selected from among patients who visited the Department of Orthodontic Dentistry of our University Hospital. Various face silhouettes were then constructed using image processing software (GIMP 2.10.12) by moving the chin in a front to back direction parallel to the FH plane, with a total of 9 patterns created. We enrolled 325 undergraduate students, general dentists, and orthodontists as subjects, who were asked to select what they considered to be the ideal profile.
Results and Discussion: The fourth-year students had the highest ratio (61.0%) of students who selected the reference model, which was about the same as the general dentists. We concluded that since knowledge of dentistry is limited until the third year of university, the proportion of students in the first three years who chose the ideal side profile was low, while the percentage was increased in the fourth year because of the introduction of orthodontics study.

側貌シルエットに対する主観的評価に関する研究

【はじめに】顔貌の調和を改善することは矯正歯科治療の重要な目的の1つであり治療計画を立案する上で顔貌の評価は大変重要である。そこで今回、歯学部生と歯科医師を対象とし、側貌シルエットに対し主観的評価を目的に研究を行った。
【対象と方法】側貌評価のための基準モデルは、本学附属病院矯正歯科に来院した患者の中から、ANBが3.7° に近似し、E-lineが良好な男性1名を選出し、側貌シルエットを作成した。これをコンピュータ上で画像処理ソフト（GIMP 2.10.12）を用いてオトガイをFH平面と平行に前後方向に動かし、9パターン作成した。対象は本学歯学部生、一般歯科医および矯正専門医の合計325名を用いて理想的な横顔を選択させた。
【結果および考察】基準モデルを選択した学生の割合は、第４学年が最も高く（61.0%）、一般歯科医とほぼ同程度であった。これらより第３学年までは歯科の知識が乏しいため、理想的な側貌を選ぶ割合は少なく、第４学年では歯科矯正学を学習したことで、理想的な側貌を選ぶ割合が高くなったと考えられる。

Subjective Evaluations of Profile Silhouettes

Problem

In the planning of orthodontic treatment, the patient's face must be evaluated properly. Patients who require orthodontic treatment are frequently affected by cosmetic disturbances, which include not only malalignment of teeth but also asymmetrical acial features. Thus improved facial harmony is an important factor to consider in orthodontic therapy. For proper morphological analysis of the face, Tweed, as well as other orthodontists, used soft tissue subject profiles to suggest a variety of analytical methods and ideal values from the standpoint of facial aesthetics. Among those values, the H-angle of Holdaway, the S-line of Steiner, the Z-angle of Merrifield, and Ricketts's E-line and the golden section ratio are widely used in the analysis of soft tissue profiles. Facial attractiveness and beauty criteria are based on experience and training as a specialist and do not match the perception of the patient or the general public.

Hypothesis

In this study, we prepared profile silhouettes for the purpose of subjective evaluations by students of University School of Dentistry, as well as professional general dentists with the hypothesis that dentistry education might make a difference in recognition of ideal facial features.

Methods

A standard silhouette model for profile evaluation was modeled after a male patient with an A-point–nasion–B point (ANB) angle of approximately 3.7° and a good E-line who was selected from among patients who visited the Department of Orthodontic Dentistry of our University Hospital. Image processing software (Adobe Photoshop Elements 15; Adobe Inc., San Jose, CA, USA) was then used to move the chin in the image so as to be parallel with the Frankfort horizontal (FH) plane in an anteroposterior direction by a total of 4.0 mm. Then the chin was moved at 1.0-mm intervals to produce different profile silhouette patterns. A total of 325 subjects were queried: 276 first- to sixth-year students (193 men and 83 women) of the School of Dentistry at our University, 37 professional general dentists (27 men and 10 women), and 12 orthodontists (3 men and 9 women). They were asked to select the profile silhouette they thought was most ideal among the nine profile silhouette patterns (Fig. 1).

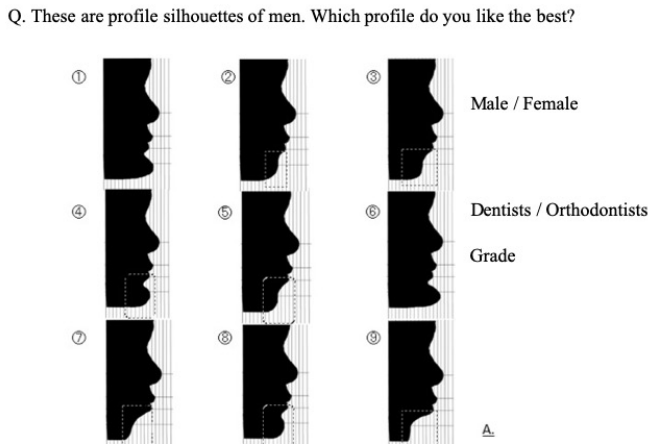


Figure 1. Answer sheet for selection of ideal profile from nine patterns.

Results

The highest proportion of students who selected the standard model as ideal was 61% of the fourth-year students, in comparison with 54.0% of the dentists and 83% of the orthodontists (Fig. 2). On the basis of these findings, we presumed that the reason why lower proportions of first- to third-year students chose the standard model was that they had less knowledge about orthodontic dentistry than did the more advanced students, who had studied that specialty. The proportions of fifth- and sixth-year students were each 50%, similar to that of the dentists, possibly because of their subsequent study of other related fields allowed them to acquire more comprehensive knowledge.

The ideal profile silhouette used in this study was based on values presented in past studies as a reference range (2–6). It is therefore likely that the ideal profile might be somewhat different from that chosen by students and professionals today. In clinical practice, treatment is performed by applying ideal values learned in dental school. In the future, however, it may be necessary to update those ideal values according to the needs of the current patient population.

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Dentists	Orthodontists
First	⑤③ 33.3%	② 45.0%	②38.6%	② 61.0%	② 50.0%	②58.5%	② 54.1%	② 83.3%
2nd	33.3%	③ 27.5%	⑤27.3%	⑤ 26.8%	③ 25.0%	③17.1%	③ 29.7%	⑧ 16.7%
3rd	② 27.5%	⑤ 15.0%	③15.9%	⑧ 9.8%	⑧ 16.7%	⑧14.6%	⑧ 10.8%	0 %
4th	⑧ 5.9%	⑧ 12.5%	⑧ 9.1%	⑦ 2.4%	⑤ 8.3%	⑤ 9.8%	⑤ 5.4%	0 %

Figure 2. Ideal profile selected by students in each year of dental school, dentists, and orthodontists.

Conclusion

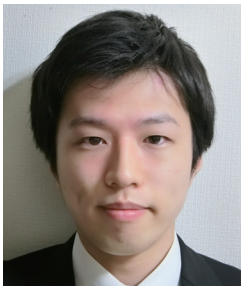
Differences regarding ideal face were noted by students in different years of dental school in accordance with their level of education about orthodontic dentistry. In a future study, we intend to compare past and current ideal values.

Growth differentiation factor-5 expression indicates heterogeneity in epithelial rests of Malassez

東京医科歯科大学歯学部 6年生 Tokyo Medical and Dental University Faculty of Dentistry Class of 2020

渡部 準也 Junya WATANABE

ファカルティー・アドバイザー：田畑 純　硬組織構造生物学分野 准教授
井関 祥子　分子発生学分野 教授



Purpose: Epithelial rests of Malassez (ERM) is epithelial network structure in periodontal membrane. ERM seems to have reproductivity and kinds of functions, but little is known about it. ERM is derived from inner and outer enamel epithelium of tooth germ which characters are different, however the difference is not paid attention. Previous study indicated that ameloblasts, inner enamel epithelium, express Growth differentiation factor 5 (GDF5). This study examines the gene expression of GDF5 in ERM of rat molar in order to explore characteristic and composition of ERM. Our research is admitted by the institutional ethics committee of our university.
Methods: Mandibular molars of 8-week-old Wister rat was sectioned and used for double staining for in situ hybridization (ISH) for GDF5 and immunohistochemistry (IHC) for cytokeratin 14 (CK14). DAPI was used for nuclear staining. The sequence of GDF5 sense and anti-sense RNA probes were examined by subcloning and sequencing. ERM was identified by the morphology and IHC for CK14.
Results and Discussion: In ISH, ERM cells were positive in GDF5 antisense probe. No mRNA signals were detected in sense probe. GDF5 expression was observed in the ERM of rat molar. The levels differed among ERM, and some ERM cells did not express GDF5. This heterogeneity in expression may be because of the difference in origin, inner/outer enamel epithelium.

Growth differentiation factor-5の発現が示すマラッセの上皮遺残の不均一性

目的 マラッセの上皮遺残（ERM）は歯根膜中に存在する網目状の上皮の構造である。ERMは様々な機能や増殖性をもつことが示唆されているが、よく分かっていない。ERMは歯胚の、性質の異なる内外エナメル上皮に由来し、先行研究により内エナメル上皮がGDF5を発現することが示されている。本研究ではERM細胞の構成や性質を探るべく、ラット臼歯のERMにおけるGDF5の発現を検証した。本研究は倫理審査委員会の承認を得て行った。
方法 8週齢ラットの臼歯部顎骨を用いてGDF5の*in situ*ハイブリダイゼーション（ISH）ーサイトケラチン14（CK14）の免疫組織化学（IHC）の二重染色法をおこない、核染色にDAPIを用いた。ISHで用いたRNAプローブは事前にクローニングとシークエンシングを行い、配列を確認した。ERM は形態学的手法及びマーカー蛋白質であるCK14によって検出した。
結果と考察 ISHで、ERMはGDF5アンチセンスプローブにおいて陽性であった。センスプローブではmRNAによる信号は検出されなかった。ラット臼歯のERMはGDF5を発現することが示された。発現強度は細胞によって異なっており、GDF5を発現していないものも存在した。このことはERM細胞が由来の異なる内外エナメル上皮による発生学的な差異と関連している可能性がある。

Growth differentiation factor-5 expression indicates heterogeneity in epithelial rests of Malassez

Problem Epithelial rests of Malassez (ERM) is net-like epithelial structure in periodontal membrane. ERM is considered to take a role in kinds of functions, and have reproductivity. However, the function or reproductivity of ERM is still unknown. It is because characteristic or composition of ERM itself is not well known. Research for ERM itself would lead to solve clinical problems such as origin of dental diseases and development of periodontal regenerative therapy.

Hypothesis ERM derives from enamel epithelium in tooth germs via Hertwig's epithelial root sheath (HERS). Enamel epithelium consists of inner enamel epithelium (IEE) and outer enamel epithelium (OEE). IEE form enamel by producing enamel protein. OEE proliferate rapidly and form outline of tooth germ. During root formation, enamel epithelium transit to HERS. HERS continue to grow during root elongation. Recent researches indicate that the growth of HERS is mainly due to OEE cell proliferation. Also, HERS is known to induce cementum on root surface. This task to secrete enamel protein may be mainly conducted by cells from IEE. Thus, in ERM, there are possibilities that there are two kinds of cells, cells derived from IEE which have roles in function and cells derived from OEE which have roles in proliferation, as same as in tooth germ and HERS.

Methods In order to distinguish cells from IEE/OEE in ERM, I studied GDF5 expression if periodontal membrane of rat. In a previous study, Growth differentiation factor 5 (GDF5) expression is reported in ameloblasts (IEE), stellate reticulum, and periodontal fibroblasts. If two kinds of cells exist in ERM, cells from IEE may express GDF5 and cells from OEE may not. GDF5 is one of BMPs. GDF5 is recently paid attention in periodontal regenerative treatment fields and its efficacy is reported in many pre-clinical and clinical trials.

Tissue preparation Wistar rats (8 weeks old) were fixed with 4 % paraformaldehyde (PFA). Dissected molar parts of the mandible were decalcified in 10% ethylenediaminetetraacetate (EDTA), then embedded in paraffin blocks. Seven-micrometer-thick serial sections were cut in mesial- distal plane. This sample preparation was proceeded in RNase free and preservation of RNA in periodontal membrane tissue was confirmed by using methyl green pyronine (MGP) stain solution (Muto Pure Chemicals, Tokyo, Japan).

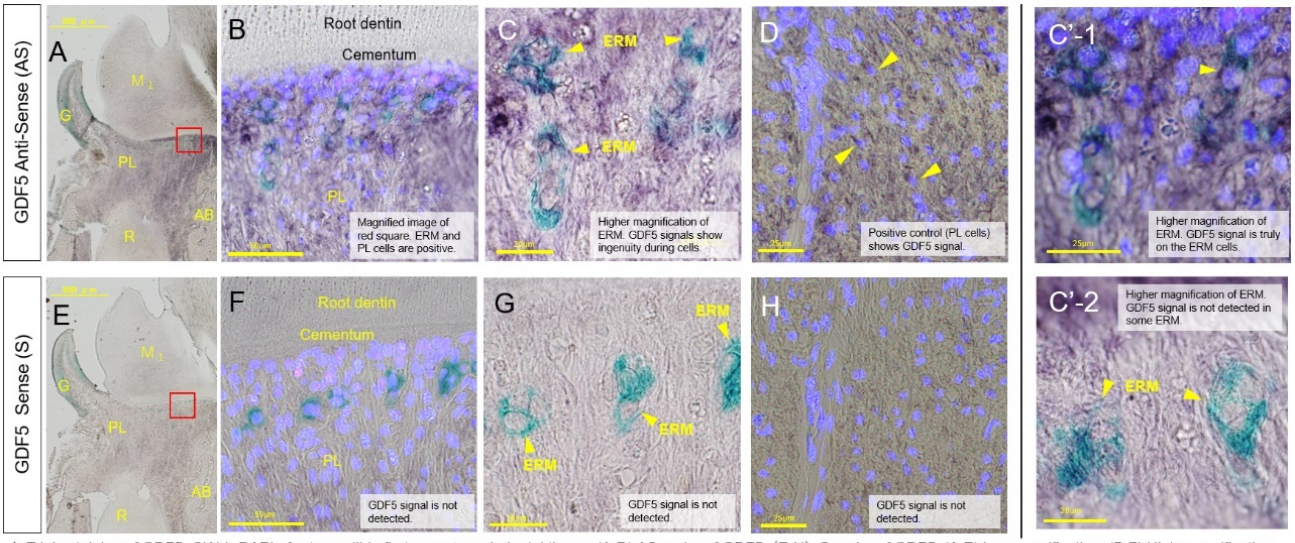
Preparation for Probe Rat GDF5 mRNA probes were made by PCR product with T3/T7 promoter. PCR products were reverse transcript into RNA and labeled with Digoxigenin (DIG) – UTP by DIG RNA labeling Kit (Roche Diagnostic GmbH, Mannheim, Germany). For PCR template, cDNA prepared from extracted RNA of rat liver (negative control) and salivary grands (positive control) samples. The cDNA products were synthesized from total RNA by The Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). The GDF5 primer sets were designed by us and synthesized outsource (Hokkaido System Science, Hokkaido, Japan). Purified DNA subcloned into the pTA2 vector (Target CloneTM, TOYOBO, Osaka, Japan), transformed into competent E.coli, competent high DH5 α (TOYOBO, Osaka, Japan), and cultured. The plasmids including PCR products were purified and sequenced using 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA). Result sequence was confirmed as rat GDF5 sequence by basic local alignment search tool (BLAST).

GDF5 expression by in situ hybridization Sections were deparaffinized and digested with proteinase K (5 μg/mL) (Roche Diagnostics GmbH), post-fixation with 4% PFA. After hybridization, endogenous tissue alkaline phosphatase (ALP) was inhibited using 10mM levamisole. For immunodetection, sections were reacted with a diluted anti-DIG Fab fragment conjugated with ALP (Roche Diagnostics GmbH). We visualized hybridized signals by nitroblue tetrazolium (NBT; Roche Diagnostics GmbH) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP; Roche Diagnostics GmbH).

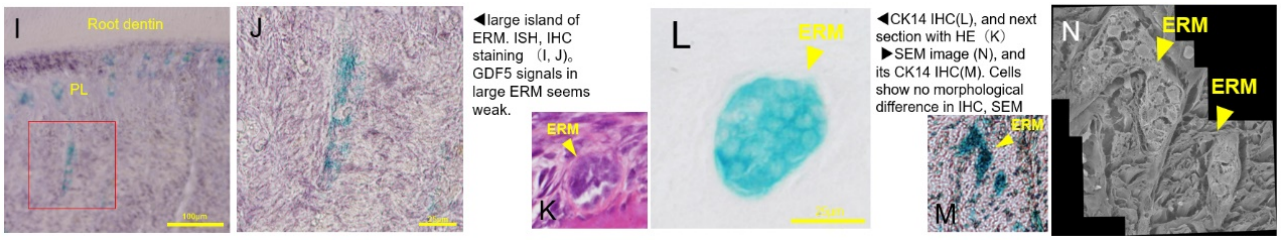
ERM detection by immunochemistry After in situ hybridization, Immunohistological staining was performed by labeled streptavidin-biotin (SAB) method, and color was generated by use of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranose (X-gal) as the substrate for β-gal.

Additional cell detection by Nuclear staining Finally, Nuclear staining was performed by 4′ ,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, MO, USA). The fluorescent images were photographed and were merged to optical images of ISH and IHC.

Results



▲ Triple staining of GDF5, CK14, DAPI of rat mandible first morar's periodontal tissue. (A-D) AS probe of GDF5（E-H） S probe of GDF5. (A,E) low magnification. (B,F) High magnification figure of periodontal tissue around cervical area. DAPI image is merged (C,G) Higher magnification of B,F. (C'-1) C with DAPI. (C'-2) Higher magnification of ERM (D,H) Higher magnification of periodontal membrane without ERM. G: gingiva PL: periodontal ligament R:root AB: alveolar bone



Images in upper line(A-D) is sections treated with antisense GDF5 probe. Purple color development of GDF5 is confirmed in cervical area of periodontal membrane (A). Green colored cells which are ERM cells are detected, and purple color is over the green color of cytoplasm of ERM (C, C'-1). The purple color levels differed among cells and some do not show the purple color (C, C'-2). GDF5 color development is confirmed on fibroblast cells which are positive control of GDF5(D), and color development is not detected in sections with sense probe (E-H). It shows hybridization is done without problem. Large island of ERM seems showing lower color development with antisense probe (I, J). In morphology, heterogeneity among ERM cells is not detected by CK14 IHC (L), and Scanning electron microscope (SEM)(N) observations.

Conclusion GDF5 expression was observed in the ERM of rat molar. The levels of expression differed among cells, and some did not express GDF5. This heterogeneity in expression may be because the difference in cell origin, cells derive from inner enamel epithelium which have roles in function and cells derive from outer enamel epithelium which have roles in proliferation. Some researchers reported that ERM composed of clear and dark cells from Transmission Electron Microscope (TEM) observation. These observed two kinds of cells by TEM might support my conclusion that there are two origins in ERM cells. About ingenuity of GDF5 expression, GDF5 is functional protein and it may be regulated by the necessity. In previous study, GDF5 expression in tooth germ is not even, but high level in lingual cusp. GDF5 expression seemed low in large island may be because population of OEE derived cells are larger than IEE derived cells. Then cell cluster became large by cell proliferation, on the other hand GDF5 expression was low.

Experiments, including sample preparation of rat and artificial recombination of gene to colon bacillus, is admitted by the institutional ethics committee of our university (approval number A2018-211A, G2018-035A).

Forensic considerations for fluorescence changes of tooth colored material due to heating

東北大学歯学部　6年生　Tohoku University School of Dentistry　Class of 2020

古内 聖弓　Satomi FURUUCHI

共同研究者：中村 直太郎（6年生）
ファカルティー・アドバイザー：鈴木 敏彦　歯科法医情報学分野 准教授
小坂 萌　歯科法医情報学分野 助教

Recording dental status for identification could be difficult, especially in the case of charred body. The difference of fluorescence emission between enamel and dental composite resin under UV-LED light are used to judge the presence of dental treatment. However, there are few reports discussing about the heating changes of fluorescence characteristics of tooth-colored products. The purpose of this study was to examine the changes in quantitative fluorescence wavelength and visual fluorescence before and after heating, in four types of tooth-colored products that are widely used in the domestic dental field, assuming the case of the oral cavity of a charred body. Commonly available brands of composite resin and glass ionomer cement were analyzed; heating was performed at temperatures of 200℃, 300℃ and 400℃. Then the waveforms of fluorescence spectrum were measured using spectrometer under a UV-LED light. The fluorescence energy was quantitatively compared by the products of height by half-width value at the peak wavelength. There was a difference in the fluorescence energy between the glass ionomer cement and the composite resin caused by heating. The upper temperature limit for distinguishing tooth-colored materials was thought to be around 300℃. These differences may be an indicator for distinguishing tooth-colored materials after heat treatment, and could be useful for dental identification of charred bodies at the actual fire scene.

法医学的観点における歯冠色成形材料の熱による蛍光変化

歯科的身元確認における口腔内の視認性は照明や対象の状態によって常に良好であるとは限らない。特に焼死体の場合は困難さを増す。歯冠色歯科材料の識別方法としてUV-LEDライトの蛍光を利用する手法が知られているが、加熱時の蛍光性変化についてまとめた報告は少ない。本研究では焼死体の口腔内を想定し、歯冠色歯科材料の加熱前後の視覚的な蛍光変化を蛍光波長測定により定量的に検討することを目的とした。国内製品の充填用コンポジットレジンおよびグラスアイオノマーセメントの円板状試料を、200℃、300℃、400℃で加熱した後、分光計を用いて蛍光波長と蛍光のエネルギーを比較した。その結果、各材料、シェード、温度による肉眼所見および蛍光強度の違いが認められ、歯冠色歯科材料が判別可能な被熱上限温度は300℃前後と推定され、蛍光強度を歯冠色歯科材料の判別に用いる可能性と条件が示唆された。これは実際の歯科的身元確認現場にフィードバック可能な、実践的で有用な情報であると考えられた。

Forensic considerations for fluorescence changes of tooth colored material due to heating

(Problem)

Due to the increasing attention to dental identification in recent years, not only forensic dentists, but also general dentists have more opportunities to fill out a dental chart. It is vital to record the findings of the oral cavity accurately in unusual situations, as compared to routine dental treatment. This can be very difficult sometimes, especially in the case of a charred body, where it is more difficult to distinguish dental findings.

Based on the increasing demand for aesthetic treatment, many dental products with excellent aesthetics, strength, surface smoothness, gloss persistence, and wear resistance have been developed as tooth-colored dental materials. On the other hand, from the viewpoint of dental identification, it is a herculean task to distinguish these superior tooth-colored dental materials from natural teeth. This may lead to oversights or mistakes.

