Potential of amelogenin protein in emdogain as a trigger factor for reparative dentine formation

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Abstract

Background: Profound caries with perforations due to iatrogenic factors should be treated with direct pulp capping (DPC) immediately before infection occurs due to bacterial contamination so that the prognosis will be better. Currently the material that is often used and becomes the gold standard is calcium hydroxide. The author wants to provide a material selection solution for DPC that can reduce tunnel defects, reduce inflammation, form more reparative dentin, and make work easier. One of the materials that have this ability is emdogain.

Aim: This undergraduate thesis aims to explore the potential of amelogenin as the main component of emdogain material in increasing the formation of reparative dentine.

Methodology: Literature search begins by compiling problem, intervention, comparison, and outcome (PICO). The inclusion criteria used were journals published within the last 10 years (2013-2022), types of journals research journals, case reports, and reviews, full paper accessible, and using Indonesian and English.

Results: 44% of the literature supports the efficacy of emdogain as a direct pulp capping material, 72.2% of the literature supports that amelogenin protein can induce the formation of reparative dentin, and 27.7% of the literature supports minimal levels of inflammation/hypersensitivity.

Conclusion: Amelogenin in emdogain has the potential to increase reparative dentin formation by forming initiation signals resembling normal dentinogenesis processes.

Keywords: profound caries, reparative dentin, emdogain, and amelogenin

Introduction

Dental caries is a non-communicable disease characterized by progressive demineralization of teeth which is influenced by various factors, namely the host, agent, environment, and time [1-6]. In deep caries, there will be damage to the dentine and death of the primary odontoblast cells so that these odontoblast cells will be replaced by progenitor cells/stem cells which will differentiate into odontoblast-like cells which will trigger the formation of reparative dentine.

Deep caries with perforation can occur due to iatrogenic perforation caused by operator error or pathological perforation caused by an extensive caries process. This often causes deep caries conditions with perforations, direct pulp capping treatment must be carried out immediately before infection occurs due to bacterial contamination so that the prognosis will be better [1, 3, 5, 6, 8].

Direct pulp capping treatment is a simpler, less time consuming and economical treatment option for patients compared to pulpotomy or root canal treatment. Several indications for direct pulp capping (DPC) treatment are asymptomatic and vital teeth, small exposed pulp size <0.5 mm and no bacterial contamination, and easily controlled bleeding within 10 minutes [6, 9-11].

There are many pulp capping materials have been used for DPC treatment. The selection of pulp capping material needs to be considered because it will affect the success of the pulp capping treatment. This pulp capping material must have several requirements, namely biocompatible, antibacterial, radiopaque in nature, preventing microleakage and having bioactive ingredients that can stimulate the formation of reparative dentine [6, 9, 12].

Currently, calcium hydroxide is often used as a gold standard material because it is relatively inexpensive, easy to obtain, and has good antibacterial properties in direct pulp capping treatments. These materials have drawbacks, namely their bond to dentin is weak, there are tunnel defects in the dentinal bridge, and poor sealing quality. Calcium hydroxide is also known to cause severe irritation to the pulp tissue and form a necrotic layer. Reparative dentine produced by calcium hydroxide material has a porous structure which will increase the risk of bacterial infection. Other materials such as mineral trioxide aggregate (MTA) are also often used in direct pulp capping treatments. This material has the advantages of good biocompatibility, minimal pulp inflammation, more predictable hard tissue barrier formation, and antibacterial properties. While the disadvantages are that it is more

expensive, the process is more difficult, the setting time is longer, discoloration occurs, and the solubility is high [13-17].

The author wants to provide a material selection solution for DPC that can reduce tunnel defects, reduce inflammation, form more reparative dentine, and make work easier. One of the materials that have this ability is emdogain. Emdogain has the main content of amelogenin (about 95%) and other non-amelogenin content (tuftelin, ameloblastin, enamelin, and other non-amelogenin proteins). This biomaterial is in the form of an injectable gel solution consisting of enamel matrix protein (amelogenin), water and a carrier (propylene glycol alginate). According to several studies, amelogenin was allegedly able to stimulate the formation of reparative dentine. Amelogenin can mimic epithelial-mesenchymal interaction by triggering the release of autocrine pleiotrophic growth factor (TGF-β) and cytokines (BMP) which will regulate the regenerative process by resembling the process of odontogenesis through the differentiation of pulp mesenchymal stem cells into odontoblast-like cells.

