様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　３ 年　 ５ 月 １２ 日現在

機関番号：３０１１０
研究種目：研究活動スタート支援
研究期間：2019～2020
課題番号：１９Ｋ２４１２７

研究課題名（和文）The in vitro/in vivo study of CCN2 on dentin regeneration

研究課題名（英文）The in vitro/in vivo study of CCN2 on dentin regeneration

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究課題では、MDPC‐23細胞、ヒト歯髄幹細胞およびWistarラットに対する新規生物活性物質のin vitro／in vivo効果を検討した。新規接着性モノマーCMETを用いたin vitro研究では、CMETがMDPC-23細胞の増殖、分化および石灰化を促進することが明らかになり、Int Endod Jに掲載された。またヒト歯髄幹細胞に対してCMETは低細胞毒性を有し、象牙芽細胞分化と石灰化に関して高い活性を示すことがわかった。in vivo研究では、CMETによる直接覆髄が象牙質橋形成を効果的に誘導することが明らかになり、特許出願中であり、論文もSCI雑誌に投稿中である。

研究成果の学術的意義や社会的意義
The successful completion of these studies will provide valuable results for the investigation of the development of novel dental materials. Besides, the result of these studies may change the current situation of direct pulp capping, and also provide new ideas for clinical treatments.

研究成果の概要（英文）：I investigated the in vitro/in vivo effect of novel bioactive materials on MDPC-23 cells, human dental pulp stem cells (hDPSCs), and wistar rats as planned. The investigation of a novel adhesive monomer CMET on MDPC-23 cells has revealed that CMET stimulated MDPC-23 cells proliferation, differentiation and mineralization. The results have been published on on International Endodontic Journal (Int Endod J. 2020 Oct; 53(10):1413-1429). The investigation on hDPSCs has showed that CMET has low cytotoxicity and exhibits high activity on stimulation of odontogenic differentiation and matrix mineralization. The in vivo study on wistar rats showed that direct capping with CMET can effectively promote dentin bridge formation.The results of the effect of CMET on dentin regeneration is under submission to SCI journal and patent application.

研究分野：口腔科学およびその関連分野

キーワード：dental materials　dentin regeneration　odontoblasts　direct pulp capping　adhesives

１．研究開始当初の背景

Direct pulp capping has been used for the treatment of pulp exposed by deep dental caries or traumatic injury. Under conditions of severe damage, newly differentiated odontoblast-like cells may migrate to sites of injury, where they are thought to form reparative dentin that protects pulp tissue from stimuli.

In 1930, Hermann discovered that calcium hydroxide (CH) is effective in repairing an exposure site. Since then, CH has been widely used for direct pulp capping treatment because of its broad antimicrobial spectrum, strong sterilization function, minute irritation, strong penetration and convergence capacity. However, the long-term success rate of CH-based treating modality appears to be low as the follow-up period increases at least partly due to its poor adhesion to dentin tissue, tunnel defects formed in dentin bridges and dissolution away shortly after placement.

Mineral trioxide aggregate (MTA) is one of the most popular material recently. The mechanism of MTA is similar to CH. Although its therapeutic performance is better than that of CH, but it still has some disadvantages including expensive price, long setting times, poor handling, and coronal tooth discoloration.

Adhesive system has been one of the most important success elements for endodontic treatment. They exhibit several advantages, including their high mechanical strength, easy application, and ability to be light cured immediately, providing superior adhesion to peripheral hard tissues and an effective seal against microleakage. Most of the monomer in the composite resin and bonding agent has been proved to be cytotoxic to pulp tissue, which means the penetration of these monomers from dentin-bond interface to pulp tissue will cause inflammation even cell necrosis.

２．研究の目的

① To study the in vitro effects and possible mechanism of novel bioactive materials on odontoblast-like cells and human dental pulp stem cells.

② To study the therapeutic effect of novel bioactive materials on dental pulp cells and to explore a new effective method for clinical treatment of direct pulp capping.

３．研究の方法

This research was divided into three independent experiments. In experiment 1, we studied the in vitro effect of novel bioactive materials on odontoblast cells. In experiment 2, we studied the effect of novel bioactive materials on human dental pulp stem cells, and explored the possible mechanism of these materials on pulpitis development and prognosis. In experiment 3, animal experiment was carried out to investigate the therapeutic effect of novel bioactive materials on dental pulp cells and to explore a new effective method for clinical treatment of direct pulp capping.