It is well established that there is a difference in the fluorescence spectrum between enamel and dental composite resin under UV-LED light, which is used to judge the presence of dental treatment. Previous studies have reported the fluorescence characteristics of dental products of various companies. However, to the best of our knowledge, there are no reports discussing the heat changes of fluorescence characteristics of domestic tooth-colored products in Japan. Therefore, basic data on the fluorescence characteristics of tooth-colored dental materials under specific environments are currently desirable in the field of forensic dentistry. The purpose of this study was to examine the changes in quantitative fluorescence wavelength and visual fluorescence before and after heating, in four types of tooth-colored products that are widely used in the domestic dental field, assuming the case of the oral cavity of a charred body.

(Hypothesis)

The laboratory with which the presenter is associated has previously reported on the differences in fluorescence wavelength between the several shades of composite resin, and the most suitable commercial UV-LED lights for dental identification. The members of the laboratory routinely engage in dental identification, and the presenter has assisted in dental identification during the training program. According to reports on fire science, the temperature near the floor has been reported to be between 150°C and 200°C even when the room is over 800°C. Since burned bodies are often found on the floor, it is assumed that the temperature of the oral cavity is similar to that near the floor. Furthermore, it has been reported that in most cases of fire in Japan, the fire was extinguished within 20 minutes. According to these reports, the heating conditions in the present study were set.

The null hypothesis of this study was that "the fluorescence of composite resins and glass ionomer cements, which is representative of tooth-colored dental materials, does not change with heating" According to a previous study, it was predicted that organic substances, such as Bis-GMA and TEGDMA, would decompose as the temperature increases, but inorganic substances, such as fillers, would remain in the composite resin. In addition, the color tone and fluorescence intensity were expected to become darker and weaker, respectively. At 400°C, the entire composite resin was expected to get carbonized, such that the fluorescence wavelength could not be measured. Moreover, as the temperature of glass ionomer cements would increase, the liquid components would evaporate and cracks would occur. On the other hand, inorganic components were expected to remain. Additionally, the fluorescence intensity was expected to become brighter due to the evaporation of the dye.

To verify the above hypothesis, the experiments were carried out using the following method.

(Methods)

1. Sample preparation Commonly available brands of composite resin and glass ionomer cement were analyzed: Estelite Universal Flow Medium (Tokuyama Dental) shade A2 and A4, Estelite ∑ Quick (Tokuyama dental) shade A2 and A4, Fuji Ionomer Type Ⅱ pale yellow (GC), and Fuji ionomer Type ⅡLC blue (GC). All samples were prepared according to the manufacturer's instructions. The samples were fabricated in 15.0 mm diameter and 1.0 mm height using a metallic split ring.

2. Heating experiment Heating was performed in an electric furnace (KDF009G, Denken, Japan) at temperatures of 200 °C, 300 °C, and 400 °C. At 200 °C, and 300 °C; the time intervals were set to 10, 20, and 30 min. At 400 °C, the surface of the samples was checked every minute and the samples were removed when the surface was visibly carbonized. Following heating, all samples were placed in a stainless steel tray. After removal from the furnace, the samples were allowed to cool to room temperature. The fluorescence measurement was performed as follows.

3. Measurement of fluorescence wavelength. The samples were set on a black flock paper and the peak wavelength of the fluorescence spectrum was measured using a spectrometer (Qmini spectrometer, RGB photonics, GmbH, Germany) under a UV-LED light. This UV-LED light is easily available and has been reported as a suitable light to distinguish composite resin in our oral presentation. The probe of the spectrometer was covered with an ultraviolet absorption filter (SC-50, FUJI FILTER). The waveforms of the fluorescence were observed, and the peak wavelength, height, and half-width value were measured. The intensity of the fluorescence was quantitatively compared by considering the height × half-width value at the peak wavelength as the energy of fluorescence. The pre-heating fluorescence wavelengths of each material were also measured as a control in the same way.

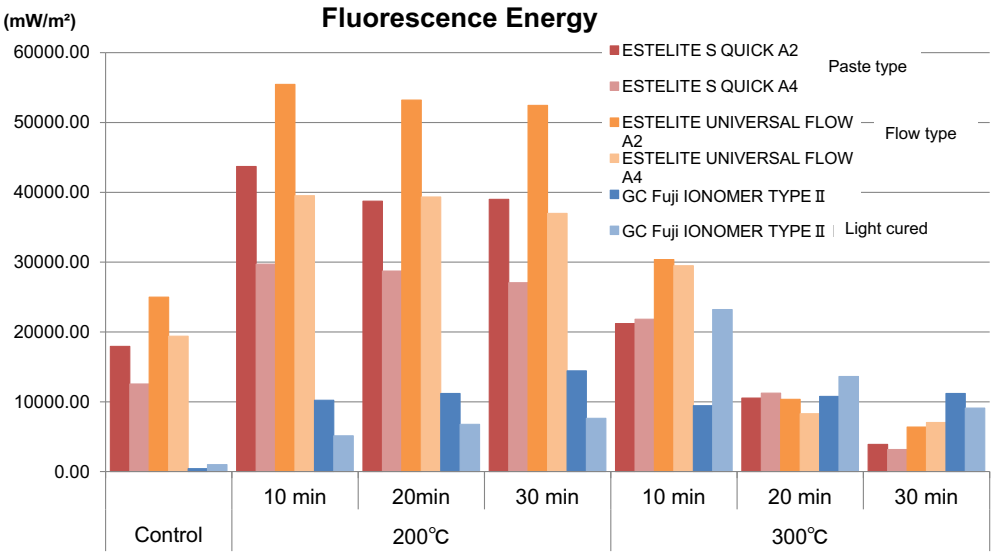
(Results)

There was a difference in the fluorescence energy between the glass ionomer cement and the composite resin caused by heating.

1. Composite resin: At both 200 °C and 300 °C, the fluorescence energy was the highest after 10 min of heating, and reduced as the heating time increased. There were no clear changes in color and fluorescence at 200 °C. On the other hand, at 300℃, the resin samples became browner when the heat duration was long, and the decrease in fluorescence was clearer than at 200 °C. There were blotchy parts on the surface of the 10 and 20 min samples.

Cracks were also observed on the surface of the samples. The cracks of the flow type were smaller than those of the paste type. At 400 °C, all resin samples were burnt black in 2 min, and fluorescence measurement was not possible. In addition, the type and shade of the composite resin affected the fluorescence energy. The fluorescence energy of the flow-type resin was larger than that of the paste type, and shade A2 was larger than that of shade A4.

2. Glass ionomer cement: The fluorescence energy increased with prolonged heating at 200 °C. At 300 °C, there was a difference between the chemically polymerized type and the light-cured type. The fluorescence energy of the chemically polymerized type increased with heating time at 300 °C, but that of the light-cured type decreased. We believe this was because we used a blue colored, light-cured glass ionomer cement. It was inferred that the blue color disappeared after heating at 300 °C, and the whiteness of the sample led to an increase in the fluorescence energy. At 400 °C, the glass ionomer cement did not become black, so weak fluorescence could still be seen.



(Conclusion)

1. The upper temperature limit for distinguishing tooth-colored materials was thought to be around 300 °C.

2. The fluorescence energy differed depending on the material. The composite resin was larger than the ionomer cements. The flow type was larger than the paste type, and shade A2 was larger than A4.

These differences may be an indicator for distinguishing tooth-colored materials after heat treatment, and could be useful for dental identification of charred bodies at the actual fire scene. Further studies are desirable for more versatile data.

Generation of genome-edited mice by *i*-GONAD -challenged by dental school students-

大阪大学歯学部 4年生 Osaka University School of Dentistry Class of 2022

都原 志穂 Shiho TOHARA

共同研究者：中谷 鞠子（4年生）
ファカルティー・アドバイザー：村上 智彦 生化学教室 講師

Recently, an innovative technology for generating knockout (KO) mice, termed *i*-GONAD was developed. The *i*-GONAD enables genome editing to be performed directly in zygotes *in vivo* without special skills (e.g. ES cells, embryo handling, microinjection) and generates KO mice in around 20 days, relieving the bottlenecks of the classical protocols with the special skills. However, *i*-GONAD is a method developed by researchers skilled in genome engineering. Whether *i*-GONAD is actually technically effective for generating KO mice was not yet verified. Targeting *Rankl*, our purpose for this experiment was to verify whether *i*-GONAD can be used to generate KO mice efficiently by students with hardly any genome-editing skills. After training for a month, we performed the *i*-GONAD procedure using 8 pregnant females in total. Four mice delivered a total of 17 offspring. Results of PCR and DNA sequence analyses using genomic DNA from the offspring indicated the success in the disruption of the target region in 5 pups. Out of the 5, tooth eruption occurred in 1 pup, suggesting that it is a *Rankl* heterozygous mouse. Failure of tooth eruption and other phenotypic characteristics of *Rankl* KO mice were observed in the other 4 pups with genomic changes and 2 more pups, even though results of electrophoresis did not show evident changes. Thus, we conclude that *i*-GONAD is a highly effective and useful method for generating KO mice.

i-GONAD法による遺伝子組み換えマウスの作製 ー歯学部生による挑戦ー

近年、*i*-GONAD法と呼ばれるノックアウト（KO）マウス作製法が開発された。*i*-GONAD法は生体内の受精卵に直接ゲノム編集を行う技術で、ES細胞や胚操作などの高度な技術を必要とせず、また約20日でKOマウスを作出できる画期的な技術とされた。一方、*i*-GONAD法は遺伝子改変技術に熟練した研究者らが開発したため、実際にKOマウスの作製に有効であるかは未検証であった。そこで、技術や経験のない歯学部生でも*i*-GONAD法を用いてKOマウスが作製できるか、*Rankl*遺伝子を標的として検証することにした。トレーニングを1ヶ月行った後、合計8匹の妊娠マウスを用いて本実験を行った。うち4匹から仔マウス（合計17匹）が産まれ、それぞれのゲノムDNAを用いてターゲット領域のゲノムPCRと電気泳動を行った。5匹の仔マウスにおいてRANKL遺伝子領域の破壊が確認された。5匹のうち、ヘテロの変異と想定される1匹の歯牙は萌出したが、他4匹と、泳動パターンではゲノム編集が明らかでない2匹の歯牙萌出が障害されていた。以上より、*i*-GONAD法を用いれば、私たち歯学部生でもKOマウスが作製できることを実証した。

Generation of Genome-edited Mice by *i*-GONAD　—Challenged by Dental School Students—

(Problem) Ever since the development of gene targeting technologies using ES cells, knockout (KO) mice have been generated and analyzed. However, generating KO mice was a challenge for many researchers because the process was time-consuming and required special skills such as ES cells, embryo handling, and microinjection. Recently, two innovative technologies that relieve the bottlenecks of classical protocols were developed. The first technology is genome editing, represented by the CRISPR/Cas9 system reported in 2013. This system easily and rapidly makes the direct editing of specific sites within a genome possible. The second technology is *i*-GONAD (improved genome editing via oviductal nucleic acids delivery), reported in 2018. The *i*-GONAD method enables genome editing to be performed directly in zygotes in vivo without specialized skills (Figure 1). However, *i*-GONAD is a method developed by researchers skilled in genome engineering, and many reports state that there are problems with genome-editing efficiency. Whether *i*-GONAD is actually technically effective for generating KO mice was not yet verified.

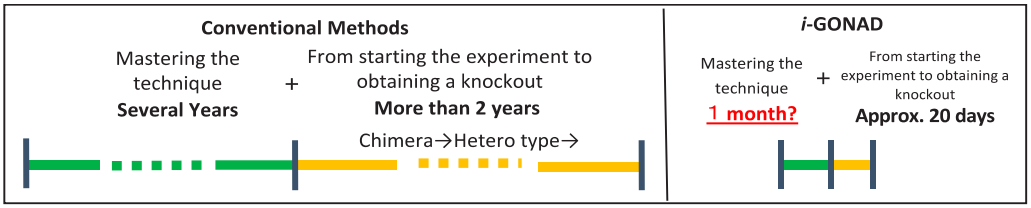


Figure 1　Period to generate knockout mice

(Hypothesis) Our purpose was to verify whether *i*-GONAD can be used to generate KO mice efficiently by students with hardly any genome-editing skills. We targeted *Rankl*, an osteoclast differentiation factor closely associated with dental diseases, in order to generate KO mice by using the *i*-GONAD technique.

(Method)

Gene editing by CRISPR/Cas9 (Figure 2): We injected a genome-editing mixture, containing CRISPR components and two designs of guide RNAs, into the oviduct of a pregnant female and then conducted electroporation of the entire oviduct. The gRNAs were designed to delete the region coding the functional domain (exon 3 and 4) of the *Rankl* gene. All animal experiments were approved by the Animal Experiment Committee of our university and performed in accordance with the regulatory guidelines.

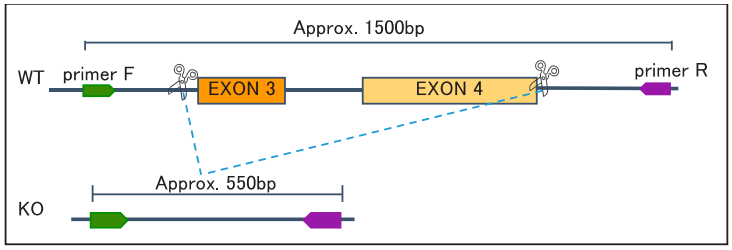


Figure 2　Outline of RANKL gene-targeted genome editing
Scissors represent targeted regions CRISPR will cut、primer F, R represent location of primers for PCR

i-GONAD procedure (Figure 3): *i*-GONAD is a method for generating genetically engineered mice by performing in vivo electroporation of the oviduct to introduce CRISPR genome-editing reagents into zygotes in the ampulla. The method involves the following steps: ①Make a dorsal incision of an anesthetized ICR day-0.7 pregnant mouse. Pull out the ovary by grasping the adipose tissue. Then, anchor the tissue with an aorta clamp. ②By observing under a dissecting microscope, insert the capillary into the lumen of the oviduct and inject the genome-editing solution in the direction of the ampulla. ③Perform in vivo electroporation of the entire oviduct. Repeat the procedure on the other oviduct. ④After completing the procedure on both oviducts, close the skin at the dorsal incision with suture wound clips.
Approximately 20 days after performing *i*-GONAD, we recorded the number of offspring and collected genomic DNA from tissue samples of the newborn mice. We performed PCR using primers illustrated in Figure 2 (primer F, R) and investigated gene editing effects. Because it is expected for *Rankl* KO mice to have a defect in tooth eruption, we observed for tooth eruption at 3 weeks of age.

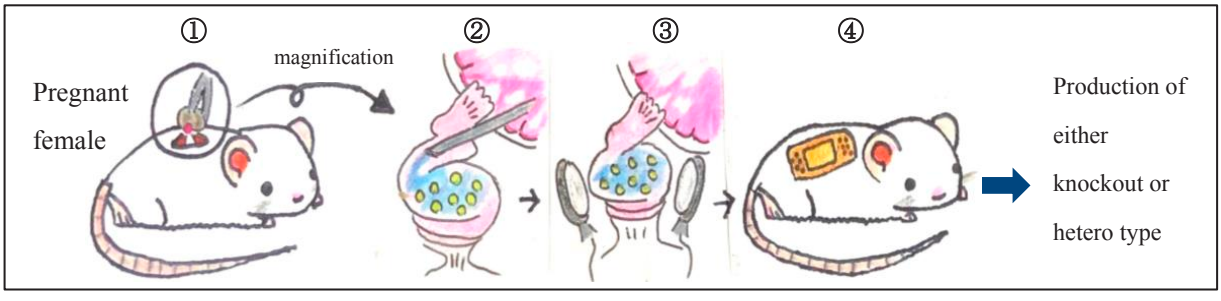


Figure 3　Outline of the *i*-GONAD method

(Results) After training for each step in the *i*-GONAD procedure for a month, we performed the experiment using 8 pregnant females (A-H) in total. The number of offspring recorded for each female is shown in Figure 4A. Mouse ABFG did not deliver by the expected due date, and no fetuses were found in the abdominal cavity the next day. We collected genomic DNA from the offspring of mouse C, D, E and H and performed genomic PCR of the *Rankl* gene sequence (Figure 4B). The electrophoretic patterns framed in yellow clearly have more than 2 bands, indicating the success in the disruption of the target region by genome editing. From these results, genome editing efficiencies were 50% (3/6), 0% (0/1), 16.7% (1/6), 25% (1/4), respectively (Figure 4A). Then, we observed for tooth eruption at 3 weeks of age. Symptoms of severe osteopetrosis reported in *Rankl* KO mice, including the failure of tooth eruption and growth retardation, were observed in mouse C2, C3, C4, C6, E1, E5 and H4 (Figure 4B, D). However, tooth eruption occurred in the rest of the living offspring (Figure 4D). Out of the mice in which gene editing clearly occurred, tooth eruption was observed in mouse H2 (Figure 4B), suggesting that mouse H2 is a *Rankl* heterozygous mouse. Also, the same phenotypic characteristics of *Rankl* KO mice were observed in mouse C3, E1, and H4, even though results of electrophoresis for these mice did not show evident genomic changes. This suggested that either translocation or single/several base pairs of nucleotide deletions occurred, disrupting the *Rankl* gene. Therefore, the exact genome editing efficiencies obtained with the *i*-GONAD method were most likely higher than the efficiencies determined from PCR analysis shown in Figure 4B. Focusing on the offspring of mouse C, we then analyzed the genomic sequence of mouse C2, C4, and C6. Sequencing analysis revealed that genome editing occurred in these mice (Figure 4C). We then determined the oral cavity of mouse C2 by using μCT analysis to examine further details of tooth eruption failure. Incisors and molars of the WT mouse were observed; however, it was clear that no teeth had erupted in mouse C2. Because this characteristic was consistent with the phenotype of *Rankl* knockout mice, we concluded that *i*-GONAD is a highly effective method for generating *Rankl* KO mice.

A.

mouse	number of pups	efficiency of genome editing
A	0	-
B	0	-
C	6	50%
D	1 (0*)	0%
E	6 (5*)	16.7%
F	0	-
G	0	-
H	4	25%

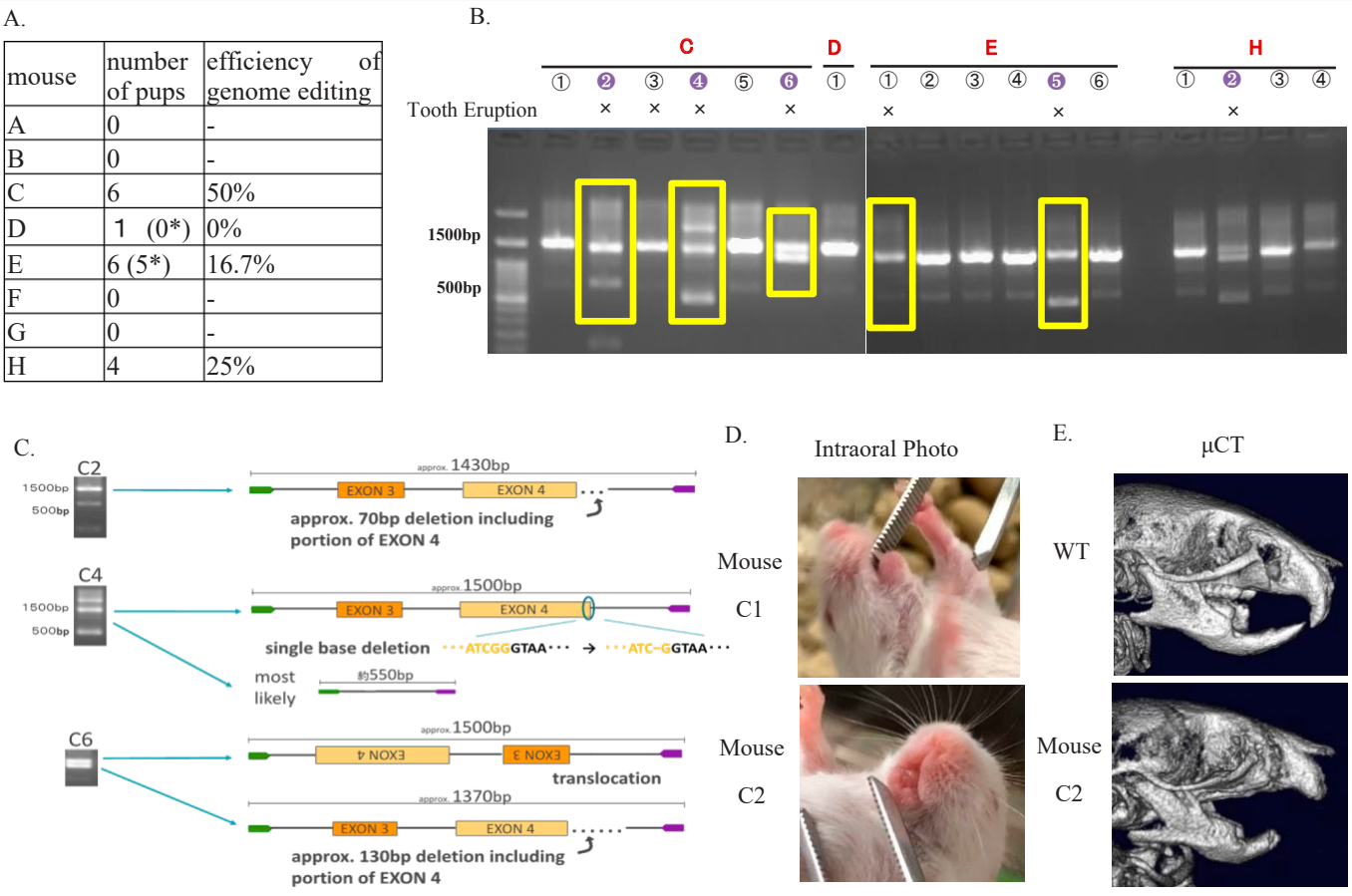


Figure 4 Generation of *RANKL* KO mice by *i*-GONAD

A. Number of off spring and genome editing efficiencies. B. Electrophoresis results of genomic PCR of target region. Lanes in which genome editing clearly occurred are framed in yellow. X indicates mice in which tooth eruption was not observed. C. Theoretical patterns of genome editing from DNA sequencing results. D. Intraoral photos and μCT images. Intraoral photos: Incisors are observed in the oral cavity of mouse C1 but not in mouse C2. μCT: Incisors and molars are both observed in WT mouse. Tooth eruption is not observed in mouse C2.