However, this emdogain has several drawbacks, namely the high price, poor sealing quality, the hard tissue barrier formed is ineffective and still weak, and the high protein content which may cause hypersensitivity [14, 15, 18-20].

Emdogain gel when applied to exposed pulp without the additional use of pulp capping materials has proven to be ineffective in producing a hard tissue barrier due to poor sealing quality, so it can be used in combination with existing pulp capping materials such as MTA. Mineral trioxide aggregate (MTA) produced a better quality reparative hard tissue response with the addition of emdogain compared to calcium hydroxide. Mixing emdogain with MTA can reduce tunnel defects in calcium hydroxide because it can result in the formation of carious tissue with better sealing quality [13, 15, 18-20].

Although several studies have revealed the role of amelogenin as the main compound of emdogain which can stimulate reparative dentin formation, the number of human studies is still limited, so the potential of emdogain compared to calcium hydroxide as a pulp-capping material is still being debated. In addition, as far as the authors know, there has been no literature review that can draw conclusions from all studies regarding the potential for amelogenin in emdogain as a pulp capping material, so the authors need to study further regarding the potential for amelogenin in emdogain to be used as a pulp capping material.

Materials and methods Inclusion criteria

- Journals published within the last 10 years (2013-2022).
- Types of research journals, case reports, and reviews.
- The journal is fully accessible.
- Discusses the effect of the amelogenin protein contained in the emdogain material resulting in the formation of reparative dentin.
- The journal is presented in Indonesian and English.

Exclusion criteria

Journals that do not meet the inclusion criteria are declared as exclusion criteria.

Research strategy

A literature search was conducted through the PubMed, Science direct, Proquest and Scopus databases. Some of the keywords used to refer to the title are "profunda caries", "reparative dentine", "emdogain", and "amelogenin". After entering the keyword, the first number of literature searches will appear, then a duplication screening is carried out followed by filter applications available in the database in the form of year of publication and type of literature. Literature that passes is then subjected to selection of abstracts and full papers that are adjusted to the inclusion criteria to test the eligibility of the literature. Literature that has passed through the whole process is then used as a reference for writing essays. The information obtained is then collected and synthesized into a new conclusion.