４．研究成果

First, the investigation of a novel adhesive monomer CMET, calcium salt of 4-methacryloxyethyl trimellitic acid (4-MET) on MDPC-23 cells has revealed that CMET

not only stimulated MDPC-23 cells proliferation but also augmented odontogenic potential and mineral nodule formation. 4-MET is one of the commonly used adhesive monomer, the calcium salt of 4-MET eliminates its strong acidity which is cytotoxic in high concentration. Inhibition of the p38 MAPK signaling pathway significantly depressed CMET-induced ALPase activity and mineralization, suggesting that CMET can promote intracellular Ca^{2+} homeostasis and provide a signal for activation of downstream events that promote odontoblast differentiation and matrix mineralization. These results have been published on International Endodontic Journal (Int Endod J. 2020 Oct; 53(10):1413-1429).

Second, the investigation of CMET on hDPSCs has showed that this novel bioactive monomer CMET has low cytotoxicity, and exhibits high activity on stimulation of odontogenic differentiation and matrix mineralization. The data showed that inhibition of p38 and JNK signaling completely suppressed the matrix mineralization of hDPSCs, which accelerated by CMET. On the other hand, inhibition of p38 signaling also suppressed the CMET-induced ALPase activity to a relatively low level through the whole culture period. Inhibition of JNK pathway reduced the CMET-enhanced ALPase activity by one third on day 14, and to the control level on day 21. Besides, inhibition of the NF-κB pathway dramatically suppressed the CMET-induced odontogenic differentiation of hDPSCs, as indicated by downregulation of odontogenic markers and reduced hDPSCs mineral deposition. The findings confirm the underlying signaling pathways that involved in this process.

Third, the in vivo study of CMET on wistar rats was performed. A mechanical pulp exposure model was established on 8-week-old male wistar rats. The histomorphological changes of dental pulp tissue of wistar rats at different time points after direct capping with CMET was compared to that of calcium hydroxide. After 14 days of operation, dentin-associated partial hard tissue bridge was found near the exposure site of the calcium hydroxide treated group, with a local inflammatory process. The CMET group exhibited the presence of thin complete bridge invading the pulp space to the opposite dentin wall, adjacent pulp tissue appeared normal. At day 28, irregular hard tissue bridge without obvious tubular structure was observed in the calcium hydroxide group. Continuous thick dentin bridge and tertiary dentin were seen in the CMET group, with well-distinguishable dentinal tubules. Intact odontoblastic layer was observed aligned along the periphery of the pulp, and adjacent to the dentinal bridge. Hard tissue bridge was formed faster, thicker, and with a better structure in the CMET group compared to that in the calcium hydroxide group. The findings herein provide evidence that direct capping with CMET can effectively stimulates the odontogenic differentiation potential of rat dental pulp stem cells and promote dentin-like hard tissue formation, and the cells secreting this structure displayed a polarized odontoblastic characteristic. The results of the in vitro and in vivo effect of CMET on dentin regeneration is under submission to SCI journal and patent application.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　1件）

１．著者名 Qiu Y. J.、Tang J.、Saito T.	４．巻 53
２．論文標題 A novel bio‐active adhesive monomer induces odontoblast differentiation: a comparative study	５．発行年 2020年
３．雑誌名 International Endodontic Journal	６．最初と最後の頁 1413～1429
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/iej.13365	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Altankhishig Bayarchimeg、Polan Mohammad Ali Akbor、Qiu Youjing、Hasan Md Riasat、Saito Takashi	４．巻 14
２．論文標題 Dentin Phosphophoryn-Derived Peptide Promotes Odontoblast Differentiation In Vitro and Dentin Regeneration In Vivo	５．発行年 2021年
３．雑誌名 Materials	６．最初と最後の頁 874～874
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ma14040874	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

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３．学会等名 第151回日本歯科保存学会2019年度秋季学術大会
４．発表年 2019年

〔図書〕　計0件

〔出願〕　計1件

産業財産権の名称 象牙芽細胞増殖・分化誘導剤	発明者 斎藤隆史、邱友靖 等	権利者 同左
産業財産権の種類、番号 特許、2019-193019	出願年 2019年	国内・外国の別 国内

〔取得〕　計0件

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関