(Conclusion) By using the *i*-GONAD method, we demonstrated that even undergraduates, with no experience in generating KO mice and hardly any genome-editing skills, can generate KO mice with only a month of practice. Furthermore, this was accomplished in a short period of time and gene editing efficiencies obtained were higher than those in references. We conclude that the *i*-GONAD method is a powerful and effective approach for generating KO mice. Currently, we have succeeded in the breeding of a *Rankl* KO strain from mouse H2. We are planning to investigate the formation of roots when tooth eruption fails using *Rankl* KO mice.

Immunohistochemical localization of DMP-1, FAM20C, and FGF23 during dentinogenesis in comparison with bone

松本歯科大学　6年生　Matsumoto Dental University　Class of 2020

小野 亜美　Ami ONO

ファカルティー・アドバイザー：中村 浩彰　口腔解剖学講座 教授

Recent studies have shown that expressions of DMP-1 and FAM20C start at different stages of odontogenesis. It is also reported that defect in DMP-1 or FAM20C causes the elevation in serum FGF23 level and hypophosphatemia, which could lead to rickets, osteomalacia, or dentinogenesis imperfecta. However, localization of FGF23 during odontogenesis remains unclear. Therefore, I built up the following hypothesis: DMP-1 and FAM20C, which are products of pulp cells including odontoblasts, control FGF23 production and they are responsible for mineralization of hard tissue. The localizations of these proteins during odontogenesis may clarify why dental dysplasia and congenital missing tooth occur. In this study, mice mandible was used as a material. Immunohistochemical localizations of DMP-1, FAM20C and FGF23 during dentinogenesis were investigated. Their localizations in mature molar dentin and bone were also detected to compare with these in incisor. The results show DMP-1, FAM20C, and FGF23 expression at different stages during dentinogenesis, suggesting that there seem to be a time-specific regulating system for expressions of three proteins during dentinogenesis. In addition, FGF23 was positive in molar odontoblasts and dental pulp cells underneath odontoblastic layer. Therefore, mature dentin odontoblasts and dental pulp cells produce FGF23, and production of FGF23 by those cells could be partially responsible for controlling serum phosphate concentration.

象牙質形成過程と骨組織におけるDMP-1、FAM20C、FGF23局在の免疫組織化学的検討

近年、歯の発生過程においてDMP-1、FAM20Cの発現開始時期は象牙芽細胞分化と関連することが示唆されている。また、これらのタンパク異常により血中FGF23が上昇し低リン血症となり、くる病、骨軟化症、象牙質形成不全症をまねくことから、FGF23の調節を介した硬組織の石灰化への関与が考えられている。しかし、象牙質形成過程におけるFGF23局在は明らかではない。そこで、本研究では象牙芽細胞などの歯髄細胞が発現するDMP-1、FAM20CがFGF23産生を調整して硬組織形成に重要な役割を担っているという仮説を立てた。出生後4週齢のマウスの下顎を用いて免疫組織染色を行い、DMP-1、FAM20C、FGF23の役割を明らかにする目的で、象牙質形成過程におけるこれらのタンパクの局在を、成熟象牙質、骨と比較検討した。これらのタンパクの相互の関連は不明であるものの、象牙質形成過程における発現の時期の違いから時期特異的な制御機構があると考えられた。また、FGF23は切歯では象牙芽細胞層の下層に弱い発現が見られ、成熟臼歯象牙質では象牙芽細胞と象牙芽細胞層下層の歯髄細胞に発現が認められた。したがって、成熟した象牙芽細胞や他の歯髄細胞によるFGF23の産生は血中リン酸濃度調節に部分的に関与する可能性が示唆された。

Immunohistochemical localization of DMP-1, FAM20C, and FGF23 during dentinogenesis in comparison with bone

(Problem)

Human dentin occupies the major part of human tooth. Its organic components mainly consist of collagenous and non-collagenous proteins. Although the amount of non-collagenous proteins in mature dentin is significantly small compared to collagenous proteins, deficiencies of genes encoding non-collagenous proteins cause impairment of tooth development. It is also reported that expressions of DMP-1, DSP, and FAM20C start at different stages of odontogenesis. Therefore, it is essential to examine the localization of these proteins during odontogenesis in order to clarify why dental dysplasia and congenital missing tooth occur.

Recent studies show that DMP-1 involves in mineralization of hard tissues via FGF23 regulation. Mutation or deletion of DMP-1 gene induces the elevation of FGF23 concentration and decrease in serum phosphate level, which could lead to rickets, osteomalacia, or dentinogenesis imperfecta. However, localization of FGF23 during odontogenesis remains unclear.

(Hypothesis)

Odontoblasts and ameloblasts as well as osteocytes are reported to produce FGF23. Additionally, FGF23 KO mice shows hyperphosphatemia, which results in ectopic mineralization. Moreover, defect in DMP-1 or FAM20C causes the elevation in serum FGF23 level and hypophosphatemia. Therefore, I built up the following hypothesis: DMP-1 and FAM20C, which are products of pulp cells including odontoblasts, control FGF23 production, and they are responsible for mineralization of hard tissue.

(Methods)

In this study, incisors of mice were used as materials because the process of odontoblast differentiation is observable in a single incisor (Fig.1). Mice mandible was fixed with 4% paraformaldehyde at postnatal days (P)28. Samples were embedded in paraffin and sectioned in the thickness of 4 μ m. Immunohistochemical localizations of DMP-1, FAM20C and FGF23 during dentinogenesis were investigated. Their localizations in mature molar dentin and bone were also detected to compare with those in incisor.

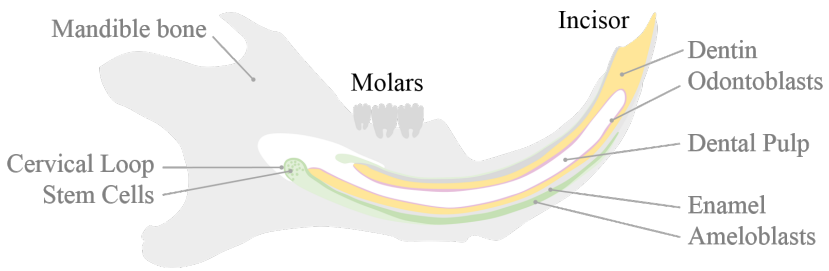


Fig. 1 Mice incisor

The process of odontoblast differentiation is observable in a single incisor because stem cells in the root continuously supply cells, which are needed for tooth formation.

Ethical codes ✓This study was approved by The university research ethics committee (Permission number: 299)

(Results)

The results of immunohistochemical stainings for DMP-1, FAM 20 C, and FGF 23 are shown in Fig.2: during dentinogenesis, mature molar dentin and bone. At the early stage of dentinogenesis, localizations of DMP-1, FAM20C, and FGF23 were rarely detected. At the middle stage of dentinogenesis, the immunoreactivity for DMP-1 was positive both in odontoblasts and peritubular dentin. FAM20C was localized in Golgi area of odontoblasts. Additionally, weak immunoreactivity for FGF23 was observed underneath odontoblastic layer. The expressions of DMP-1 and FAM20C at the late stage of dentinogenesis were seen at the same area as the former stage, and their expressions were intense. FGF23 was also positive under odontoblastic layer. In bones, DMP-1 showed strong expressions both in osteocytes and bone matrix. FAM20C and FGF23 were seen in some osteocytes. These results suggest that phosphorylation of DMP-1 by FAM20C is done in Golgi apparatus and FGF23 is produced by osteocytes as previously reported. In mature molar dentin, DMP-1 was positive both in odontoblasts and peritubular dentin. FAM20C was detected in odontoblasts. However, localization of FGF23 in molar was different from that in incisor. FGF23 in molar was found in odontoblasts and underneath odontoblastic layer, indicating that not only osteocytes but also mature dentin odontoblasts are likely to produce FGF23. Moreover, expressions of DMP-1, FAM20C, and FGF23 during dentinogenesis and mature molar dentin suggest that productions of these proteins are likely to be controlled for a certain period of time.

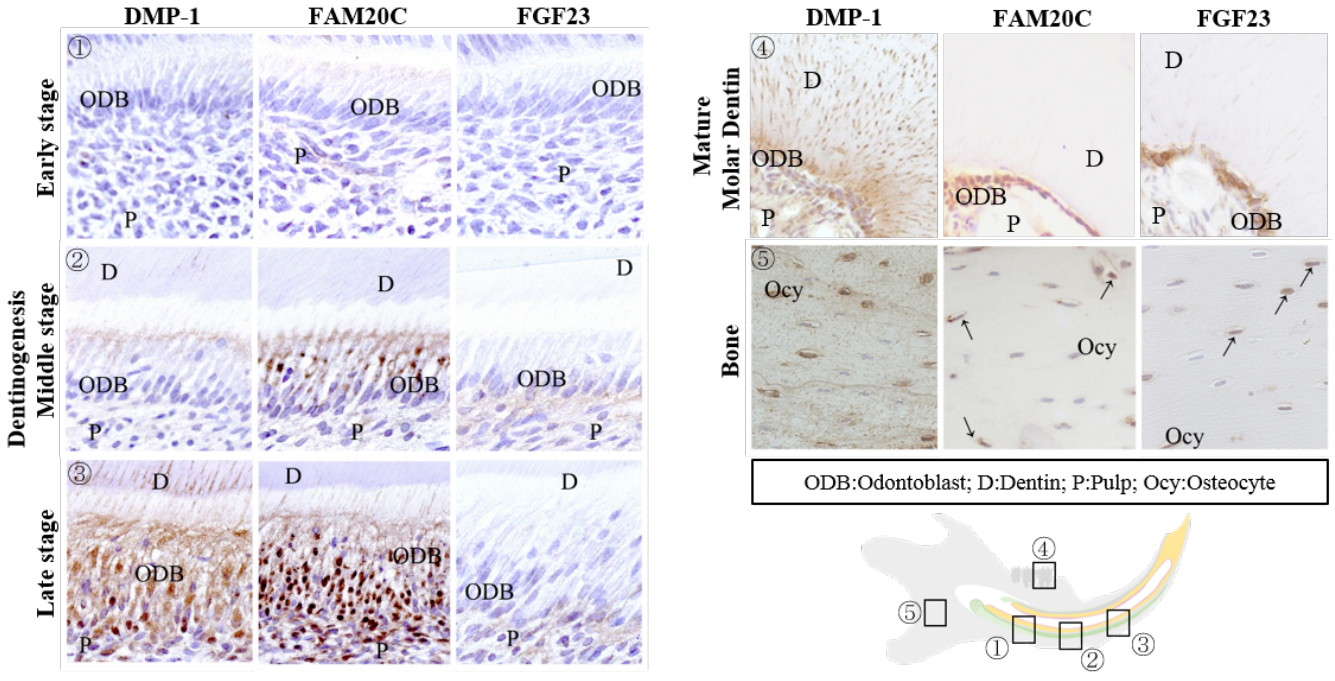


Fig.2 Immunohistochemical localization of DMP-1, FAM20C, and FGF23 during dentinogenesis (early, middle, and late stages), mature molar dentin and bone

(Conclusion)

DMP-1, FAM20C, and FGF23 expression at different stages during dentinogenesis, suggesting that there seem to be a time-specific regulating system for expressions of three proteins during dentinogenesis. In addition, FGF23 was positive in molar odontoblasts and dental pulp cells underneath odontoblastic layer. Therefore, production of FGF23 is done by mature dentin odontoblasts or other dentin pulp cells, and it could be partially responsible for controlling serum phosphate concentration.

An analysis of the genes related to the survival of biofilm bacteria

徳島大学歯学部 5年生 Faculty of Dentistry Tokushima University Class of 2021

喜田 悠太 Yuta KITA

ファカルティー・アドバイザー：藤猪 英樹 口腔微生物学分野 教授

Biofilm bacteria are known to cause chronic infection, even though they are sensitive to antibiotics. Recent years, "Antibiotic Tolerance" has been attracting attention, but the mechanism are still unclear. In this study, we focused on the PA2384 gene and analyzed its role in order to elucidate the mechanism of antibiotics tolerance using *Pseudomonas aeruginosa*, a typical biofilm bacteria. We found the expression of the PA2384 gene was significantly induced in the biofilm cells of *P. aeruginosa* wild-type strain PAO1. In biofilm cells killing assay against biapenem, the survival rate of the PA2384 over-expression strain was significantly higher than that of the vector control strain, in contrast the PA2384 gene deletion mutant strain was previously reported to show significantly reduced antibiotic tolerance in the biofilm cells killing assay. The phenotypes of the PA2384 over-expression strain showed that flagellar motility was suppressed and the production of the virulence factors, pyocyanin, pyoverdine were significantly reduced. Our data suggest that PA2384 would be a key player which can lead to chronic infections through these functions in *P. aeruginosa*. PA2384 is a potential target of new drugs against *P. aeruginosa* chronic infection caused by biofilm.

バイオフィルム細菌の生存戦略に関わる遺伝子の解析

細菌がバイオフィルムを形成した場合、適切な抗菌薬を使用しても十分な効果が得られず、慢性化することから問題となることが多い。この原因として抗菌薬抵抗性が重要であると考えられているが、そのメカニズムは不明な点が多い。本研究ではバイオフィルム細菌として代表的な緑膿菌を用い、抗菌薬抵抗性のメカニズムを明らかにすることを目的として、PA2384遺伝子に着目し、その役割について解析した。緑膿菌標準株PAO1においてPA2384遺伝子はバイオフィルム形成時に発現が誘導されることが分かった。これまでにPA2384遺伝子欠損株でバイオフィルム形成時に抗菌薬抵抗性が低下することを報告してきたが、今回PA2384遺伝子強発現株を用いて抗菌薬抵抗性を調べたところ、バイオフィルム形成菌ではカルバペネム系抗菌薬のビアペネムに抵抗性を示した。また強発現株では鞭毛運動が抑制され、病原因子のピオシアニン、ピオベルディンの産生が有意に低下した。これらの結果よりPA2384遺伝子の発現は病原因子産生を抑制し、バイオフィルム形成時の抗菌薬抵抗性に重要な役割を果たすことが示された。以上よりPA2384遺伝子はバイオフィルム形成による慢性難治性疾患治療の標的として有望であることが示された。

An Analysis of the Genes Related to the Survival of Biofilm Bacteria

[Problems]

Biofilm bacteria are known to cause chronic infection, even though they are sensitive to antibiotics. In recent years, “antibiotic tolerance”, which is a phenomenon where bacteria cannot grow in the presence of antibiotics but does not die, has been attracting attention as a cause thereof. Antibiotic resistance is a typical survival strategy for bacteria exposed to antibiotics, which is based on gene mutation or acquisition of resistant genes. Unfortunately, the mechanism of antibiotic tolerance are still unclear.

We previously reported that the PA2384 gene is related to antibiotic tolerance by transposon mutagenesis in *Pseudomonas aeruginosa*, a typical biofilm bacteria, and the PA2384 deletion mutant strain shows significantly reduced antibiotic tolerance in biofilm cells.

By the genome information, the PA2384 gene is a small size DNA consisting of operon, but its function is unclear.

[Hypothesis]

We hypothesized that PA2384 was induced in biofilm and played an important role in antibiotic tolerance of biofilm cells, and this would allow the biofilm cells to escape the immune system and cause chronic infections.

[Method]

To clarify the function of this gene, we generated the PA2384 over-expression strain and the vector control strain using the *P. aeruginosa* wild-type strain PAO1.

For biofilm cells killing assay, we used peg biofilm method. A 96-well plate was filled with bacterial culture, and by inserting the pegs in the wells and shaking the plate for 24 hours, biofilm was formed on the surface of the pegs. We exposed it to biapenem, a carbapenem antibiotics, for 24 hours. The biofilm was then detached using sonication, and viable cells were counted.

Pyocyanin and pyoverdine production were confirmed by these colors using natural light or Blue LED after 24 hour incubation.

Flagellar motility was measured by spotting these strains on 0.3% LB culture plates and incubating them for 24 hours and measuring the diameter of the colonies.

The expression of the PA2384 gene was measured by real-time PCR. The biofilm cells which were incubated 24 hours using peg biofilm method, and the planktonic cells which were incubated 24 hours, these mRNAs were extracted and measured by real-time PCR after reverse transcription.

[Result]

The expression of the PA2384 gene was significantly induced in the biofilm cells of PAO1 after 24 hours of incubation compared to the planktonic cells. (Fig.1)

In biofilm cells killing assay against biapenem, the survival rate of the PA2384 overexpression strain was about 40 times higher than that of the vector control strain, although the biofilm-forming ability remained unchanged. (Fig.2)

So, PA2384 plays an important role during biofilm to further enhance antibiotic tolerance.

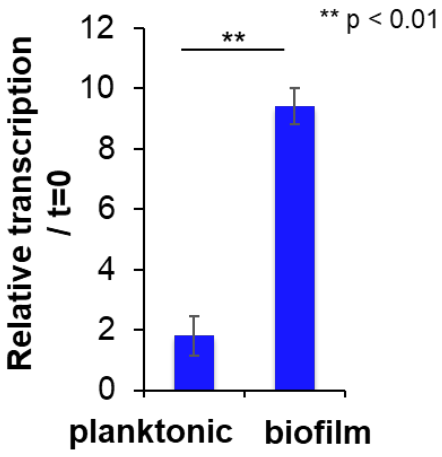


Fig. 1 Gene Expression of PA2384 in PAO1

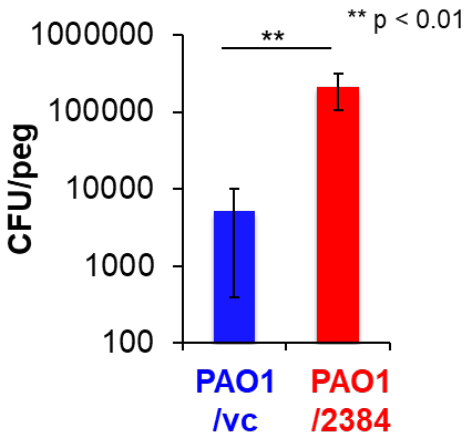


Fig. 2 Effect of PA2384 Over-expression on Bacterial Killing

The phenotypes of the PA2384 overexpression strain showed that the production of virulence factor, pyocyanin, pyoverdine were significantly reduced. Moreover flagellar motility, which is involved in pathogenicity, was significantly suppressed. (Fig3)

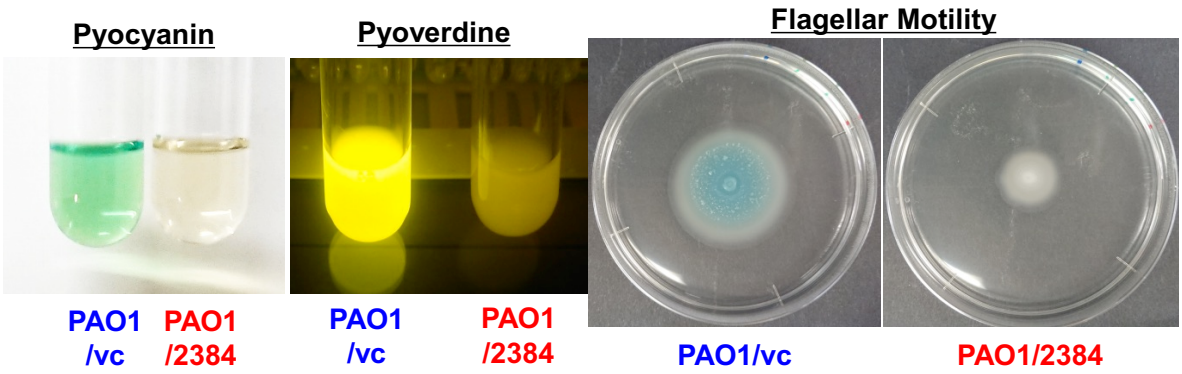


Fig.3 Effect of the PA2384 Over-expression on Virulence

[Conclusion]

The PA2384 gene is induced in biofilms, and increased antibiotic tolerance during biofilm formation, and decreased the expression of virulence factors. PA2384 would be a key player which can lead to chronic infections through these functions in *P. aeruginosa*.

PA2384 is a potential target of new drugs against *P. aeruginosa* chronic infection caused by biofilm.

Evaluation of cell differentiation and proliferation potential using neural network

岡山大学歯学部　4年生　Okayama University Dental School　Class of 2022

村田 志穂　Shiho MURATA

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

「Hopfield Network」is a new analysis method that evaluates the differentiation and proliferation ability of each cell as "cell potential" objectively. This method seems to be useful to analyze the mechanisms of cell development and differentiation, as well as to evaluate and control the quality of stem cells used in regenerative medicine. In this study, we analyzed the "cell potential" of monocytes, macrophages, and osteoclasts inherent in the ribs of the living body using this new method. For analysis, we used the gene expression profiles of single types of cells obtained from an open database “Mouse Cell Atlas”. Analysis of Hopfield network showed no significant difference in the energy averages of three types of cells. The results also showed that there is a wide range of difference in the monocyte potential *in vivo*. Until now, only *in vitro* samples of these types of cells have been utilized in Hopfield network analysis, and this study was the first to be analyzed using *in vivo* data. The results of this study suggest that "cell potential" is considerably different in *in vitro* and *in vivo* environments, and it is thought to be useful for objectively evaluate the specificity of cells in tissues.

ニューラルネットワークを使用した細胞の分化増殖能の評価

ニューラルネットワークの一種であるホップフィールドネットワークは，細胞個々の分化増殖能を「細胞のポテンシャル」として数値化し，客観的に評価する解析手法である。この解析手法は，細胞の発生や分化のメカニズム解析，さらには再生医療に用いる幹細胞の評価や品質管理ができる可能性を秘めている。我々は今回，この解析手法を用いて生体内の肋骨に存在する単球・マクロファージ・破骨細胞のポテンシャルを解析した。解析材料には、オープンソースの“マウス細胞アトラス”の単一細胞データを使用した。これまでホップフィールドネットワーク解析では，*in vitro*試料の単球・マクロファージのみが解析されており、これら3種類の*in vivo*試料を用いた解析は本研究が初めてである。これら3種類の細胞のポテンシャルの数値において比較を行ったところ，有意な差は認められなかった。さらに、生体内の細胞のポテンシャルには大きなばらつきがみられた。本研究結果は*in vitro*，*in vivo*環境下では細胞のポテンシャルが異なることを示唆しており，生体内の各組織に存在する細胞の特異性を客観的に判断する上で有用と考えられる。

Evaluation of Cell Differentiation and Proliferation Potential using Neural Network

(Problem)

The human body is composed of cells with various differentiation stages: from embryonic- and hematopoietic- stem cells (high differentiation/proliferation ability) to nerve cells and cardiomyocytes (low differentiation/proliferation ability). It is extremely important to quantify and evaluate the differentiation/proliferation ability of individual cells as "**Cell potential**" for understanding the mechanism of cell development and differentiation and evaluating the quality of stem cells used in regenerative medicine.