Results

Search results and article selection

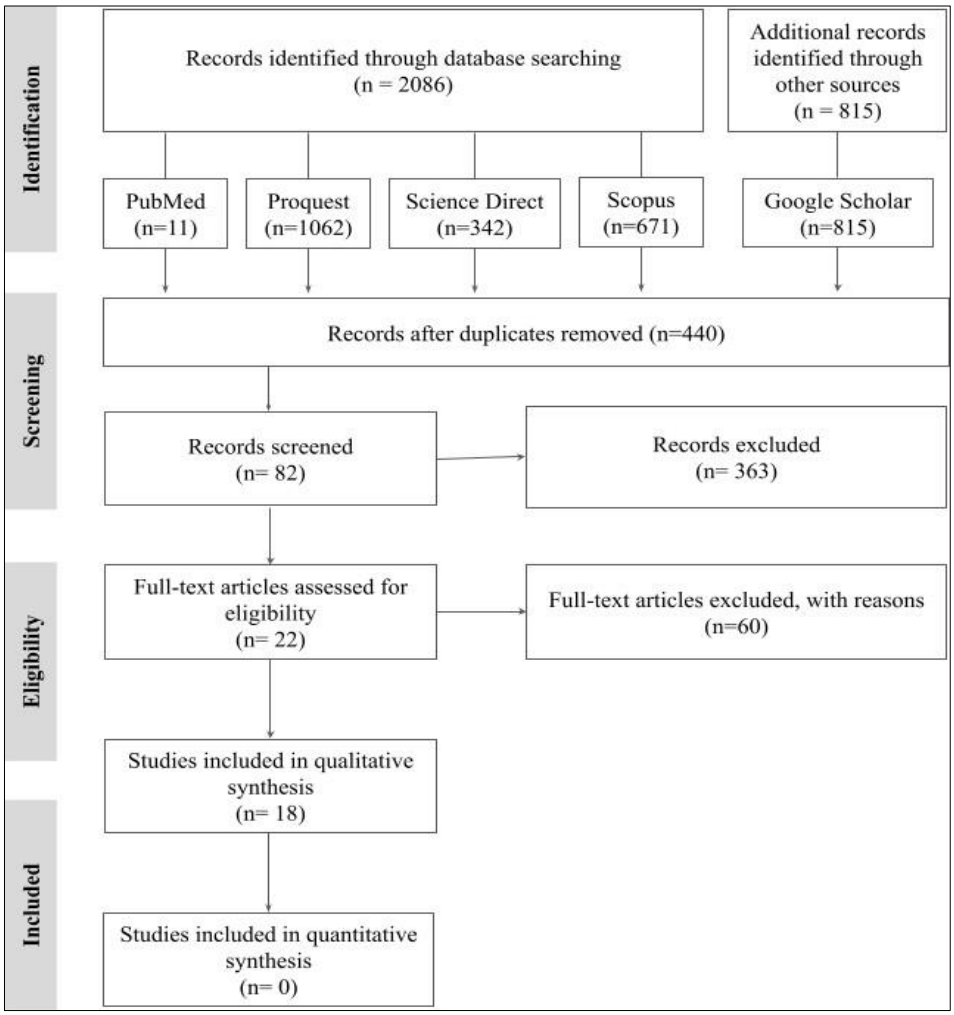

Fig 1

From searching articles with predetermined keywords, namely "deep caries", "reparative dentine", "emdogain", and "amelogenin", the article identification stage obtained the number of articles from identification through the PubMed database = 11, Science-direct = 342, ProQuest = 1062, and Scopus = 671 and through manual identification (Google Scholar) there were 815, so that a total of 528 articles were obtained. The screening stage was carried out with the filter features available in the database in the form of year of publication (2013- 2022) and type of article (research journals, case reports, and reviews), a total of 528 articles were obtained. After duplication was removed, 440 articles were collected which were then selected based on title. A total of 82 articles were selected based on the title. A total of 36 articles were selected based on abstracts. A total of 18 articles were used. Figure 1 shows the results of searching and selecting articles through the PRISMA flowchart.

Search results 18 articles

The search results for 18 articles yielded 10 literature reviews consisting of 3 systematic reviews and 7 narrative reviews and there were 8 original research articles. Data extracted from 18 articles yielded 8 supporting hypotheses, 1 unsupported hypothesis, 7 questionable hypotheses, and 2 undetermined data regarding the efficacy of EMD as a DPC. Data extracted from 18 articles yielded 13 supporting hypotheses, 2 unsupported hypotheses, and 3 undetermined data regarding amelogenin protein that can induce the formation of reparative dentine. Data extracted from 18 articles yielded 5 supporting hypotheses, 2 unsupported hypotheses, 1 questionable hypothesis, and 10 indeterminate data regarding minimal levels of inflammation/hypersensitivity. The experimental animals used were 2 rats, 1 rabbit, 2 pigs, and 2 dogs from 4 journals. Available markers are BMP, TGF- β, FGF, CC, DSP, collagen I, DMP 1, Interleukin (IL- 1, IL-6, IL-8, IL-11), MCP-1, TNF- α, ANKH, OCN, RUNX2, BSP, ALP, OPN, VEGF, and ERK 1/2.

Discussion

The protein amelogenin is the main enamel matrix protein and makes up about 90% of the total enamel matrix protein and plays a role in mineralization and changes in enamel morphology. Amelogenin will be expressed during embryonic development, caries occurs, and injury after dental cavity preparation. Amelogenin can also be found in odontoblasts that differentiate during predentin formation (at the early bell stage) and will be lost in mature odontoblasts and distributed in dentinal tubules (T.A. Mitsiadis *et al*., 2014) [21]. This indicates that amelogenin derived from the ameloblastic layer can be distributed and translocated to the odontoblast and dentine

layers. In caries teeth, it shows that newly formed odontoblasts facing injury/caries will express de novo amelogenin, this shows that amelogenin is involved in the process of dentine repair. Odontoblasts damaged during cavity preparation will be in the dentinal tubules and will die by apoptosis, so that the amelogenin will go to the dentin matrix and form the initiation signal for the formation of reparative dentin (T.A. Mitsiadis *et al*., 2014) [21]. The protein amelogenin stimulates mesenchymal progenitor cells which will become odontoblast-like cells [15,21,22]. Studies regarding amelogenin expression in dentine are still limited.

Amelogenin protein is the main component contained in the enamel matrix derivative material (Emdogain) which is thought to induce reparative dentinogenesis and is involved in the growth and maturation of dental pulp cells during odontogenesis [17, 23-25]. Amelogenin can play an important role in the formation of dentin during dentinogenesis by mimicking normal dentinogenesis (K. Al-Hezaimi, *et al.,* 2013) [26]. According to Song *et al*., 2017 [14], this amelogenin component can mimic epithelial-mesenchymal interactions by triggering the release of several growth factors (TGF-β) and cytokines (BMP) which will trigger the differentiation of human dental pulp stem cells (HDPSC) into odontoblasts like cell. TGF-β plays a role in cell signaling that stimulates matrix formation and mineralization. BMP plays a role in stimulating odontoblast differentiation and formation of reparative dentin. Amelogenin plays an important role in dentinogenesis by inducing cell signaling that stimulates matrix formation and mineralization [22, 23, 25-28]. However, in a study conducted by Frasheri *et al*., 2016 [29], amelogenin cannot induce the formation of mineralized nodules in HDPSC, amelogenin can enhance the mineralization process. In several studies, high expression of dentin sialoprotein (DSP) and collagen type I was found which are indicative biomarkers of odontoblast proliferation and dentine formation [16, 19, 27, 29-31].

According to the journal (da Rosa *et al*., 2018 and Gaber, 2022) [24, 15], Enamel matrix derivative (EMD or emdogain) can be suggested as a pulp capping material due to the presence of amelogenin in its composition which is involved in the growth and maturation of dental pulp cells during odontogenesis. Emdogain also contains transforming growth factor-beta 1 (TGF-β1) which is involved in cell signaling to stimulate matrix formation and mineralization (K. Al-Hezaimi, *et al.*, 2013) [26]. Emdogain can prolong dentine mineralization and facilitate reparative dentine formation, as well as reduce proinflammatory cytokine production [13, 14, 17, 32, 33]. EMD can activate the expression of genes involved in pulp cell proliferation and differentiation (E.A. Riksen *et al*., 2014) [27]. The regenerative process of EMD consists of odontoblast differentiation with dentine formation and pulp healing without affecting the vitality of the remaining pulp in a manner similar to normal dentinogenesis (K. Al-Hezaimi, *et al.,* 2013) [26]. Emdogain has a positive effect on genes that regulate tooth hard tissue mineralization and facilitate pulpal innervation (E.A. Riksen *et al*., 2014) [27]. This supports the clinical results of EMD inducing less pain/hypersensitivity experienced after its use and increasing reparative dentine formation. In the use of EMD material it was observed that there were newly formed blood vessels just below the odontoblast-like cell zone and were detected in the center of the pulp which indicated remodeling of the pulp tissue (M.P. Bajić *et al*., 2015) [23]. In a study of human premolars in which calcium hydroxide and EMD pulp capping materials were treated, it was shown that these two materials had the ability to induce the formation of tertiary dentine (M.P. Bajić *et al*., 2015) [23].