A "**Hopfield Network**", which is a type of neural network, has been recently developed as an evaluation method for cell differentiation and proliferation ability. In the Hopfield Network, the energy value is high in undifferentiated and proliferative cells and low in terminally differentiated cells, therefore, the "Cell potential" is expressed as a numerical scale.

Monocytes, macrophages, and osteoclasts are derived from hematopoietic stem cells. So far, analysis using *in vitro* samples showed that there is a significant difference in the "Cell potential" between monocyte/macrophage lineage cells according to their differentiation stages. However, the "Cell potential" of monocytes, macrophages, and osteoclasts inherent in the living body has not been analyzed. In this study, we analyzed the "Cell potential" of monocytes, macrophages, and osteoclasts existing in the ribs *in vivo* using the Hopfield Network.

(Hypothesis)

Y: There is significant difference in the "Cell potential" among monocytes, macrophages, and osteoclasts present in bone *in vivo*, like *in vitro* samples.

N: There is no significant difference in the "Cell potential" among monocytes, macrophages, and osteoclasts present in bone *in vivo*, unlike *in vitro* samples.

(Methods)

For analysis, we utilized the single cell data (GSE108097, scRNA-seq) of "Mouse Cell Atlas" from NCBI Gene Expression Omnibus (GEO), which has provided an open source gene expression database. We used the sample data of monocytes, macrophages, and osteoclasts isolated from the ribs of neonatal mice.

In order to confirm that the cells were sampled appropriately, we compared the expression levels of representative genes in each cell type with the gene expression data. We also evaluated the "Cell potential" of individual types of cells using the Hopfield Network.

(Results)

1. Expression levels of representative genes (table 1)

The numerical value (logFC*) is strongly related with the specificity and quantity of gene expression, and the positive value indicates that the expression of the interest gene is higher than that in other cell types.

The osteoclast-specific genes Nfatc1 and Dcstamp were specifically expressed in osteoclasts, whereas the pattern recognition receptor gene Tlr2 (immunocompetent cell-specific) was expressed in monocytes and macrophages, but not in osteoclasts. In addition, the gene Csf1r, which is an important factor in the process of differentiation from monocyte to osteoclast, was expressed in all three types of cells. These results support that the sampling of cells was properly performed.

Table 1

Gene	Monocyte	Macrophage	Osteoclast
Nfatc1	—	—	0.9183
Dcstamp	—	—	1.5408
Tlr2	1.6099	0.6198	—
Csf1r	0.8929	1.9582	1.3655

*Log FC: logarithmic change ratio. Calculated $\log_2$ (sample/control).

Ex) 2 = $\log_2(4)$: The expression level of the gene is 4 times higher in the sample than in the control.

2. The Hopfield network energy values of monocytes, macrophages, and osteoclasts (Table 2, Figure 1).

Welch's t-test was used to determine whether there were any significant differences among the mean energy values of the three cells. (Bonferroni's correction, $P < 0.0167$). There was no significant difference in the mean values (Figure 2). This result indicates that there is no significant difference in the "Cell potential" of monocytes, macrophages, and osteoclasts in bone.

In addition, large variations in Hopfield Network energy were observed in monocytes (Figure 2). It is considered that the potential difference between individual cells was quantified and expressed as the difference in energy value. This result suggests the presence of heterogeneity in monocytes in bone.

Table 2

Cell type	Number of samples	Hopfield network energy (Average)
Monocyte	47	-2,385,439
Macrophage	24	-2,399,924
Osteoclast	7	-2,344,338

Figure 1

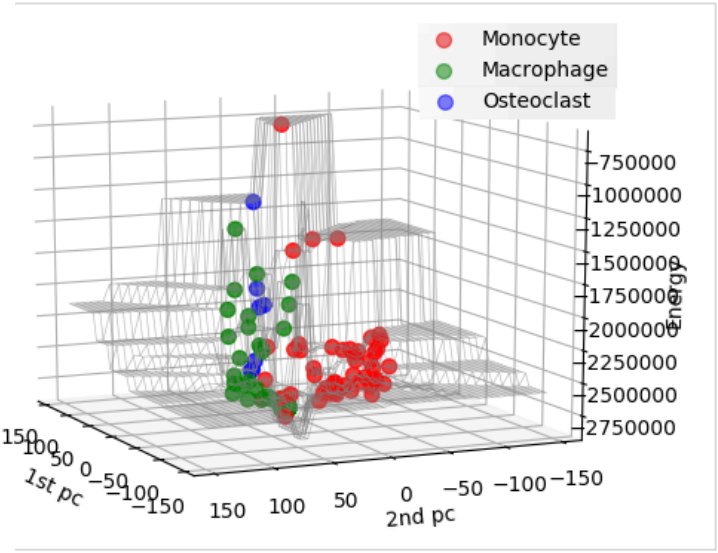
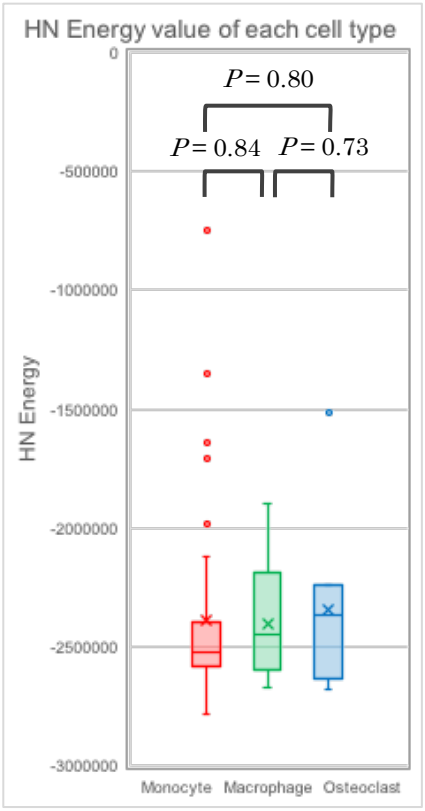


Figure 2



(Conclusion)

Unlike the result of analysis used *in vitro* samples, the analysis used *in vivo* samples showed that the “Cell potential” of monocyte, macrophages, and osteoclasts is comparable. These results indicate that the evaluated “Cell potential” of monocyte, macrophages, and osteoclasts is quite different between *in vitro* and *in vivo* condition. The results of this study will provide important notice that analysis using “Hopfield Network” should be carefully done with consideration of the condition of the obtained samples. Improvement of the analysis using neural network system would be very useful to establish a method for selecting stem cells from tissues for regenerative medical treatment.

Trehalose suppresses osteoclast formation

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

根岸 宗一郎 Soichiro NEGISHI

ファカルティー・アドバイザー：吉村 健太郎 口腔生化学講座 講師

It is well known that dietary sugars are involved in the pathogenesis of dental caries. On the other hand, the role of sugars in that of periodontal disease has not been fully clarified. We are interested in the effects of dietary sugars on alveolar bone resorption in periodontitis. In this study, we examined the effects of various monosaccharides and disaccharides on the differentiation of mouse bone marrow macrophages into osteoclasts induced by RANKL *in vitro*. Trehalose, composed of two glucose moieties with α1→α1-glycosidic bond, strongly suppressed the osteoclast differentiation through inhibition of the expression of *Nfatc1*, the gene for the master transcription factor of osteoclast differentiation. On the other hand, the same concentration of mannitol, a sugar alcohol that clinically used for modulation of osmolarity of the body fluid, did not affect the osteoclast differentiation, indicating that the suppressive effect of trehalose on the osteoclast differentiation was not due to the change in osmolarity. Trehalose is a palnt-derived edible sugar widely used for preparation of various foods including sweets, and its safety has been confirmed. We believe that trehalose is a promising candidate of the novel therapeutic or prophylactic agent for periodontal bone resorption.

トレハロースは破骨細胞形成を抑制する

糖質が齲蝕の発症に関わる事がよく知られている一方、歯周病の病態へ糖質の関与は不明な点が多い。我々は、歯周病における歯槽骨吸収に対する種々の食用糖質の影響に興味を持った。そこで、RANKLよって誘導されるマウス骨髄マクロファージの破骨細胞分化にたする種々の単糖類および二糖類の効果を*in vitro*培養系で解析した。その結果、グルコースどうしがα1→α1グリコシド結合した、植物由来の二糖であるトレハロースが、破骨細胞分化のマスター転写因子NFATc1の遺伝子発現を低下させることで、破骨細胞分化を強力に抑制することを見出した。その他の単糖や二糖には、有意な破骨細胞分化抑制作用はなかった。また、同じ濃度のマンニトールにも破骨細胞分化抑制作用が認められなかったことから、トレハロースの破骨細胞分化抑制作用は、浸透圧の変化によるものではないと考えられた。トレハロースは、様々な食品に添加されており安全性が確立していることから、新しい骨吸収抑制剤の候補物質となる事が期待される。

Trehalose Suppresses Osteoclast Formation

(Problem) Dental caries and periodontal disease are the two major diseases that worsen our dental health. The role of sugars in the pathogenesis of dental caries can be relatively simply explained. Oral bacteria such as *Streptococcus mutans* metabolize various dietary sugars and produce organic acids that cause decalcification of the teeth. On the other hand, the role of dietary sugars in the development of the periodontal disease has been poorly documented. Periodontal bacteria not only directly destroy our cells but also they and their components such as lipopolysaccharide stimulate our inflammatory and immune systems, which induces inflammation in the periodontal tissue. Inflammation in the periodontal tissue causes the loss of alveolar bones via induction and activation of osteoclasts, a type of cells specialized for bone resorption. Clinical control of differentiation of osteoclasts in periodontal disease is an important issue for the quality of life of the patients. What we are interested in is whether or not edible sugars affects differentiation of osteoclasts. If some of the sugars promote it, we can pay attention to the intake of such sugars. If some sugars suppress osteoclast differentiation, we will have an opportunity to develop a novel method to control alveolar bone loss in periodontal disease. In this study, we investigated the effects of various sugars on the differentiation of osteoclasts *in vitro*.

(**Hypothesis**) Our hypothesis is that some of the dietary sugars would up-regulate or down-regulate osteoclast differentiation. Especially, we expected to find a specific sugar or sugars that can negatively control osteoclast differentiation.

(**Methods**) We used an *in vitro* culture system to evaluate the differentiation of macrophages into osteoclasts. Bone marrow cells were obtained from the femurs and tibias of 5- to 6-week male ddY mice. Bone marrow cells were cultured for 3 days in the medium supplemented with 10% fetal bovine serum and 4000 units/mL of M-CSF (macrophage-colony stimulating factor) to propagate the macrophage fraction (bone marrow macrophages). Bone marrow macrophages were collected by trypsinization, plated in 96-well culture plates at a density of 1×10^4 cells/well, and further cultured for 3 days in the same medium containing 4000 units/mL of M-CSF and 100 ng/mL of RANKL (receptor-activator of NF-kappa B ligand) to induce their differentiation into osteoclasts. RANKL is a cytokine required for differentiation, activation, and survival of osteoclasts. Sugars were added to the cultures of bone marrow macrophages in the presence of M-CSF and RANKL to examine the effects of the sugars on the differentiation of macrophages into osteoclasts. We performed the following three experiments.

(1) Experiments to examine the effects of various monosaccharides and disaccharides on osteoclast differentiation.
The effects of a fixed concentration (25 mmol/L) of monosaccharides (D-glucose, D-fructose, D-galactose, and D-mannose), disaccharides (maltose, lactose, sucrose, and treharose), and D-mannitol, the sugar alcohol of D-mannose clinically used for regulation of osmotic pressure of body fluids, on osteoclast differentiation were examined. Differentiation of osteoclasts was evaluated by activity staining of TRAP (tartrate-resistant acid phosphatase), a marker enzyme of osteoclasts. TRAP-positive multinucleated cells are regarded as osteoclasts.

(2) Experiments to examine the concentration-dependent effects of trehalose and mannitol on osteoclast differentiation.
In the first experiment (1), trehalose strongly suppressed osteoclast differentiation. Hence, we examined the concentration-dependent effect of trahalose on osteoclast differentiation using the same experimental system described above. Trehalose at the concentration of 0, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, or 100 mmol/L was added to the cultures. The effect of the same concentrations of mannitol was also examined to exclude the possible effect of the change in osmolarity by the addition of treharose.

(3) Experiments to examine the effects of treharose, sucrose, and maltose on the expression of NFATc1, the master transcription factor for osteoclast differentiation.

It is known that up-regulation of the expression of NFATc1, the master transcription factor of osteoclast differentiation, is the first step of the differentiation of macrophages into osteoclasts induced by RANKL. Hence, we examined the effects of several disaccharides (trehalose, sucrose, and maltose) on the expression of *Nfatc1* mRNA by real-time RT-PCR (reverse transcription-real-time polymerase chain reaction) using that of *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase) mRNA for normalization of RNA extraction. Total RNA was extracted from the cells cultured for 48 hours in the presence of RANKL and the sugars (25 mmol/L) using TRIzol reagent (Thermo Fisher Scientific).

(Results) As described below, we found that trehalose, a disaccharide consisting of two α-D-glucose units with α1→α1 glycosidic bond, suppresses differentiation of macrophages into osteoclasts induced by RANKL.

(1) Trehalose suppressed osteoclast differentiation.

Among the dietary sugars tested, trehalose (25 mmol/L) almost completely suppressed the formation of osteoclasts. Even though statistically insignificant, sucrose tended to reduce the number of osteoclasts. The other sugars did not affect the formation of TRAP-positive multinucleated cells.

(2) Treharose but not mannitol efficiently suppressed osteoclast differentiation.

Trehalose at 5 mmol/L or higher suppressed osteoclast differentiation in a concentration-dependent manner (Fig. 1). On the other hand, mannitol at 50 mmol/L or lower did not affect it. Hence, suppressive effect of trehalose on osteoclast differentiation cannot be explained by change in osmolarity.

(3) Trehalose suppressed the expression of *Nfatc1* gene.

Trehalose significantly suppressed the expression of *Nfatc1* mRNA. Sucrose showed a tendency to suppress it, but the effect was not statistically significant. Maltose, a disaccharide consisting of two α-D-glucose units with α1→4 glycosidic bond, did not affect the expression of *Nfatc1* mRNA.

(Conclusion) We found that trehalose suppresses osteoclast differentiation (Fig. 2). Tehalose is a plant-derived dietary sugar widely used for preparation of cakes and Japanese sweets. It is also drawing attention for its phamacological activities such as suppression of blood sugar level and prevention of neurodegenerative disease. Its high safety to human body seems preferable for development of anti-bone resorptive medicine. Trehalose, as described above, is a disaccharide consists of two α-D-glucose units with α1→α1 glycosidic bond. The ring structure of each glucose unit of trehalose does not open because the anomer carbons of both glucose units are connected by glycosidic bond. The results suggest that α-D-glucose moieties with α1→α1 glycosdic bond is important for suppression of osteoclast differentiation. It is also known that trehalose lipids produced by some kind of bacteria stimulate monocytes and macrophages. We would like to continue this study to elucidate the mechanism by which trehalose inhibits osteoclast differentiation.

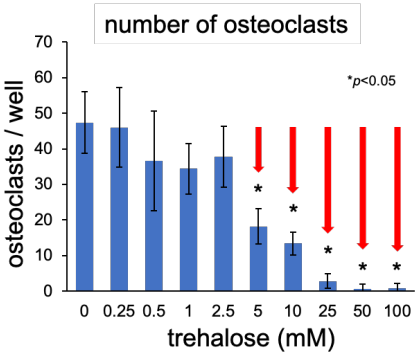


Fig. 1. trehalose suppressed osteoclast differentiation in a concentration-dependent manner.

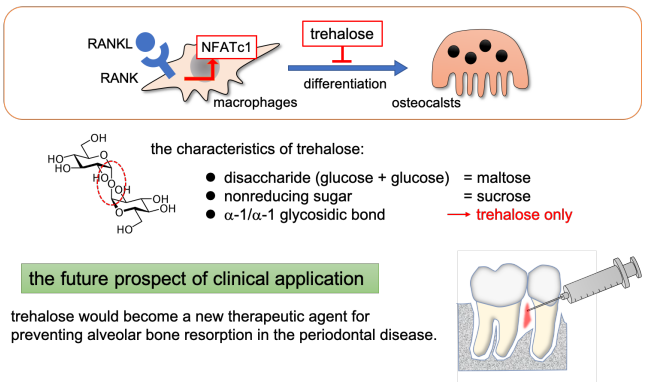


Fig.2. trehalose suppressed osteoclast differentiation *in vitro*.

Screening and identification of human oral bacteria that inhibit growth of periodontal pathogenic bacteria

福岡歯科大学　5年生　Fukuoka Dental College　Class of 2021

池本 梨央南　Riona IKEMOTO

共同研究者：畳屋 有希（5年生）　中村 麻衣（3年生）
ファカルティー・アドバイザー：稲井 哲一朗　機能構造学分野 教授
田中 芳彦　感染生物学分野 教授

The human oral cavity consists of over 600 bacterial species and has well-balanced microbiota. However, dysbiosis of the balanced oral microbiota due to the increase of periodontal pathogens is associated with periodontal disease. Oral microbiota make various symbiotic relationships with microorganisms. It has been known that some bacteria in the oral cavity produce inhibitory compounds to compete with other bacteria and grow dominantly in the oral cavity. We hypothesize that the oral microbiota of healthy human contains specific bacteria that exhibit competition against periodontal pathogen *Porphromonas gingivalis*. The aim of this study is to screen and identify the candidate bacteria derived from dental plaque of healthy human that inhibit the growth of *P. gingivalis*. We characterized 11 strains of oral bacteria by Gram-staining which showed an inhibitory activity against *P. gingivalis*. Seven of the 11 strains were Gran-positive, rod. We focused on 2 strains, RI-19 and RI-22 that showed clear inhibitory activity against *P. gingivalis*. The 16SrRNA sequence of two strains showed that RI-19 and RI-22 were identical to *Actinomyces sp. oral* and *Actinomyces viscosus*, respectively. We propose that, in healthy oral cavity, *Actinomyces species* with inhibitory activity against *P. gingivalis* may prevent colonization of late colonizer *P. gingivalis*.

歯周病原細菌の増殖を抑制するヒト口腔内常在微生物の探索と同定

デンタルプラークには口腔微生物叢が形成されている。しかし清掃不良などにより口腔微生物叢のバランスが崩れ、*Porphromonas gingivalis*などの歯周病原細菌が増殖することで歯周病を発症すると考えられる。我々は、健常なヒトの口腔微生物叢には、歯周病原細菌の増殖を抑制する細菌が存在するのではないかと仮説を立てた。本研究では、健常なヒトの口腔微生物叢から、*P. gingivalis*の増殖を抑制する細菌を探索し、その菌を同定することを目的とした。健常なヒトのデンタルプラークから培養法により細菌を単離し、増殖阻害を示す細菌を11株見出すことができた。11株の中で、特に増殖阻害活性が高かった2株は、グラム陽性桿菌で、16SrRNA遺伝子のシーケンス解析の結果、デンタルプラークの初期定着菌である*Actinomyces sp. oral*および*Actinomyces viscosus*であった。健常なヒトの口腔微生物叢では、*P. gingivalis*の増殖を抑制する*Actinomyces*により、*P. gingivalis*の増殖が阻害されている可能性が考えられる。本研究は、研究倫理規定関連委員会の承認を得た。

Screening and identification of human oral bacteria that inhibit growth of periodontal pathogenic bacteria

Problem

The human oral cavity consists of over 600 bacterial species and has well-balanced microbiota. However, dysbiosis of the balanced oral microbiota, such as an increase of periodontal pathogens, is associated with periodontal disease. In fact, a periodontal pathogen *Porphyromonas gingivalis* is frequently isolated from the active site of periodontal patients. Periodontal disease is a leading cause of tooth loss and also increases the risk of systemic disorders such as aspiration pneumonia, infective endocarditis, and diabetes. Tooth brushing to remove dental plaque is one way to prevent periodontal disease. However, as *P. gingivalis* is resident in oral cavity, removal of periodontal pathogens from the oral cavity is transient, and they increase in number over time. Therefore, new strategies to limit colonization of *P. gingivalis* are required for the prevention of periodontal disease.

Hypothesis

Oral microbiota make various symbiotic relationships with microorganisms. We hypothesize that oral microbiota of healthy human contains specific bacteria that exhibit competition against periodontal pathogen *P. gingivalis*. The aim of this study is to screen and identify the candidate bacteria derived from dental plaque of healthy human to inhibit the growth of *P. gingivalis* (Fig. 1). The final goal of my study is to develop new strategies to prevent colonization of *P. gingivalis* in oral cavity by using the competitive bacteria isolated in this study.

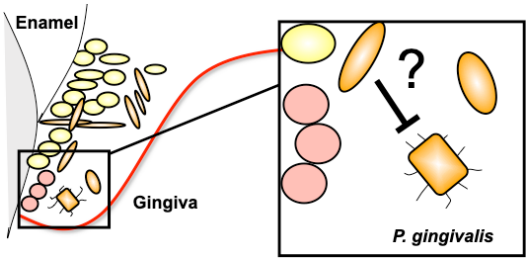


Fig. 1. Hypothesis of this study

Methods

Isolation of oral bacteria from dental plaque: Dental plaques of the buccal surface of a submaxillary right first molar were collected from 2 healthy young women, sample #1, sample #2. The dental plaques were spread on Nutrient agar (NA) or GAM agar plates and were incubated at 37°C for 5 days under aerobic or anaerobic conditions, respectively. The isolated colonies were counted and colony-forming unit (CFU) was determined.

Screening of oral bacteria with potential to inhibit the growth of *P. gingivalis*: The colonies with different morphology and color from GAM agar plate were picked up and inoculated on GAM agar plate. The plates were incubated at 37°C for 5 days under anaerobic conditions. The plates were overlayed with Tryptic Soy agar medium containing *P. gingivalis* W83. After 6 days of incubation at 37°C under anaerobic conditions, bioactivities of the colonies were assessed by measuring the inhibition zone.

Characterization of the oral bacteria with potentiall to inhibit the growth of *P. gingivalis*: The isolated bacteria that inhibit the growth of *P. gingivalis* were characterized by Gram-staining and identified based on 16SrRNA sequencing. Parts of 16S rRNA gene (the conserved region 10 to 800) of 6 strains were amplified by PCR using universal primers. The PCR products were cloned into pGEM-T vector. The resulting sequences were used to search the DDBJ database using BLAST.