Emdogain is considered to be better than calcium hydroxide when used as pulp capping [17, 26, 27, 30]. Several animal studies have shown that EMD is more effective than calcium hydroxide and MTA (S. Najeeb *et al*., 2017) [30]. Teeth treated with EMD in the first two weeks showed less sensitivity (M.P. Bajić *et al*., 2015) [23]. Emdogain can give good results after the first month of administration, but its efficacy will decrease over time which could be due to the inflammatory effect on EMD (S. Najeeb *et al*., 2017) [30]. In the material test study for the EMD group and the MTA group the results were similar and showed that a dentine bridge was formed (S. Najeeb *et al*., 2017) [30]. Emdogain could be a promising alternative to MTA and Biodentine in increasing pulp repair capacity after dental pulp injury. However, further future studies are needed to assess clinical outcomes and compare them with in vitro findings (T.A. Mitsiadis *et al*., 2014) [21]. (A.R. Youssef *et al*., 2019) [16]. However, according to (Najeeb *et al*., 2017) [30] the results of a histological evaluation of EMD materials as human pulp capping materials still found no evidence of being superior to other materials and the level of inflammation in the use of EMD was also known to increase (such as calcium hydroxide and MTA). Emdogain when used on exposed pulp without the use of additional pulp-capping materials proved to be ineffective in producing a hard tissue barrier due to poor sealing quality (M. Singhal *et al*., 2015) [32]. According to (Najeeb *et al*., 2017) [30], in his literature study it was stated that the exposed pulp involved for direct pulp capping (DPC) was no larger than 2 mm. Assessment of direct pulp capping (DPC) in larger pulp exposures (more than 2 mm in diameter) will require further clinical examination (K. Al- Hezaimi, *et al.*, 2013) [26]. Small exposed pulp will prevent bacterial contamination which means it will reduce the failure of dentin bridge formation. This shows that the amount of exposed pulp will affect the success rate of DPC. Recent studies have shown that the combination of capping materials with EMD will improve capping quality by increasing the biocompatibility of capping materials such as Ca(OH)2 to induce healing of pulps with calcification (E.A. Riksen *et al*., 2014) [27].

The results of the data extracted from 18 articles regarding the efficacy of EMD as a DPC, amelogenin protein which can induce the formation of reparative dentin, and minimal levels of inflammation/hypersensitivity of EMD materials. The supporting hypothesis can be influenced by the presence of amelogenin, propylene glycol alginate (PGA), and TGF-β content of EMD. This amelogenin can trigger signaling of HDPSC cells which will turn into odontoblast-like cells. These odontoblast-like cells will induce the process of reparative odontogenesis to form reparative dentine. Propylene glycol alginate (PGA) has antibacterial properties that can prevent bacterial contamination so that it will facilitate proper dentin bridge formation. TGF-β plays a role in cell signaling that stimulates matrix formation and mineralization. In addition, the efficacy of this EMD material is also influenced by good cavity sealing using glass ionomer cement under aseptic working conditions, good immune status of experimental animals/humans, and minimal exposed pulp size. The hypothesis that does not support it can be caused by bacterial

contamination that occurs in the pulp chamber caused by the expansion of the exposed pulp due to caries and iatrogenic and is influenced by the anatomical conditions of primary teeth that are different from permanent teeth. The pulp chamber of primary teeth is larger and the pulp horns are closer to the outer surface of the tooth than permanent teeth, so that bacterial contamination can occur more easily, where the inflammatory effect that occurs can inhibit the formation of dentine bridges. The data is still questionable because not many clinical trials have been conducted and the effect of EMD is unclear. The data cannot be determined due to the absence of any of the three discussions regarding the efficacy of EMD as a DPC, amelogenin protein which can induce reparative dentine formation, and the minimal level of inflammation/hypersensitivity of EMD material in the literature.

As far as the authors know, there are still few studies in humans, so there is still debate about the efficacy of emdogain and further research/testing of EMD materials is necessary. In several literature and research studies, the amelogenin component in EMD has been shown to be a triggering factor for the formation of reparative dentin in minimally exposed pulps. Emdogain has the potential to be used for direct pulp capping. However, there are still few studies regarding EMD materials for DPC in humans so that the efficacy and safety cannot be concluded and if EMD is used as the 'material of choice' for DPC, further clinical trials with a long-term duration are still needed.

Conclusion

Thus, it can be concluded that amelogenin in emdogain has the potential to increase reparative dentine formation by forming initiation signals resembling normal dentinogenesis processes, namely by epithelial-mesenchymal interactions by triggering the release of several growth factors (TGF-β) and cytokines (BMP) which can stimulate differentiation. human dental pulp stem cells (HDPSC) to become odontoblast-like cells. These odontoblast-like cells induce the process of reparative odontogenesis to form reparative dentine. However, the emdogain material is still not effective as the material of choice for DPC.

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