Results

The CFU of isolated bacteria on GAM agar medium per 1 g plaque were 2.6 x 10^{10} CFU in sample #1 and 1.8 x 10^{8} CFU in sample #2. We picked up 45 colonies with different morphology and color and then screened candidate bacteria with potential to inhibit the growth of *P. gingivalis*. We demonstrated that 11 strains showed an inhibitory activity against *P. gingivalis* (Fig. 2).

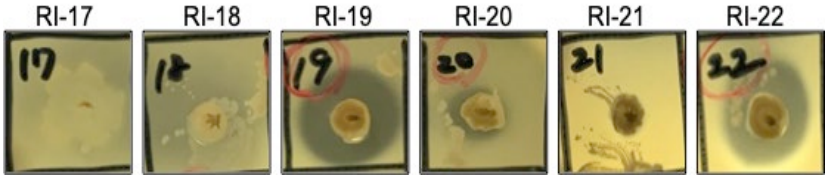


Fig. 2. Representative data of screening of oral bacteria that inhibit the growth of *P. gingivalis.*

We characterized 11 strains by Gram-staining. Seven of 11 strains were Gram-positive, rod. We focused on 2 strains, RI-19 and RI-22 that showed clear inhibitory activity against *P. gingivalis*. The 16SrRNA sequence of two strains showed that RI-19 and RI-22 were identical to *Actinomyces sp. oral* and *Actinomyces viscosus,* respectively (Fig. 3). The inhibitory activity of RI-19 and RI-22 against periodontal pathogens *P. intermedia* and *F. nucleatum* were not clear compared to that against *P. gingivalis* .

Machigashira *et al*. (Journal of the Japanese Society of Periodontology, 37;618-627, 1992) reported that *Actinomyces viscosus* ATCC15987 isolated from oral cavity produced approximately 58 kDa protein showing actimicrobial activity against *P. gingivalis*. The *Actinomyces* strains with inhibitory activity against *P. gingivalis* isolated in this study may produce similar antimicrobial proteins. It has been known that *Actinomyces* is colonized early in the dental plaque maturation process. We propose that, in healthy oral cavity, *Actinomyces* species with inhibitory activity against *P. gingivalis* limit colonization of late colonizer *P. gingivalis* and therefore prevent periodontal disease.

Strain	Inhibition zone	Gram-staining	Form	16S rRNA sequence
RI-3	+	positive	rod	*Actinomyces johsonii*
RI-5	+	positive	rod	
RI-8	+	positive	rod	
RI-11	+	positive	rod	*Actinomyces naeslundii*
RI-19	**++**	**positive**	**rod**	***Actinomyces sp. oral***
RI-20	+	positive	rod	
RI-22	**++**	**positive**	**rod**	***Actinomyces viscosus***
YT-9	+	negative	rod	
YT-19	+	positive	cocci	*Streptococcus oralis*
YT-20	+	positive	cocci	*Streptococcus oralis*
YT-23	+	positive	cocci	

Fig. 3. Summary of oral bacteria that inhibit the growth of *P. gingivalis.*

Conclusion

The bacterial strains isolated from dental plaque of healthy subjects, which were Gram-positive rod, *Actinomyces,* showed inhibitory activity against periodontal pathogen *P. gingivalis*. Further investigation of inhibitory mechanism of the isolated strains will provide new strategies to prevent periodontal disease.

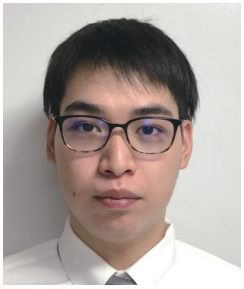
This study was approved by the ethics committee.

A study for the induction of activating innate immune responses in the oral cavity

日本大学松戸歯学部 4年生 Nihon University School of Dentistry at Matsudo
Class of 2022

植草 信乃介 Shinnosuke UEKUSA

ファカルティー・アドバイザー：小林 良喜 感染免疫学講座 助教



The benefits of probiotics are numerous and well known. According to the report in which joined the research group, they mention that Lactic acid bacteria (LAB) had inhibited periodontitis in the murine model. However, the inhibitory mechanism that extends from the intestinal tract to the oral cavity is still unknown. In order to maintain homeostasis, the nervous and vascular systems are distributed throughout the body to form systemic structural networks. Through the vascular networks, various signal molecules are transported throughout the body to form local functional networks. From these, we hypothesized that factors in the vascular system might lead to the oral cavity. In order to prove these, the following methods were used. Pooled plasma was collected from LAB-administered (LAB-plasma) mice and transferred systemically from the tail vein eight times in three weeks. An elevated level of β-defensin 3 (bD3) was detected of mice systemically injected with LAB-plasma compared to the control mice group. Further, bD3-specific mRNA levels were significantly increased in the oral in these mice. bD3 is known to be produced mainly by epithelial cells. Therefore, the production of the bD3 may have been induced by epithelial cell-mediated production of factors in the blood. These results suggest that the spillover of LAB to the oral cavity, a distant organ originating from the intestinal tract, is mediated by blood humoral factors. (The University's IACUC has approved all animal experiments)

口腔内自然免疫応答の活性化に関する研究

プロバイオティクス乳酸菌が生体に与える影響は多数報告されている。これまでに、乳酸菌投与により歯周炎の予防効果を動物実験により報告されているが腸管と口腔をつなぐ因子はまだ不明であった。全身の臓器間を結ぶネットーワークである神経系と脈管系により生体恒常性が保たれていることから、乳酸菌により腸内環境が活性化することで神経系や脈管系を通じて抗炎症や免疫活性作用が口腔周囲組織に至るのではと考え、これらを解明するために本研究に至った。トレハロース溶液に懸濁した乳酸菌（Lactic acid bacteria; LAB）を胃内投与させたマウスから採取した血漿（LAB-plasma）を尾静脈から移入し、対称群と比較することで口腔組織への免疫応答を検討した。興味深いことにLAB-plasma投与群では唾液中のβ-ディフェンシ3（bD3）の産生が増加し、歯肉、舌、唾液腺においてbD3特異的mRNAの発現が顕著に増加していた。bD3は主に上皮細胞から産生されることが知られていることから、LAB-plasma中の液性因子による上皮細胞を介したbD3の産生誘導が考えられる。以上の結果から、LABの投与により腸管を起点した遠隔臓器である口腔への波及は血液中の液性因子を介することが示唆される（本動物実験は本学動物実験委員会の承認済み）。

A study for the induction of activating innate immune responses in the oral cavity

(Problem)

Numerous studies have reported on the effects of probiotic lactic acid bacteria on the body. In particular, it is known to inhibit or improve not only the intestinal environment but also systemic diseases by activating the intestinal microbiota and intestinal immune system. According to the previous reports, lactic acid bacteria effectively prevented periodontitis originating from the intestinal tract in animal experiments. However, the factors linking the intestinal tract and the oral cavity were still unknown.

(Hypothesis)

According to the lectures, the nervous system and the vascular system are the networks that connect organs of the whole body and that neurotransmitters, cytokines, chemokines, and immune cells regulate the systemic organs through the vascular system to maintain homeostasis. We hypothesized that the administration of lactic acid bacteria (LAB) stimulated the intestinal environment. The nervous system or fluid factor reached the oral through the vascular system. It activated the anti-inflammatory and immune responses, which led us to the present study to elucidate these factors.

(Methods)

Following previous studies, BALB/c mice were orally administered with Lactobacillus gasseri O3-2, which was suspended in a 25% trehalose, for one week. Only 25% of trehalose was used for the control group. One day after the last administration, blood was collected under general anesthesia.

To prove our hypothesis, the plasma was systemically injected by tail vein to sex-matched and age-matched BALB/c mice for eight times over three weeks. One day after the last injection, saliva was collected by pilocarpine stimulation, and the gingiva, tongue, and salivary glands were isolated from mice euthanized with carbon dioxide gas (Figure 1).

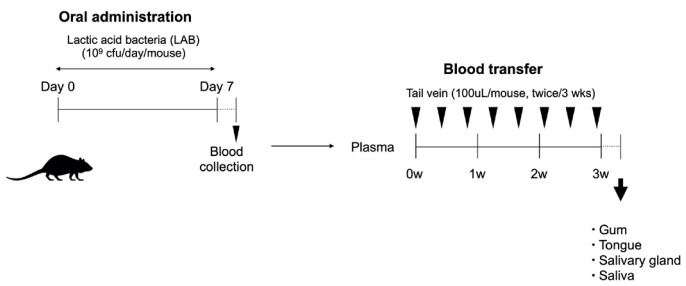
1. measurement of beta-defensin 3 in saliva

Salivary bD3 levels were analyzed using a Mouse beta Defensin 3 ELISA Kit. Briefly, mouse saliva was collected one day after the last systemically administration.

2. analysis of ß-defensin 3 mRNA expression

After collected the saliva, mice were sacrificed, and gingival tissue, tongue, and salivary gland were carefully removed. Total RNA was isolated and subjected to quantitative real-time PCR for bD3 mRNA.

Figure 1. Experimental protocol



(Results)

1. Production of ß-defensin 3 in saliva

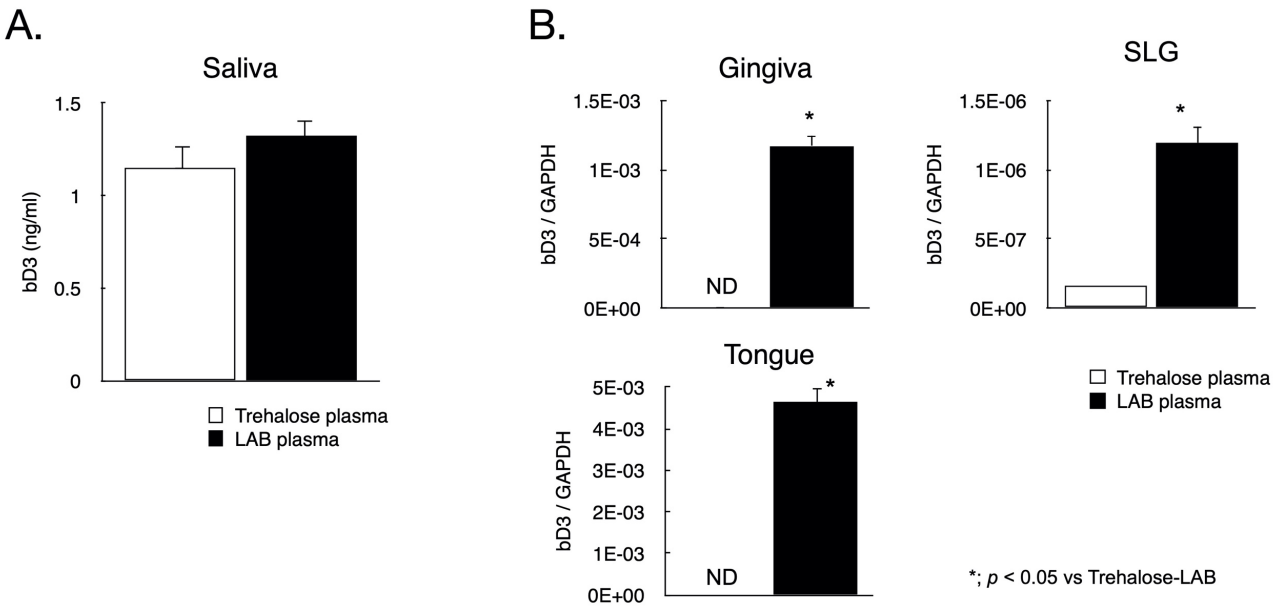
One day after the systemic administration, elevated levels of β-defensin 3 (bD3) were detected in the saliva of mice administered with LAB-plasma (Figure 2A).

2. Analysis of bD3 mRNA expression

Furthermore, significant expression of bD3-specific mRNA was detected in the gingiva, tongue, and salivary gland compared with the control group (Figure 2B).

It is well known that bD3 is produced by epithelial cells. It may be possible that the factor is stimulated by blood to be produced through the oral mucosa and salivary gland.

Figure 2. bD3 expression and protein in mucosal components of mice administered LAB-plasma



(Conclusion)

These results suggested that increase the possibility that the induction of bD3 levels in the oral mucosa (such as distant mucosal surfaces) due to the factors in plasma.

Effectively conditioned peripheral blood mononuclear cells restore salivary gland function in mice with Sjögren-like disease

長崎大学歯学部　5年生　Nagasaki University School of Dentistry　Class of 2021

園木 美結　Miyu SONOKI

ファカルティー・アドバイザー：住田 吉慶　硬組織疾患基盤研究センター 准教授

Objective: Sjögren's syndrome (SS) is an autoimmune disease that causes loss of secretory function in salivary glands which have focal lymphocyte infiltration composed of T and B cells. Currently there is no effective therapy for SS, therefore new treatments are required. We previously showed that effectively conditioned peripheral blood mononuclear cells (E-MNCs) improved salivary gland function in radiation-injured mice. Thus, we investigated if E-MNCs could also restore salivary gland function in a mouse model of SS.
Methods: To obtain E-MNCs, peripheral blood mononuclear cells (PBMNCs) from CB6F1 mice were cultured in serum-free medium with five recombinant proteins (5G culture) for 7 days. To evaluate E-MNC efficacy in SS onset, E-MNCs were transplanted into salivary glands of 8-week-old NOD mice and salivary secretion was measured over time. Salivary glands were harvested, followed by analyses of specific genes and proteins, and lymphocyte infiltration.
Results: After 5G culture, E-MNCs were characterized by an increase in $CD11b^{+}/CD206^{+}$ and CCR2-/$Galectin3^{+}$ cells (M2 macrophages). Moreover, the M2c macrophage marker MSR1 was increased in E-MNCs indicating anti-inflammatory and immunoregulatory characteristics. E-MNC-treated NOD mice maintained their salivary secretion, and had reduced lymphocyte infiltration and mRNA expression of chemokines such as CCL8 and CXCL13, that attract T and B cells, respectively.
Conclusion: These results showed that E-MNC treatment improved salivary gland function in mice with SS-like disease, possibly by inhibition of signaling for lymphocyte infiltration.

シェーグレン症候群による障害唾液腺の機能を修復する

目的：口腔の難治性疾患を対象に、われわれは慢性炎症除去や免疫適正化に働く免疫細胞を利用する細胞治療の開発を企図し、末梢血単核球からM2型マクロファージを主体とする抗炎症型末梢血濃縮細胞群（E-MNC）の誘導法開発に取り組んできた。今回、シェーグレン症候群（SS）を対象としたE-MNC治療の可能性を検討するため、マウスモデルの唾液腺に対する病態是正の有効性を検討した。
方法：CB6F1マウスの末梢血単核球を5種の増殖因子を含む無血清培地で培養し、E-MNCの培養条件を至適化した。その後、E-MNCをSSモデルのNODマウスの顎下腺に移植し、経時的に唾液分泌機能とリンパ球浸潤の制御に対する影響を解析した。
結果：E-MNCでは、CD11b/CD206陽性、CCR2 /Galectin3陽性細胞（M2様マクロファージ）が増幅するが、これらの分画中ではMsr1陽性細胞（M2c型マクロファージ）が増加していた。E-MNCを投与したマウスでは、分泌機能障害は軽減傾向を示し、投与初期ではリンパ球遊走に関連するCCL8やCXCL13などケモカインの発現抑制を認め、リンパ球浸潤が減少した。
結論：NODマウスの唾液腺において、E-MNCがリンパ球浸潤を抑制し、分泌機能の障害抑制に機能する可能性が示唆された。現在、その詳細な機序を解析しているところである。

Effectively conditioned peripheral blood mononuclear cells restore salivary gland function in mice with Sjögren -like disease

Problem

Salivary glands play an important role in protecting and lubricating the oral cavity through the secretion of saliva. Sjögren's syndrome (SS) is an autoimmune disease characterized by salivary gland destruction leading to loss of secretory function and complications such as aggressive periodontitis and dysphagia. Consequently, quality of life is greatly affected. Although the etiology of SS is still unknown, studies have identified key mechanisms that lead to SS development. A hallmark of the disease is the presence of focal lymphocyte infiltration in the salivary glands which are composed of mostly T and B cells.

Hypothesis

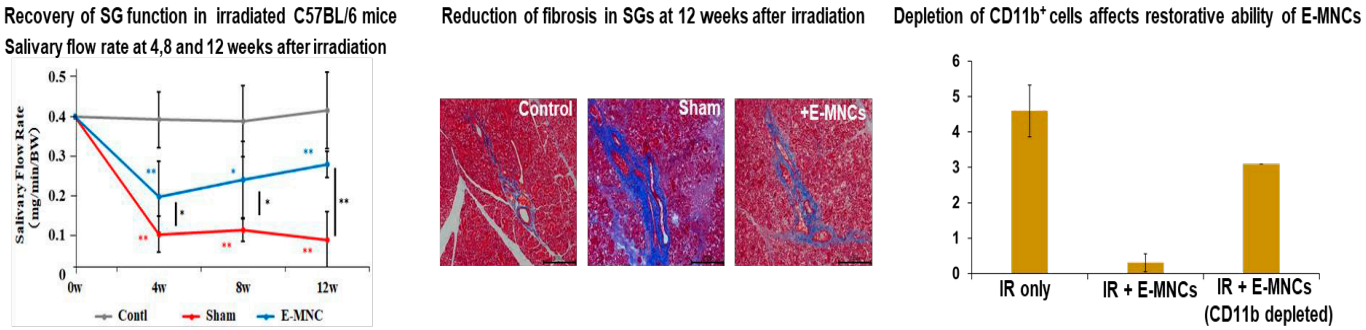


Figure 1

We worked to develop a cell therapy that enhances the anti-inflammatory and regenerative properties of PBMCs. We previously showed that effectively conditioned peripheral blood mononuclear cells (E-MNCs) improved salivary gland function in radiation-injured mice and the key components of E-MNCs therapy may be in M2 macrophages ($CD11b^+/CD206^+$) (Figure 1). Therefore, we hypothesized that E-MNC treatment may recover salivary gland function in mice with Sjögren's-like disease through its anti-inflammatory and immune-regulatory cell properties.

Methods

To obtain E-MNCs, peripheral blood mononuclear cells (PBMCs) from healthy donors were cultured in serum-free medium with five recombinant proteins (5G culture) for 6 days. Before commencing with the animal study, we analyzed the anti-inflammatory effect of E-MNCs using a co-culture system with CD3/CD28-stimulated human PBMNCs. Then, we developed the murine E-MNC culture using the CB6F1 mouse to evaluate the efficacy of E-MNCs in the onset of SS. For this, E-MNCs were transplanted into salivary glands of 8-week-old NOD mice and salivary secretion was measured over time. Salivary glands were harvested, followed by analyses of specific genes and proteins, and lymphocyte infiltration (Figure 2).

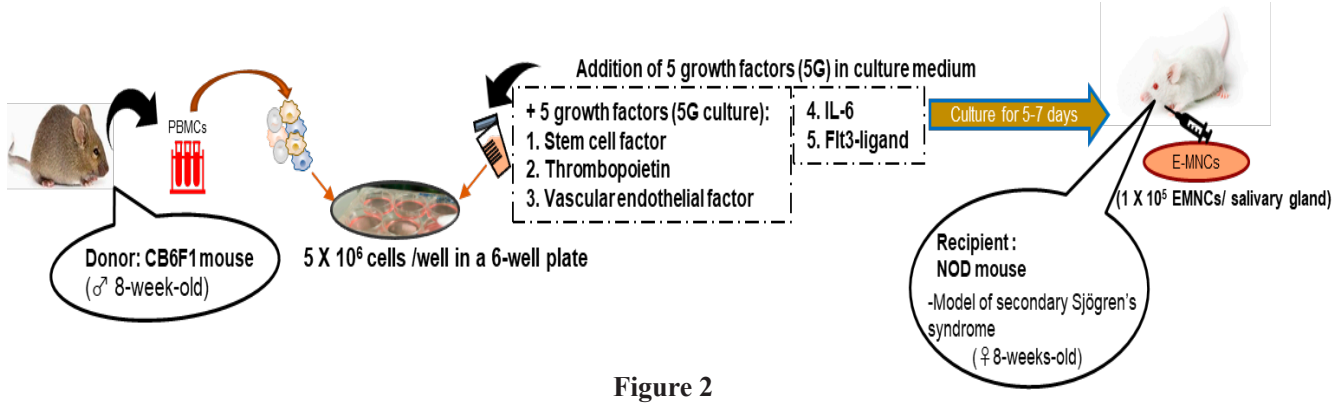


Figure 2

Results

After 6 days of human E-MNC culture, fluorescent cell sorting revealed a robust increase in CD11b^{+}/CD206^{+} cells (M2 macrophages). In addition, the PBMNCs co-cultured with E-MNCs had significantly reduced expression of inflammatory genes (Figure 3). Mouse E-MNCs were also characterized by an increase in CD11b^{+}/CD206^{+} (M2 macrophages) and CCR4^{+}/CCR6^{-} (Th2 cells) and macrophage-like shapes (Figure 4). Moreover, the M2c macrophage marker MSR1 was increased in E-MNCs indicating anti-inflammatory and immune-regulatory characteristics.

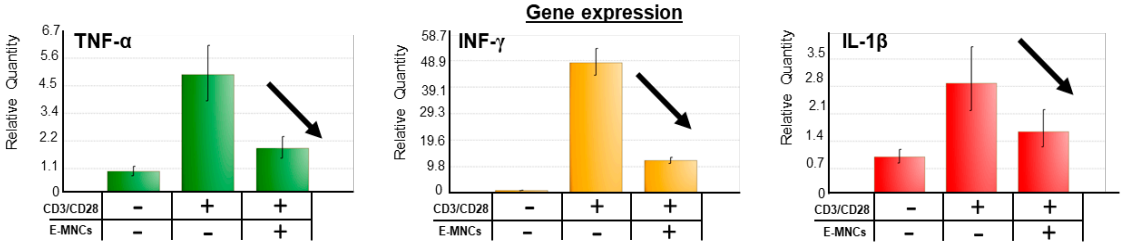


Figure 3

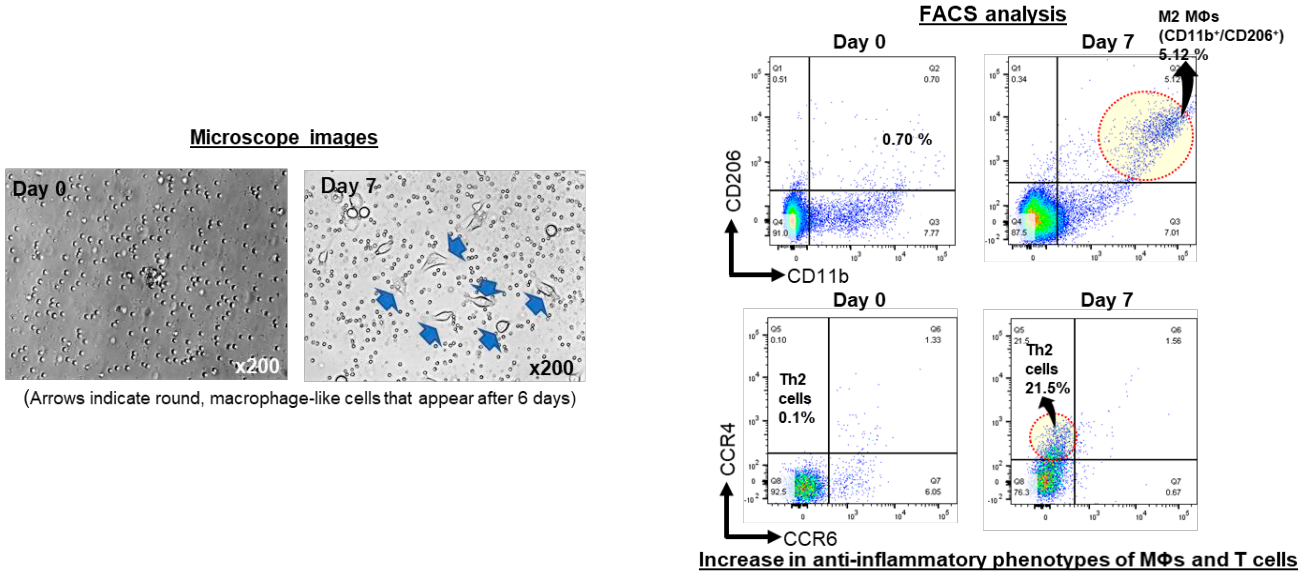


Figure 4

E-MNC-treated NOD mice maintained their salivary secretion (Figure 5) and had reduced lymphocyte infiltration (Figure 6) and mRNA expression of chemokines such as CCL8 and CXCL13, that attract T and B cells, respectively.

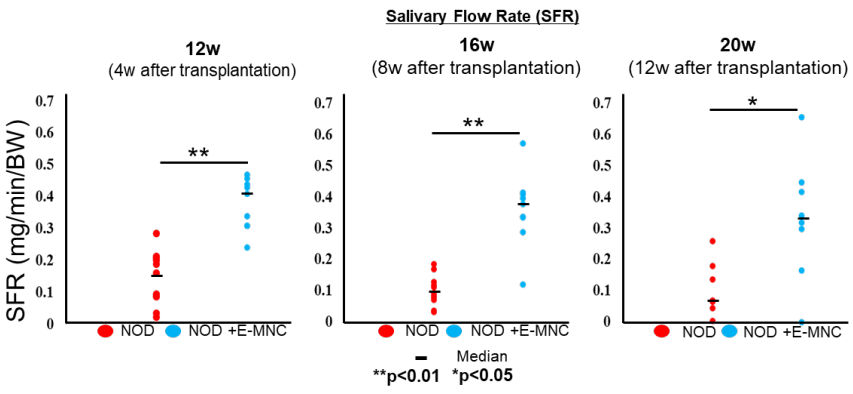


Figure 5

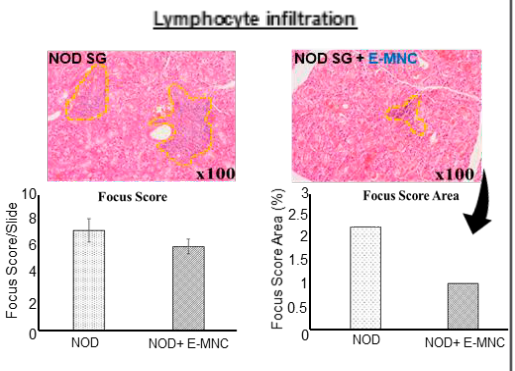


Figure 6

Conclusion

-E-MNC treatment in the onset of disease, reduced the size of lymphocyte infiltrates and maintained saliva secretion in NOD mice.

-These results showed that E-MNC treatment improved salivary gland function in mice with SS-like disease, possibly by inhibiting chemokines related to lymphocyte infiltration.

Histological alteration of bone-specific blood vessels by alendronate administration

北海道大学歯学部 6年生 Hokkaido University School of Dental Medicine Class of 2020

吉野 弘菜 Hirona YOSHINO

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

Introduction: 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.

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

【Problem】

Recently, bone-specific blood vessels were found that were strongly positive for CD31, endomucin and featured EphB4-immunoreactivity. Therefore, the endomucin-positive bone-specific blood vessels are EphB4-reactive venous sinusoids. This also suggests that bone is blood-vessel-rich tissue, and there must be some interactions between bone cells and blood vessels (Nature, 2014). Meanwhile, alendronate, the most commonly used bisphosphonate for osteoporotic treatment, accumulates in the mineralized bone matrix and consequently inhibits osteoclasts by becoming incorporated into the osteoclasts through their ruffled borders. Taken together, I wondered whether alendronate inhibits highly endomucin-positive bone-specific blood vessels, as alendronate may be incorporated into the vascular endothelial cells by means of pinocytosis. My question is whether alendronate directly inhibits bone-specific blood vessels, and if there are some interactions between osteoblasts and bone-specific blood vessels, whether osteoblasts are inhibited as well.

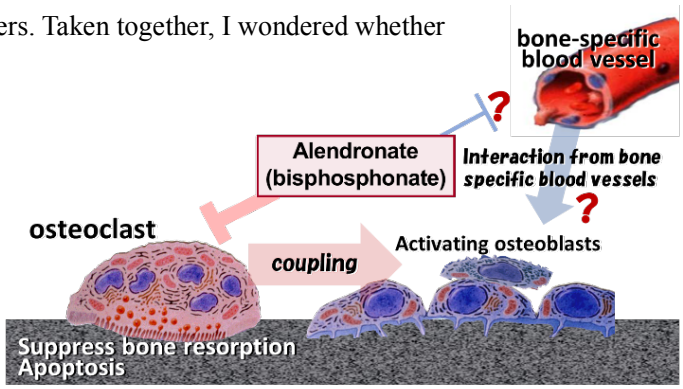


Fig. 1

【Hypothesis】

1) Alendronate directly inhibit bone specific blood vessels.

2) Bone specific blood vessels inhibited by alendronate reduce osteoblastic activity.

【Material & Methods】

Six-week-old male ICR mice received vehicle (control group) or 1mg/kg/day (once a day) of alendronate (ALN group) for 10 days. The mice were fixed with a paraformaldehyde solution, and their femora and tibiae were used for immunohistochemical and ultrastructural analyses, *e.g.*, tartrate-resistant acid phosphatase (TRAP) staining and the immunohistochemistry of alkaline phosphatase (ALP) and endomucin. Gene expressions of *Endomucin*, *Acta2* (α-*smooth muscle actin*), *Gata2*, *Cdh5* (*Ve-cadherin*), *Vash1* in the control and ALN-administered bone were examined by RT-PCR.

【Results】

1) The effect of alendronate on the bone-specific blood vessels

As shown in Fig. 2B, the alendronate-treated endomucin-positive blood vessels were markedly reduced in size, in that they appeared to have shrunk to the degree indicated by the arrows. In comparison with the control metaphyseal bone that had the huge brown-colored blood vessels (Fig. 2A), alendronate-treated bone had the very small blood vessels in the metaphysis (Fig. 2B). When examined under transmission electron microscopy, the control blood vessels showed the typical smooth surfaces of vascular endothelial cells (data not shown). However, the alendronate-treated vascular endothelial cells showed several small processes and irregularly shaped luminal surfaces (Fig. 2C). In addition, there were many small vesicles (arrows, Fig. 2C, inset) released from the luminal surfaces of the alendronate-treated vascular endothelial cells.

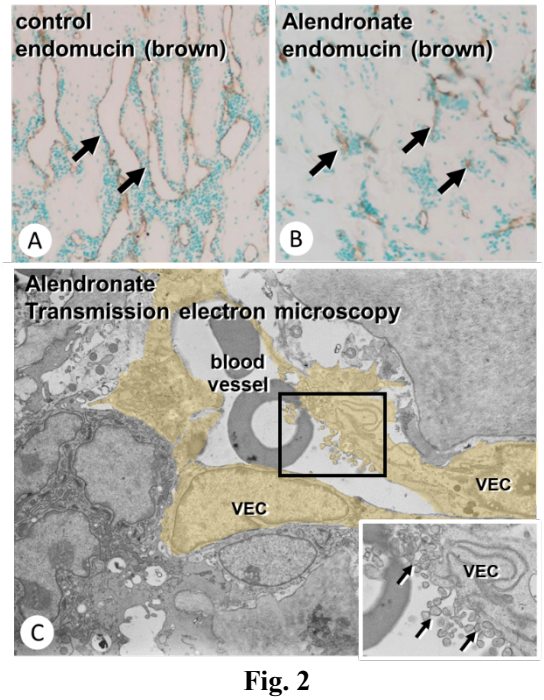
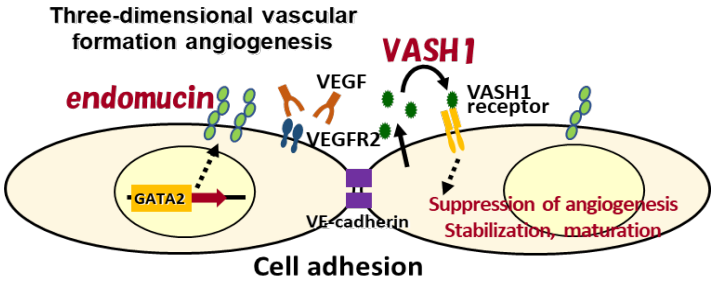


Fig. 2

When I examined the angiogenic genes and the genes for maintaining the vasculature, the mRNAs encoding *Endomucin*, *Acta 2*, *Gata2* and *Vash1* had increased after alendronate administration (Fig. 3). Taking into account that GATA2 and endomucin help to maintain the three-dimensional structure of blood vessels, and VASH1 is involved in negative feedback loops in angiogenesis, alendronate appears to inhibit endomucin-positive vascular endothelial cells.



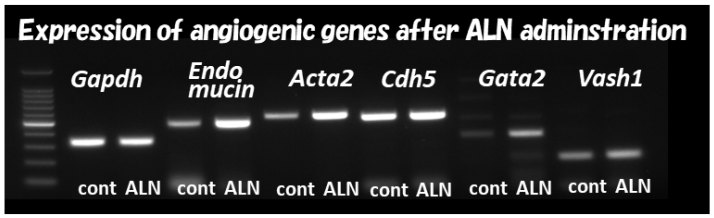


Fig. 3

2) Histological changing of bone-specific blood vessels, osteoblasts, and osteoclasts by alendronate administration

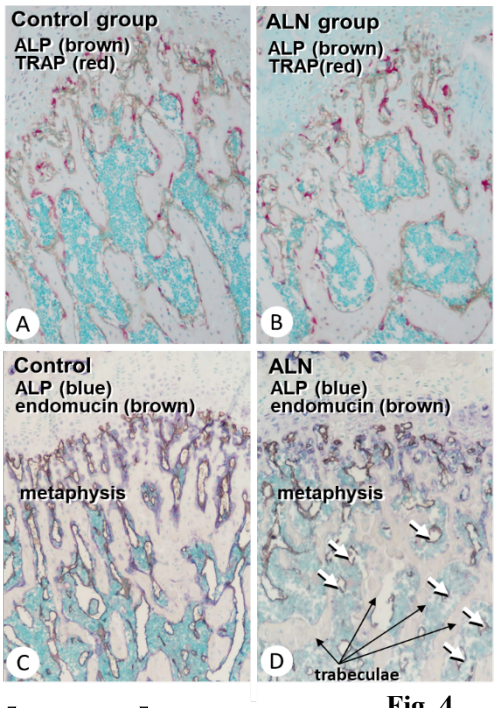


Fig. 4

The distribution of TRAP-positive osteoclasts (red color) was not as obviously affected by the regimen of alendronate administration (Fig. 4A, B), which means that cell coupling between osteoblasts and osteoclasts remained intact. However, interestingly, the blue-colored alkaline phosphatase-positive osteoblasts appeared to be only restricted to the chondro-osseous junction, and were not detectable in the secondary trabecular region, where only the shrunken and small brown-colored endomucin-reactive blood vessels were seen (arrows, Fig. 4D). Thus, the examination using the animal model suggested the existence of osteo-vascular interactions, which is a novel concept for regulating not only the bone metabolism. Further investigations into the exosome-like vesicles from the alendronate-treated vascular endothelial cells, as well as the direct interaction between osteoblasts and bone-specific blood vessels (Fig. 5), will be necessary in the near future.

【Summary】

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.

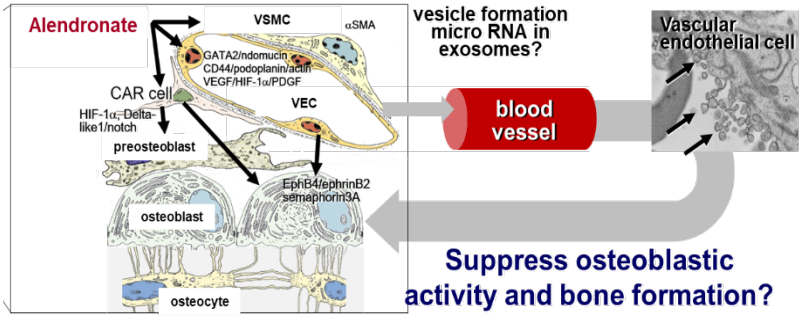


Fig. 5

【Conclusion】

Alendronate may not only affect osteoclasts but also bone-specific blood vessels, which subsequently influence osteoblasts.

The effect of hypoxia on osteoblast differentiation

鹿児島大学歯学部　5年生　Kagoshima University Faculty of Dentistry　Class of 2021

佐藤 大幹　Daiki SATO

共同研究者：林 瑶大（5年生）
ファカルティー・アドバイザー：松口 徹也　口腔生化学分野 教授

Osteoblasts contribute to form bone tissue by producing osteoid. In the field of dentistry, bone regeneration mediated by osteoblasts has become a mainstream therapy for bone defect caused by diseases and so on. However, current tissue regeneration therapy takes long time and this kind of long-term therapy often worsens the patient's QOL. Therefore, a good enhancing method of osteogenesis to shorten the term of therapy is in urgent need. It has been reported that trabecular bone volume is reduced in mice exposed to hypoxia, so it seems that environmental oxygen concentrations would affect osteoblast differentiation. Although several reports showed that oxygen levels affect osteoblast differentiation, molecular details of the effect of hypoxia on osteoblast still need further investigation. Herein, we compared degrees of matrix mineralization and the expression of osteogenic marker genes in osteoblasts placed under normoxia and hypoxia. MC3T3-E1 cells were incubated with osteogenic differentiation media under normoxia and hypoxia, and then matrix mineralization was visualized by alizarin red staining and the expression of osteogenic marker genes was analyzed by realtime PCR. Alizarin red staining indicated that matrix mineralization was significantly reduced in hypoxia compared with normoxia. The mRNA expression levels of *Runx2*, *Alpl*, *Bglap* and *Spp1* were decreased in hypoxia compared to normoxia. Unexpectedly, mRNA increase of *Sp7* was significantly enhanced in hypoxia compared with normoxia. These results suggested that hypoxia suppresses differentiation and function of osteoblast.

骨芽細胞の分化におけるHypoxiaの影響

骨芽細胞は類骨を産生し骨組織の形成に重要な働きを持つ。歯科領域では歯槽骨や顎骨欠損の修復治療として骨芽細胞による骨再生療法が主流となっているが、治療には半年以上を要するため患者のQOLに影響を及ぼす。その為、より短期間でかつ簡便な骨芽細胞による骨再生を促進する方法が必要とされてきている。これまでの報告からも、酸素環境の違いが骨芽細胞の分化にも同様に影響すると考えられるが、骨芽細胞の分化における酸素分圧の違いによる影響の分子生物学的詳細については未だ明確ではない。そこで本研究では、マウス骨芽細胞株であるMC3T3-E1を骨芽細胞分化培地でそれぞれnormoxiaとhypoxiaの環境下で一定期間培養し、それぞれの石灰化誘導能と骨分化マーカー遺伝子の発現を解析、比較した。その結果、石灰化誘導能については、normoxiaで高度な石灰化が確認されたが、hypoxiaでは石灰化の顕著な低下を認めた。骨分化マーカー遺伝子の発現については、*Runx2*、*Alpl*、*Bglap*、*Spp1*はhypoxiaで発現量の低下を認めたが、興味深いことにSp7はhypoxiaにおいて発現の増強を認めた。*Col1a1*、*col1a2*ではnormoxiaとhypoxiaで有意な差は見られなかった。以上の事から、hypoxiaは骨芽細胞の分化や機能に抑制的な影響を及ぼす事が示唆された。

The effect of hypoxia on osteoblast differentiation

(Problems)

Osteoblasts, which are short spindle shaped and similar to fibroblasts, produce osteoid and contribute to bone formation. In the field of dentistry, bone regeneration mediated by osteoblasts has become a mainstream therapy for bone defect caused by diseases such as periodontitis and oral cancer. However, current tissue regeneration therapy usually takes more than 6 months. This kind of long-term therapy often worsens the patient's quality of life (QOL). Therefore, a good enhancing method of osteogenesis to shorten the term of therapy is in urgent need.

(Hypothesis)

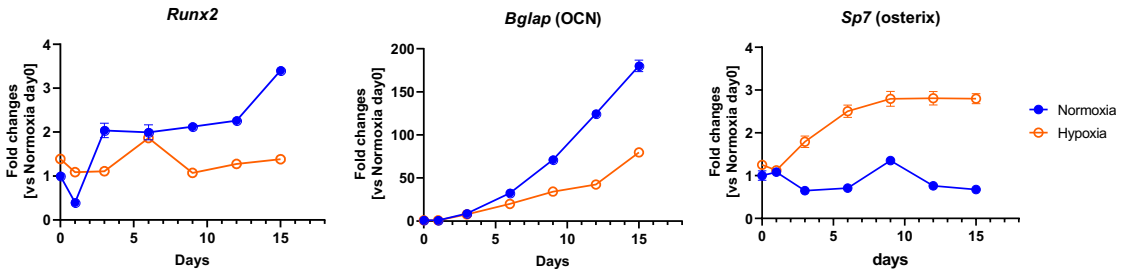
In general, cell culture experiments are usually conducted under normoxia (normal oxygen level), whereas most cells in the body are actually under hypoxia (low oxygen level). It has been reported that trabecular bone volume is reduced in mice exposed to hypoxia, so it is likely that environmental oxygen concentrations would affect osteoblast differentiation. Although several reports showed that oxygen levels affect osteoblast differentiation, molecular details of the effect of hypoxia on osteoblast differentiation still need further investigation. In this study, we examined effects of hypoxia on osteoblast differentiation and matrix mineralization in comparison to normoxia *in vitro*. Based on previous reports including both *in vivo* and *in vitro* studies, we hypothesized that osteoblast differentiation and activation are reduced in hypoxia.

(Methods)

Osteoblast differentiation is analyzed by degrees of matrix mineralization and the expression of osteogenic marker genes. An osteoblast cell line, MC3T3-E1, were incubated with osteogenic differentiation media (ODM) containing ascorbic acid and beta-glycerophosphate in normoxia and hypoxia. After 9, 12, and 15 day culture in ODM, matrix mineralization was visualized by alizarin red staining. Total RNA was collected every 3 days and the expression of osteogenic marker genes were analyzed by realtime PCR.

(Results)

Alizarin red staining indicated that matrix mineralization was significantly reduced in hypoxia compared with normoxia. The mRNA expression levels of *Runx2*, *Alpl* (alkaline phosphatase), *Bglap* (OCN) and *Spp1* (OPN) were decreased in hypoxia compared to normoxia. Unexpectedly, mRNA increase of *Sp7* (osterix), a key transcriptional factor for osteoblast differentiation, was significantly enhanced in hypoxia compared with normoxia. No significant difference of *IBSP* (bone sialoprotein), *Col1a1* (collagen type I, alpha 1) or *Col1a2* (collagen type I, alpha 2) gene expression was observed between normoxia and hypoxia.



(Conclusion)

Hypoxia suppresses overall differentiation and function of osteoblast.

The effects of butyrate-treatment on three-dimensional gingival tissue cultures

日本大学歯学部　5年生　Nihon University School of Dentistry　Class of 2021

黒澤 佑介　Yusuke KUROSAWA

共同研究者：山口 裕史（5年生）
ファカルティー・アドバイザー：津田 啓方　生化学講座 准教授

The gingival tissue consists of an epithelium-connective-tissue bilayer. Periodontal disease is caused due to mature dental plaques that contain high concentrations of bacteria-derived short-chain fatty acids, among which butyrate reportedly has some strong physiological and pathological effects. However, there is no clear information on the role of butyrate in the epithelium-connective-tissue bilayer. In this study, a three-dimensional (3D) gingival tissue system consisting of primary gingival fibroblasts and a gingival epithelial cell line was constructed, and the effects of butyrate exposure on this system were examined. Histological analysis confirmed that the 3D cultures comprised a stratified squamous epithelial layer and a connective tissue layer, in which the cells appeared not to be mixed. Moreover, it was observed that butyrate exposure to the 3D cultures led to the release of DNA, SAP130, and citrullinated histone H4 from the gingival epithelium layers. SAP130 and citrullinated histones are involved in the induction of inflammation functioning as a damage-associated molecular pattern and in autoantibody generation, respectively. Therefore, these results suggest that butyrate exposure to the 3D gingival culture systems induces necrotic cellular death along with the release of inflammation and autoimmune disease-inducible factors.
This research was approved by the Ethics Committee of Nihon University (Approved No. EP19D011).

歯周病原菌代謝産物酪酸の作用が歯肉3次元培養組織に及ぼす影響

歯周疾患は細菌由来の高濃度の短鎖脂肪酸を含む成熟プラークにより引き起こされる。短鎖脂肪酸の中でも様々な生理活性を持つといわれている酪酸が、上皮と結合組織の二層からなる歯肉組織へ及ぼす影響は知られていない。本研究では、初代歯肉線維芽細胞と歯肉上皮株化細胞を用いて歯肉三次元培養系を構築し、酪酸が本培養系に及ぼす影響を調べた。歯肉三次元培養系の切片を作成し、HE染色および免疫染色を行ったところ、重層扁平上皮細胞層と結合組織層の二層からなり、これらの細胞が混ざっていない事が確認された。この三次元培養系に酪酸を作用させたところ、歯肉上皮細胞よりDNA、SAP130、およびシトルリン化ヒストンが放出されることが確認された。SAP130はDAMPsとして炎症誘導促進作用をもち、シトルリン化ヒストンは自己抗体産生に関与することから、歯肉への酪酸の作用は歯肉上皮のネクローシス様細胞死を介して放出される因子により、歯周組織の炎症および自己免疫疾患の発症が誘導される可能性が示唆された。
日本大学倫理委員会承認番号EP19D011

The effects of butyrate-treatment on three-dimensional gingival tissue cultures

(Problem)

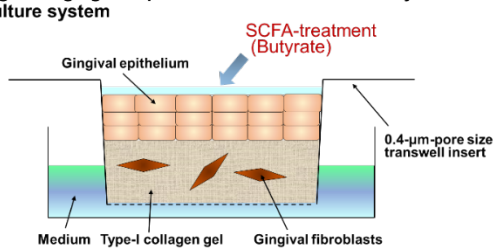
The gingival tissue consists of an epithelium-connective-tissue bilayer, and the epithelial cells and fibroblasts in the tissue communicate with each other. Furthermore, the gingival epithelium primarily obtains its nutrients from a highly vascularized connective tissue layer. In such anatomical environment, the gingival tissue establishes a barrier protecting the periodontal tissue from bacteria and other organisms.

Periodontal disease is caused due to the accumulation of mature dental plaques that contain high concentrations of short-chain fatty acids, which are produced by Gram-negative anaerobic plaque bacteria such as *Porphyromonas gingivalis*. Among the short-chain fatty acids found in dental plaques, butyrate (butyric acid) has been reported to exert a variety of effects on cells. However, the role of butyrate on the epithelium-connective-tissue bilayer has not been clearly understood.

(Hypothesis)

In this study, a three-dimensional (3D) gingival tissue bilayer system consisting of primary gingival fibroblasts and a gingival epithelial cell line was constructed, and the effects of butyrate-exposure on this 3D gingival culture system were investigated (Fig. 1).

Fig 1. 3D gingival epithelium-connective tissue hybrid culture system

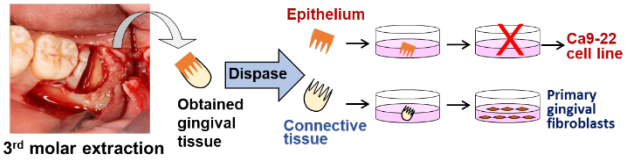


Based on research conducted using the two-dimensional gingival cell culture system that demonstrated that butyrate exposure induces not only apoptosis but also necrosis-like cellular death, **it was hypothesized that butyrate exposure to the 3D gingival tissue system may also induce necrotic cellular death and the subsequent release of intracellular molecules.**

(Methods)

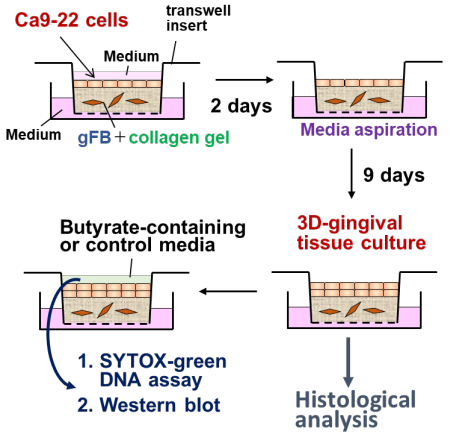
First, attempts were made to obtain primary cells from a piece of gingival tissue, which was collected during the 3rd molar extraction of a patient. After separating the gingival epithelium from the connective tissue via dispase treatment, each layer was separately cultured in the medium. Outgrowing cells were used as primary cells (Fig. 2). Although primary gingival fibroblasts could be obtained, gingival epithelial cells, unfortunately, could not be obtained. Therefore, the human gingival epithelial Ca9-22 cell line was used as a counterpart of primary gingival epithelial cells (Fig. 2).

Fig. 2. Collection of human gingival primary cells



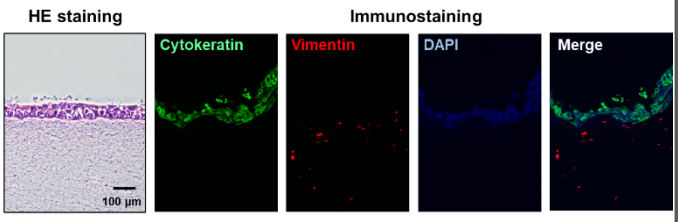
A 3D gingival culture was constructed for which primary gingival fibroblasts were mixed with type-I collagen gels and the mixture was poured into a transwell inserts having 0.4-μm pores. After the solidification of the gel, the Ca9-22 cell suspension was placed on the gel. After incubation for 2 days, the media were aspirated and incubated for another 9 days to stratify the epithelial layer (Fig. 3).

Fig. 3. Procedure to construct 3D gingival tissue culture



Histological analysis confirmed that the 3D cultures comprised a stratified squamous epithelial layer and a connective tissue layer in the hematoxylin-eosin staining (Fig. 4). Immunostaining analysis also demonstrated that cytokeratin, an epithelial marker, and vimentin, a fibroblast marker, were observed in the epithelial layer

Fig. 4. Histological analysis of 3D gingival tissue culture system



and collagen gel layer, respectively (Fig. 4).

Using these 3D gingival tissue cultures containing an epithelial-connective tissue bilayer, the effects of butyrate exposure on the cultures were investigated. Epithelial layers of the 3D cultures were treated with media containing 1%FBS in the presence or absence of butyrate. Cell culture supernatants were collected, and the amount of DNA released in the supernatants was measured using SYTOX-green dye, which emits a strong fluorescence when the dye binds to double-stranded DNA. SAP130 and citrullinated histone H4 in the supernatants were analyzed by western blotting.

(Results)

1. Butyrate exposure induced DNA release from the 3D gingival culture in a time- and dose-dependent manner (Figs. 5 and 6).

Fig. 5. The amount of DNA induced by butyrate exposure onto 3D gingival culture system

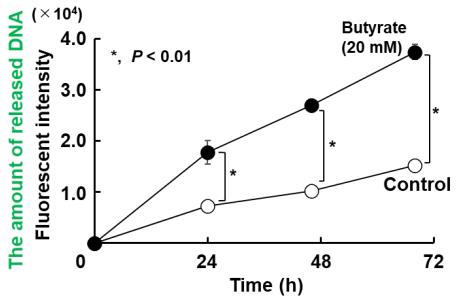
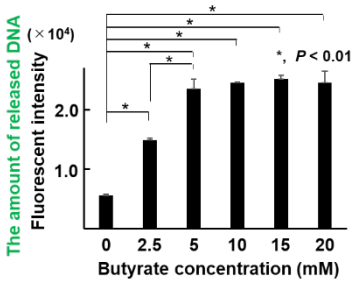
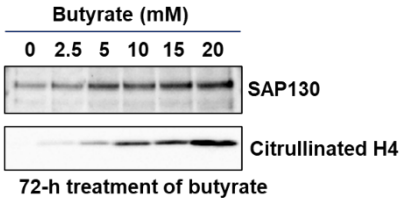


Fig. 6. Dose-dependent effects of butyrate-exposure on DNA release from 3D cultures



2. Butyrate exposure to the 3D gingival culture dose-dependently induced the release of SAP130 and citrullinated histone H4 (Fig. 7).

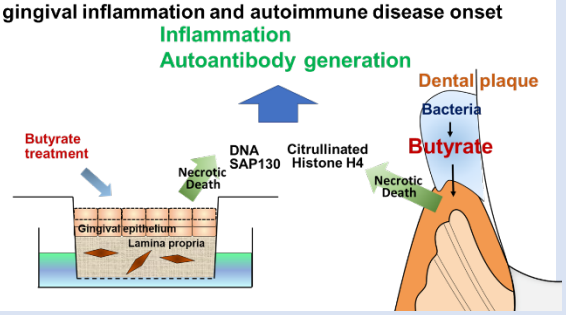
Fig. 7. Dose-dependent release of intracellular molecules from 3D cultures



(Conclusion)

- Butyrate exposure to the gingival epithelium of 3D gingival culture systems induced the release of DNA and intracellular molecules. These data suggest that butyrate exposure induced necrotic cell death.
- SAP130 and citrullinated histones are involved in the induction of inflammation functioning as a damage-associated molecular pattern (DAMP) and in autoantibody generation, respectively.
- **Therefore, the induction of necrotic cellular death by butyrate exposure, which is found in mature dental plaques, may promote gingival inflammation and autoimmune diseases (Fig. 8).**

Fig. 8. Butyrate exposure to gingival tissue may induce gingival inflammation and autoimmune disease onset



This research was approved by the ethics committee of our university (Approved #EP19D011).

Establishment of a murine salivary gland tumor model and its characterization

岩手医科大学歯学部 2年生 School of Dentistry Iwate Medical University Class of 2024

羽金 雅登 Masato HAGANE

ファカルティー・アドバイザー：入江 太朗 病態解析学分野 教授
衣斐 美歩 病態解析学分野 特任講師

Salivary gland tumors (SGTs) present with a great variety of morphological features, which make it very difficult to classify them. As a result, the pathological diagnosis of SGTs is challenging. The classification of SGTs is largely dependent on the histogenesis; however, the histogenesis of human SGTs is largely unknown. The aim of this study was to generate a SGT model, which could aid in understanding the histogenesis of these tumors. We hypothesized that the overexpression of PLAG1 in the acinar and ductal cells of normal salivary glands will lead to the generation of a SGT. Thus, a cell type-specific PLAG1 conditional knock-in mouse model was created by tamoxifen administration. Immunofluorescence revealed the co-expression of the acinar cell marker aquaporin 5 with the enhanced green fluorescent protein (EGFP) in the generated SGT. The acinar and ductal cell marker cytokeratin 18 was not detected; alternatively, weak expression of the myoepithelial marker cytokeratin 14 was observed, but it was not co-localized with EGFP. These results indicated the specific, though imperfect, differentiation of the SGTs into acinar cells, and no definite differentiation into myoepithelial cells. Thus, the generation of a SGT comprising acinar-like cells was accomplished by inducing the overexpression of PLAG1 in the acinar and ductal cells of normal salivary glands in mice.

唾液腺腫瘍モデルマウスの確立とその性状解析

唾液腺腫瘍の組織像は極めて多彩で腫瘍分類が複雑なため、病理医が診断に難渋することも多い。腫瘍分類が複雑な理由は唾液腺腫瘍の初期組織発生が未だ不明であるためである。唾液腺腫瘍の組織発生を詳細に理解するためには唾液腺腫瘍モデルマウスの実現が必須である。そこで我々は腫瘍原性を有するPLAG1を正常唾液腺組織の腺房および導管上皮細胞に過剰発現させることにより唾液腺腫瘍が作製できるのではないかとの仮説を立て、細胞種特異的にPLAG1を過剰発現するノックインマウスを作製した。Tamoxifen投与により誘導された腫瘍はaquaporin 5陽性、cytokeratin 18陰性を示した。cytokeratin 14は一部陽性像が認められたが、遺伝子組換えが生じた際に発現するEGFPとは共局在がみられなかった。これらの結果は作製された腫瘍が特異的ではあるが不完全な腺房細胞への分化を伴っており、筋上皮細胞への分化は伴わないことを示すものと考えられた。我々は正常唾液腺組織の腺房および導管上皮細胞にPLAG1を過剰発現させることにより、腺房様細胞からなる唾液腺腫瘍モデルを確立し得た。（本研究は所属機関動物実験委員会にて承認済）

Establishment of a murine salivary gland tumor model and its characterization

(Problem)

Salivary gland tumors present with a great variety of morphological features. This leads to a frequent overlap of the microscopic features among various salivary gland neoplasms, and sometimes, between benign and malignant lesions. As a result, the pathological diagnosis of a salivary gland tumor is challenging. The classification of a salivary gland tumor is largely dependent on its histogenesis. However, the histogenesis of human salivary gland tumors is largely unknown because they are generally well-developed during surgical resection. Therefore, the generation of a salivary gland tumor model, which could aid in understanding the histogenesis of these tumors, is of utmost importance.

(Hypothesis)

The PLAG1 gene, which is a crucial oncogene involved in the development of pleomorphic adenomas in salivary glands, was used to generate a salivary gland tumor model. We hypothesized that the overexpression of PLAG1 in the acinar and ductal cells of normal salivary glands will lead to the development of a salivary gland tumor.

(Methods)

A cell type-specific PLAG1 conditional knock-in mice was created to induce the overexpression of PLAG1 in the acinar and ductal cells of normal salivary glands. The mice were created by crossing PLAG1 conditional knock-in mice with Sox9-CreERT2 mice. Sox9 was expressed in the acinar and ductal cells of the normal salivary glands. Tamoxifen was intraperitoneally administered to the mice following which, the newly generated cell-type-specific PLAG1 conditional knock-in mice overexpressed PLAG1 and enhanced green fluorescein protein (EGFP) due to the removal of the floxed "stop cassette" by the tamoxifen-inducible CreERT2 in the Sox9 expressed cells (Figure 1). Thus, PLAG1 was overexpressed in the acinar and ductal cells of the normal salivary glands in the conditional mice following tamoxifen administration. Tumor formation was confirmed on the second day after tamoxifen administration, and the lesions were excised. The specimens were prepared and hematoxylin-eosin staining and immunofluorescence were performed.

Figure 1. Generation of cell type-specific PLAG1 conditional knock-in mice.

The newly generated cell type-specific PLAG1 conditional knock-in mice were created by crossing PLAG1 conditional knock-in mice with Sox9-CreERT2 mice. The mice overexpressed PLAG1 and enhanced green fluorescein protein (EGFP) after the removal of the floxed "stop cassette" by the tamoxifen-inducible CreERT2 in the Sox9 expressed cells.

(Results)

The newly generated cell type-specific PLAG1 conditional knock-in mice developed a large mass in the right cheek one week after confirmation of the development of a tumor (Figure 2a). A semi-macroscopic view of the generated tumor on the second day after tamoxifen administration revealed a well-circumscribed expansive mass in the submandibular gland indicating that the tumor had arisen from the salivary gland (Figure 2b), and was a primary salivary gland tumor. Histologically, the generated tumor presented with a fibrous capsule that separated it from the surrounding tissues (Figure 2c). The tumor cells consisted of acidophilic cytoplasm and eccentric nuclei, and formed an organoid pattern (Figure 2d). These findings suggested that the lesion was a benign epithelial neoplasm of the salivary gland.

The results of the immunofluorescence experiments showed that the acinar cell marker aquaporin 5 was co-expressed with EGFP (Figures 2e-g). The acinar and ductal cell marker cytokeratin 18 was not detected (Figures 2h-j). A weak expression of the myoepithelial marker cytokeratin 14 was noted in the cells, but it was not co-expressed with EGFP, which might be an indication of the presence of non-neoplastic myoepithelial cells in the lesion (Figures 2k-m).

(Conclusion)

The main finding of this study was the generation of a salivary gland tumor comprising acinar-like cells (acinic cell carcinoma-like tumor) by inducing the overexpression of PLAG1 in the acinar and ductal cells of normal salivary glands.

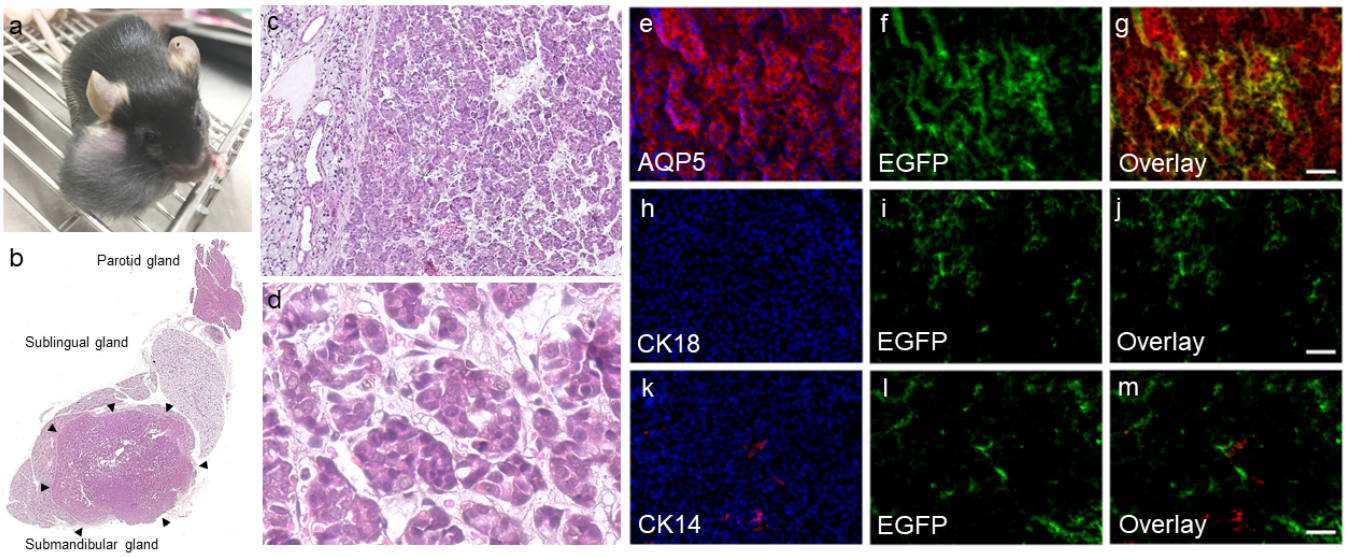


Figure 2. Characteristics of the generated salivary gland tumor.

(a) A large salivary gland tumor mass in a cell type-specific PLAG1 conditional knock-in mouse. (b-d) Semi-macroscopic (b) and microscopic (c, d) findings in the murine salivary gland tumor tissues. (e-m) Immunofluorescence showing the indicated proteins in the salivary gland tumors. AQP5; aquaporin5 (Red), CK18; cytokeratin 18 (Red), CK14; cytokeratin 14 (Red). DAPI staining (Blue) indicates nuclei. Scale bar: 50 μm.

Control of promotion/suppression for osteoclast differentiation based on identification of mechano-threshold

大阪歯科大学　4年生　Osaka Dental University　Class of 2022

鈴田 真裕　Mayu SUZUTA

共同研究者：田中 純生（4年生）
ファカルティー・アドバイザー：納富 拓也　薬理学講座 講師

Previous studies exploring bone mechano-signaling have focused on osteoblasts. However, there are several inconsistencies in reports on the relationship between mechanical stress (MS) and osteoclast differentiation. I hypothesized the presence of a mechano-threshold, which selectively supports the promotion or suppression of osteoclast differentiation. Based on this threshold, the control of osteoclastogenesis and mechano-signaling was investigated.

The ERK ratio (pERK/total ERK) increased at 5 min after exposure of RAW cells to stretch stimulus, suggesting that osteoclasts can sense MS. To identify the mechano-threshold and investigate the relationship between osteoclast differentiation and long-term MS, cells were exposed to the stretch stimulus in the presence of RANKL. MS in low repetition (1 set) promoted osteoclast differentiation, whereas that in high repetition (5 and 7 sets) suppressed the same; suggesting the existence of a mechano-threshold between the low and high repetition conditions. Further, the MS induced a long-term potentiation/suppression of the ERK ratio. These effects were diminished in the presence of inhibitors for mechano-sensitive ion channels. In case of MS above the mechano-threshold, NMDA glutamate receptors were found to affect MS-induced monotonic increase in ERK ratio, and membrane potential was depolarized. Light-induced membrane depolarization inhibited osteoclastogenesis in cells expressing channelrhodopsin.

Our findings suggest that the mechano-threshold helps discriminate between promotion and suppression of osteoclast differentiation. A mechano-memory, similar to brain-memory, could be activated in these cells on exposure to a MS above the mechano-threshold.

力学的閾値同定を主軸とした破骨細胞分化（促進/抑制）制御法の確立

骨中力学的刺激伝達機構の研究は、骨を作る骨芽細胞を中心に発展してきた。骨を壊す破骨細胞について、力学的刺激との関連性は、現在まで一致しない結果であり、不明確である。本研究では、破骨細胞分化方向を決定する力学的閾値を仮定して、閾値を活用した分化制御法の確立とその力学的刺激伝達機構について検討した。前破骨様細胞のRAW細胞は、力学的刺激（伸展刺激）に反応してERK比率が増加するが、10-15分後に刺激前のレベルに戻った。長期的な力学的刺激と破骨細胞分化の関係を検討するために、分化誘導期間を通して1日1回の伸展刺激を行った。刺激条件の違いで分化促進もしくは抑制が生じて、分化方向を逆転させる力学的閾値の存在が認められた。この作用は、力学的刺激応答性イオンチャネル阻害薬により消失し、閾値以上の刺激では、脳内記憶機構と類似してNMDA受容体の関与と細胞膜電位の脱分極が認められた。光刺激による細胞膜電位の脱分極は破骨細胞分化を抑制した。これらの結果より、破骨細胞には力学的閾値が存在し、閾値以上/以下の刺激を用いることで分化制御を可能とした。また、閾値以上の刺激では脳内記憶機構と類似した力学的刺激記憶機構の存在が示唆された。

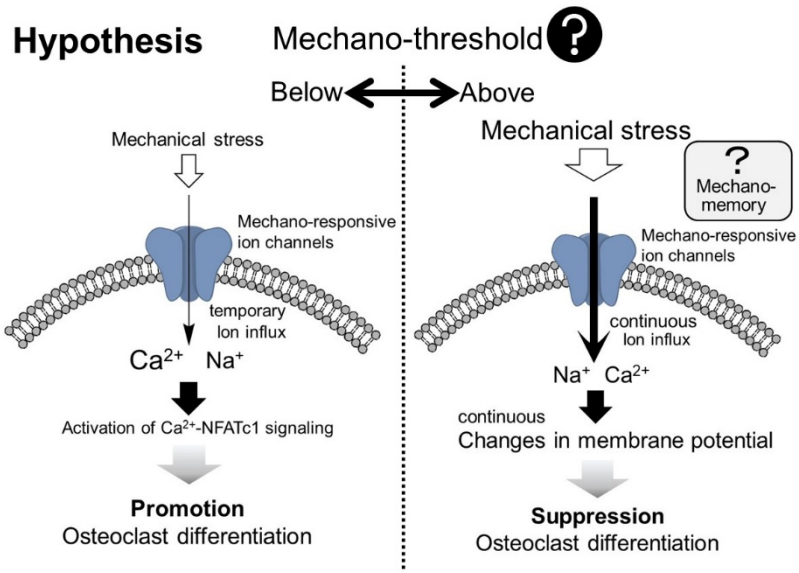
Control of Promotion/Suppression for Osteoclast Differentiation Based on Identification of Mechano-threshold

(Problem)

Bone mass is maintained by bone-forming osteoblasts and bone-resorbing osteoclasts. The imbalance between the osteoblasts and osteoclasts causes bone loss and subsequent osteoporosis. Many studies have reported on the therapeutic effect of mechanical stress, such as resistance exercise, on osteoporosis which acts by increasing bone formation and decreasing bone resorption. However, the underlying mechanism remains unclear. Although effects of mechanical stress on osteoblast differentiation and function are partly known, results from similar studies on osteoclasts are inconsistent; with mechanical stress reported to promote or suppress osteoclast differentiation. Further, some reports indicate that the decreased bone resorption in response to mechanical stress is due to global hormonal changes, and is not a direct consequence of mechanical stress. Therefore, it is unclear if osteoclasts can directly sense mechanical stress. Against this backdrop, I investigated the relationship between mechanical stress and osteoclast differentiation with the overall objective of understanding how mechano-signaling may influence the latter.

(Hypothesis)

Previous reports exploring these phenomena were inconsistent with respect to the parameters of induced mechanical stress, such as intensity, time and type of stimulus, used in the work. To account for these variations, I hypothesize the presence of a mechano-threshold for interpretation of the relationships between the mechanical stress and osteoclast differentiation (Fig. "Hypothesis"). This could be conceptualized by the following scenarios:



1: When osteoclasts sense mechanical stress below the mechano-threshold, mechano-responsive ion channels open temporally leading to ion flux. Osteoclast differentiation is promoted due to the resultant increase in intracellular Ca^{2+} concentration and the activation of the Ca^{2+}-NFATc1 signaling.

2: In reverse case (of mechanical stress above the mechano-threshold), mechano-responsive ion channels are continuously activated, and continuous ion flux occurs. In such a scenario, osteoclast differentiation is suppressed as a response to long-term membrane depolarization and the activation of mechano-signaling (mechano-LTP: mechano long-term potentiation). This phenomenon is similar to brain-LTP which is based on the learning and memory system. The long-term changes in signaling pathways in response to mechanical stress are, therefore, collectively defined as "mechano-memory".

(Methods)

Pre-osteoclast like RAW264.7 (RAW) cells subjected to the stretch stimulus (a type of mechanical stress) were used for the following experiments:

A: To confirm the short-term mechano-response, total protein was extracted from the cells at periods of 5, 10, and 15 min after a single incident of exposure to mechanical stress. The levels of phosphorylated ERK (pERK) and total ERK (ERK ratio: pERK/total ERK), which indicates activation of intracellular mechano-signaling, were quantified by western blotting.

B: Mechano-thresholds were identified based on the application of multiple types of stretch stimulus in the presence of RANKL. TRAP activity, recognized as a biochemical marker of osteoclast differentiation, was measured at 7days post culture.

C: Long-term activation of mechano-signaling was confirmed by the extraction of total proteins 24 h after the last cycle of stretch stimulus, followed by the estimation of the ERK ratio. Changes in membrane potential were measured using a membrane potential-sensitive fluorescent dye.

D: The relationship between the membrane potential and osteoclast differentiation was investigated using RAW cell stably transfected with the light-responsive ion channel, channel-rhodopsin. A light stimulus was applied to cells to induce membrane depolarization aftter addition of RANKL.

(Results)

1: To examine the short-term mechano-response, stretch stimulus ("5% intensity-5min" × 1-5 sets) was applied to RAW cells. Increase in pERK levels was detected at 5 min after the induction of mechanical stress; although pERK levels were the same as those for the control (without stimulus) at 10-15 min after the stimulus. Therefore mechano-signaling in response to short-term stimulus lasts 10 to 15 min. This suggests that osteoclasts can sense mechanical stress.

2: To identify the mechano-threshold and confirm the effects of long-term mechanical stimulus on osteoclast differentiation, stretch stimulus (once/day for 7 days) was applied to cells during the induction of osteoclast differentiation. Osteoclast differentiation was promoted in case of single exposure to the "5% intensity-5 min" condition (Fig.1; 2nd column from the left). However, osteoclast differentiation was suppressed in other condition ("5% intensity-5min" × 5 or 7 sets) (Fig. 1; 4th and 5th columns from the left). These results indicate that the mechano-threshold regulating osteoclastogenesis exists between these conditions ("5% intensity-5min" × 1-5set).

Fig. 1

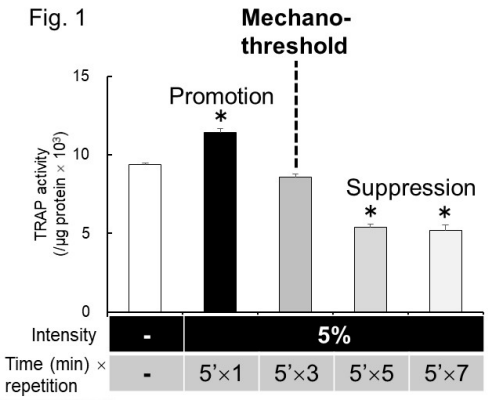


Fig. 2

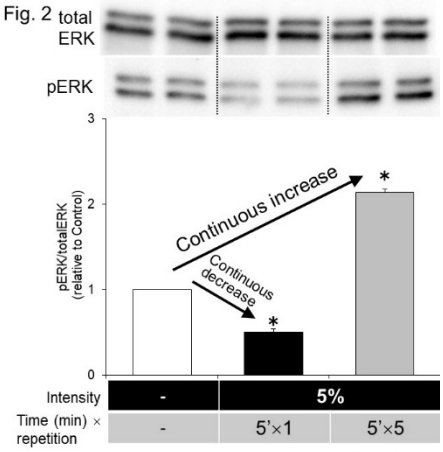
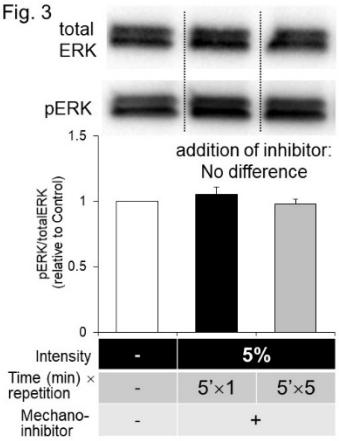


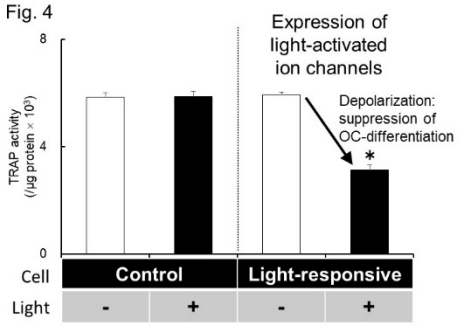
Fig. 3



3: The mechano-threshold is believed to establish a balance between the promotion and suppression of osteoclast differentiation. To clarify this possibility, the ERK ratio was quantified in RAW cells after exposure to a stretch stimulus below/above the mechano-threshold. Although the short-term mechano-response lasted for only 10-15min, long-term mechanical stress below/above the mechano-threshold (once/day for 7days) decreased/increased the ERK ratio even at 24 h after the last set of stretch stimulus application (Fig. 2; 2nd and 3rd columns from the left). These continuous changes in the ERK ratio indicate that osteoclasts possess a mechano-memory which, analogous to brain memory, memorizes exposure to mechanical stress.

4: Next I focused on the class of the membrane proteins termed as mechano-responsive channels. Membrane proteins are molecules involved in the early stage of mechano-signaling and act as the first line of response. Addition of a reversible inhibitor (Gd^{3+}; 1μM) during exposure to stretch stimulus diminished mechanical stress-induced changes in both the TRAP activity and the ERK ratio (Fig. 3; 2nd and 3rd columns from the left). These results suggest that mechano-responsive channels have important roles in the early stages of osteoclastic mechano-signaling. In brain-LTP based on learning and memory, NMDA glutamate receptors (NMDA) are known to assume critical functions. During the exposure to the mechanical stress above the mechano-threshold, the addition of a reversible NMDA inhibitor (AP5; 1μM) diminished the increase in the ERK ratio and the suppressive effects on osteoclastogenesis. Further the membrane potential in these cells was continuously depolarized, similar to that observed in brain-LTP. These results strongly suggest that mechano-LTP is analogous to brain-LTP, with important contributions in osteoclastic mechano-signaling.

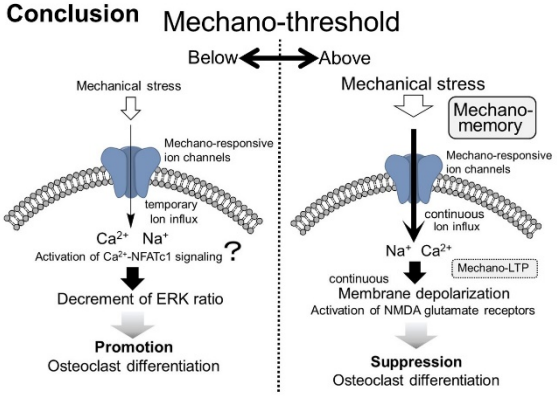
Fig. 4



5: The relationship between membrane depolarization and osteoclast differentiation was examined using optogenetics-based approaches. When a light stimulus (5 sets/day; 1set: "2sec-ON, 1sec-OFF" × 10 times) was applied to RAW cells expressing channel-rhodopsin, osteoclast differentiation was suppressed by light-induced membrane depolarization (Fig. 4). This indicates that the observed changes in membrane potential affects osteoclastogenesis.

(Conclusion)

Taken together, these results show that osteoclasts possess a mechano-threshold which discriminates between promotion and suppression of osteoclast differentiation (Fig. Conclusion). Depending on this mechano-threshold, osteoclast differentiation could be controlled by mechanical stress in a context-dependent manner. Especially in cases of exposure to mechanical stress above the mechano-threshold, mechano-LTP (similar to brain-LTP characterized by continuous membrane depolarization and the contribution of NMDA) occurs and osteoclastogenesis is inhibited. The present findings suggest that osteoclasts have a mechano-memory in the form of long-lasting intracellular mechano-signaling.



上位入賞結果

● 優勝：基礎部門 第1位

北海道大学歯学部 6年生　吉野 弘菜 さん
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

● 準優勝：臨床部門 第1位

九州大学歯学部 5年生　高本 侑立子 さん
欠損補綴治療による起立運動機能への影響

● 基礎部門 第2位

大阪歯科大学 4年生　鈴田 真裕 さん
力学的閾値同定を主軸とした破骨細胞分化（促進/抑制）制御法の確立

※臨床部門 第2位 該当なし

優勝/基礎部門第1位　北海道大学歯学部　吉野 弘菜 さん


二次審査風景

審査講評

コロナ禍の令和2年度でしたが、将来を嘱望されるスチューデント・クリニシャン（SC）のために何とかSCRP日本代表選抜大会を実現させようと何度も検討し、結論としてオンライン審査にて開催するという運びになりました。その結果、臨床部門5名、基礎部門13名にエントリーいただき、感謝申し上げます。

一次審査といたしましては、SC18名に発表ビデオと事前抄録をご提出いただき、基礎部門と臨床部門の審査員により厳正に審査いたしました。その結果、臨床部門からは1名、基礎部門からは2名を選出させていただきましたが、SC全員の研究内容は甲乙つけがたく、大変苦労したことを付け加えさせていただきます。

二次審査におきましては、審査員長および副審査員長司会のもと、3名のSCがオンラインにて発表を行ない、次いで6名の審査員による質疑応答を行いました。審査では英語での発表および研究の内容等を審査し、審査員6名の合計点を算出し、最高点と最低点（審査員2名分）を排除し、残りの審査員4名の合計点の平均点から順位を決定させていただきました。

今回オンラインによる審査を行ないましたが、様々な問題点があり、SCならびに指導された先生方に多大なるご迷惑をお掛けいたしました。今後は更なる検討を加え、より良い開催ができるようにと考えております。

副審査員長　井上　孝

審査員一覧

審査員長	平野　裕之*	京都府開業
副審査員長	井上　　孝*	東京歯科大学　名誉教授
審査員	小川　祐司*	新潟大学大学院医歯学総合研究科　予防歯科学分野　教授
審査員	林　美加子*	大阪大学大学院歯学研究科　歯科保存学教室　教授
審査員	上條竜太郎	昭和大学歯学部　口腔生化学講座　教授
審査員	澁川　義幸	東京歯科大学　生理学講座　教授
審査員	影山　幾男	日本歯科大学新潟生命歯学部　解剖学第1講座　教授
審査員	細川　隆司	九州歯科大学　口腔再建リハビリテーション学分野　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）学術大会における発表に移行し、Student Competition for Advancing Dental Research and its Application（SCADA）と更に名称変更となりました。

SCRPは約60年間に世界5大陸に拡大し、オーストラリア、オーストリア、ブラジル、カナダ、中国、デンマーク、英国、エストニア、フィジー、フィンランド、フランス、ドイツ、香港、アイスランド、インド、インドネシア、アイルランド、日本、ラトビア、リトアニア、マレーシア、メキシコ、ミャンマー、オランダ、ニュージーランド、ノルウェー、フィリピン、シンガポール、南アフリカ、韓国、スウェーデン、スイス、台湾、タイ、トルコ、ベトナムで開催されました。各国代表は米国大会においてコンペティションは行なわれず、招待発表ならびに学術交流機会を得ることができます。

日本においては1995年に日本歯科医師会主催、デンツプライジャパン株式会社（現：デンツプライシロナ社）後援により米国と同様SCPの名称で第１回日本代表選抜大会が開催され、4校の参加でスタートしました。長年に亘る同社からの後援を経て、第26回となる本年度から日本代表選抜大会は本会による単独開催となりました。新型コロナウイルス感染症に大きな影響を受けながらも18校からの参加があり、来場を伴わない2次審査方式により開催する運びとなりました。

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

1995年（平成7年度）　第1回大会　歯科医師会館
日本歯科大学歯学部（当時）5年生　河野 智子 さん

歴代優勝者/日本代表 （敬称略）

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”

第 21 回（H.27/2015 年）■東京医科歯科大学歯学部　田中 大貴

閉経後骨粗鬆症モデルにおける FactorX 発現制御機構

Daiki TANAKA – Tokyo Medical and Dental University Faculty of Dentistry

“The FactorX expression mechanisms in a model of postmenopausal osteoporosis”

第 22 回（H.28/2016 年）■鹿児島大学歯学部　神園 藍

Syk 活性阻害は間葉系幹細胞の骨分化を促進し脂肪分化を抑制する

Ai KAMISONO – Kagoshima University Faculty of Dentistry

“Syk inactivation induces to promote osteogenic differentiation and suppress adipogenic differentiation of mesenchymal stem cells”

第 23 回（H.29/2017 年）■広島大学歯学部　吉野 舞

単一細胞トランスクリプトミクスによる骨芽細胞の多様性の解析

Mai YOSHINO – Hiroshima University School of Dentistry

“Single-cell transcriptomics Uuncovers the diversity of osteoblasts”

第 24 回（H.30/2018 年）■北海道大学歯学部　阿部 未来

骨リモデリングとモデリングの骨芽細胞活性化における細胞学的相互作用

Miki ABE – Hokkaido University School of Dental Medicine

Cellular interaction activating osteoblastic bone formation during bone modeling and remodeling

---

第 25 回（R.1/2019 年）■広島大学歯学部　前川原 思惟子

***Porphyromonas gingivalis*（*P.g.*）-fimA type2 と type4 血清抗体価の上昇は歯周炎の関連する早産のマーカーとなる**

Shiiko MAEKAWARA – Hiroshima University School of Dentistry

***Porphyromonas gingivalis (P.g.)*-fimA (Type2 and Type4) serum antibody titer is a possible marker for preterm birth associated with periodontitis**

参加大学関係者一覧

大学	学長/学部長	ファカルティー・アドバイザー	研究指導協力者	スチューデント・クリニシャン	共同研究者	SC No.
北海道大学歯学部	八若 保孝	網塚 憲生 長谷川 智香	本郷 裕美	吉野 弘菜	―	14
岩手医科大学歯学部	三浦 廣行	入江 太朗 衣斐 美歩	佐藤 泰生	羽金 雅登	―	17
東北大学歯学部	髙橋 信博	鈴木 敏彦 小坂 萌	髙田 雄京 高橋 正敏	古内 聖弓	中村 直太郎	5
奥羽大学歯学部	大野 敬	福井 和徳	川鍋 仁 山野辺 晋也	今井 千穂子	―	3
日本大学松戸歯学部	小方 頼昌	小林 良喜	―	植草 信乃介	―	12
東京医科歯科大学歯学部	依田 哲也	田畑 純 井関 祥子	杉浦 真琴 坂口 もも子	渡部 準也	―	4
日本大学歯学部	本田 和也	津田 啓方	―	黒澤 佑介	山口 裕史	16
昭和大学歯学部	槇 宏太郎	吉村 健太郎	宮本 洋一 山田　篤	根岸 宗一郎	―	10
松本歯科大学	川原 一祐	中村 浩彰	―	小野 亜美	―	7
大阪大学歯学部	今里 聡	村上 智彦	西村 理行	都原 志穂	中谷 鞠子	6
大阪歯科大学	川添 堯彬	納富 拓也	野﨑 中成	鈴田 真裕	田中 純生	18
岡山大学歯学部	長塚 仁	岡村 裕彦	池亀 美華 福原 瑶子	村田 志穂	―	9
徳島大学歯学部	宮本 洋二	藤猪 英樹	村上 圭史 廣島 佑香 Omar Marianito Maningo Rodis	喜田 悠太	―	8
九州大学歯学部	中村 誠司	大木 郷資 古谷野 潔	荻野 洋一郎 竹村 陽子 今井 実喜生	高本 侑立子	小野 涼平 遠藤 真緒	1
九州歯科大学	西原 達次	角舘 直樹	―	高田 知佳	―	2

大学	学長/学部長	ファカルティー・アドバイザー	研究指導協力者	スチューデント・クリニシャン	共同研究者	SC No.
福岡歯科大学	髙橋 裕	稲井 哲一朗 田中 芳彦	永尾 潤一	池本 梨央南	畳屋 有希 中村 麻衣	11
長崎大学歯学部	村田 比呂司	住田 吉慶	Montenegro Raudales Jorge Lui	園木 美結	ー	13
鹿児島大学歯学部	西村 正宏	松口 徹也	千葉 紀香	佐藤 大幹	林 瑶大	15

SCADA-Japanへようこそ

SCADA Associates in Japan
代表　井田 有亮

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

各校代表として研究発表に臨まれたStudent Clinician・Co-Clinicianの皆様は、大変多忙な学生生活の中にあって研究活動に精励され、発表に至る貴重な経験を蓄積されたことと拝察いたします。さらに、本年は世界規模の感染症流行下において、例年に比べて一段と活動に制約が加わる状況の中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。また、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。

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。今回は集合しない新し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ました。本年10月16日（金）にオンラインによるオリエンテーションを開催し、SCADAの歴史と活動の紹介に加え、初の試みとしてSCRP大会参加各校からの研究チーム紹介、研究内容紹介、発表に対する質疑が盛んに行われました。この度の経験を活かして交流の輪を自大学以外へと広げていただきたいと考えます。

末筆ながら、20年余にわたってSCRP日本代表選抜大会を継続的に主催してこられた公益社団法人日本歯科医師会、そして、ご多忙の中にあっても熱心に学生の研究指導に当たられている各校のファカルティー・アドバイザーの先生方に、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年には日本の会員により、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。Student Clinicianは、SCADAおよびSCADA-Japanに自動入会となり、Membership Certificateを得ることができます。

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

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

開催日：2021年8月20日（金）※

場　所：歯科医師会館

※令和3年度 日本代表選抜大会は8月20日（金）の開催予定ですが、新型コロナウイルス感染症が関係機関に影響を及ぼしている場合は、令和2年度同様、日程の変更および開催形式（審査方法）が「手引き」記載の方法から変更になる場合がありますので、ご了承ください。

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

あとがき

本研究発表抄録集を取りまとめている今、この困難な状況のもと参加してくださった学生諸君、指導教官の先生方への感謝の気持ちと、一つのイベントをなんとか成功裡に終えることができた安堵感で一杯になっています。

今回はこれまでにない初めての開催形式を取りましたが、今後起こるであろう社会情勢の変化に日本歯科医師会は柔軟に対応し、若い世代が国際社会へ羽ばたく姿をこれからも喜んで応援いたしたいと考えています。参加された各位におかれましては今回の経験を糧にし、ご自身の活躍の場を拡げていただくと共に、ぜひ皆さんのDNAを後輩に紡いでいただきますようお願いいたします。

昨年末に本会が単独開催することが決まり、短い準備期間にSCRP本来の姿を残したコンペティションを開催できたのは担当部署の日本歯科医師会国際渉外委員会と事務局の尽力の賜物であることを申し添えます。

末尾になりますが、皆様の益々のご発展ご活躍を祈念し、あとがきに代えさせていただきます。

令和2年度SCRP 審査員長　平野　裕之

SCRP日本代表選抜大会 研究発表抄録集
令和2年度号 [通巻26号]

国際渉外委員会
委員長　平野　裕之
副委員長　井上　　孝
委員　倉治 ななえ
委員　岩崎 万喜子
委員　小川　祐司
委員　林　美加子

担当役員
副会長　佐藤　　保
常務理事　尾松　素樹
理事　伊藤　明彦
理事　佐藤 真奈美
（所管・学術課/国際渉外関係）

令和2年11月20日発行
編集兼発行人　尾松素樹
発行所　日本歯科医師会
〒102-0073 東京都千代田区九段北4丁目1番20号
TEL：03（3262）9212　FAX：03（3262）9885
©2020 日本歯科医師会
ホームページアドレス　https://www.jda.or.jp/

本誌掲載記事の転載・複製の際は、あらかじめ日本歯科医師会（学術課/国際渉外関係 **電話03-3262-9212**、**Eメール：scrp@jda.or.jp**）にご連絡のうえ許諾をお求めください。但し、スチューデント・クリニシャン（SC）の抄録ページについては、作成を行ったSC本人および当該抄録作成に係った大学関係者等が使用する場合を除きます。

https://www.jda.or.jp