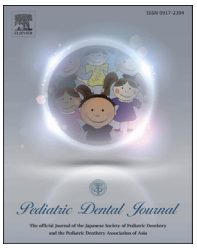
PEDIATRIC DENTAL JOURNAL XXX (2018) 1–7

Available online at www.sciencedirect.com

Pediatric Dental Journal

journal homepage: www.elsevier.com/locate/pdj



Research Paper

Preventive effects of iontophoresis on bovine enamel decalcification through enhancing uptake and transportation of fluoride -*in vitro* study

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ARTICLE INFO

Article history:
Received 9 May 2017
Received in revised form
25 January 2018
Accepted 19 February 2018
Available online xxx

Keywords:
Iontophoresis
Fluoride
Enamel
Decalcification
Decalcification depth

ABSTRACT

Background: Topical fluoride application has been performed all over the world especially in the field of Pediatric Dentistry to prevent enamel decalcification. However, the effect of the method against demineralization has not been sufficiently reported yet. This study was performed to evaluate the effectiveness of the method by measuring the uptake amount of fluorine (F) into enamel, and also to investigate the relationship between the amount of F incorporated and the decalcification depth.

Methods: Bovine incisors were immersed in 2% fluoride solution in the absence (the immersion method) or presence (the iontophoresis method) of iontophoresis. Sample teeth were then decalcified in 0.1 M lactic acid solution. The F concentration was measured using a fluorine ion meter, while atomic absorption spectrophotometry was used to determine calcium (Ca) concentrations.

Results: When the decalcification time was 15 min, the uptake of F was significantly higher in the iontophoresis method than in the immersion method. Furthermore, the decalcification depth was markedly shallower with the iontophoresis method than with the immersion method. In immersion method, no changes were observed in the uptake of F between decalcification times of 5 and 15 min regardless of immersion times. F uptake levels were dependent on the current-carrying time. A clear inverse correlation was observed between F uptake levels and the decalcification depth ($r = 0.967$).

Conclusions: Iontophoresis increased the uptake of F and enhanced its penetration into the enamel, thereby decreasing decalcification by acid. The study showed the effectiveness of the Iontophoresis method.

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https://doi.org/10.1016/j.pdj.2018.02.001
0917-2394/

1. Introduction

Fluorine (F) is an essential trace element that is required to maintain normal growth in the skeletal system [1,2] and reduce the incidence rate of caries [3–5]. Decalcification by caries is fatal for teeth because they are the most calcified tissue in the human body and hardly metabolize after being formed. Therefore, strategies to prevent caries are important. The application of fluoride has been shown to very effectively inhibit caries and is in widespread clinical use especially in the field of Pediatric Dentistry, even though several other trace elements such as strontium, aluminum, and zinc have also been identified as cariostatic agents [6–8].

The role of F and its mechanism of action in inhibiting caries have been elucidated in detail: when fluoride is applied topically, F^- ions replace OH^- ions in apatite crystals at the tooth surface and within enamel to form fluoroapatite (or fluoridated hydroxyapatite), which markedly stabilizes crystals and reduces their solubility, ultimately inhibiting caries [9]. Calcium fluoride (CaF_2) is also formed, and subsequently acts as an enamel protector against acids or as a pH-controlled reservoir of fluoride to be released during cariogenic challenges [10,11]. Moreover, F– was previously shown to decrease the growth rate of dental plaque as well as the synthesis of acid and bacterial intracellular polysaccharides in dental plaque [12].

Topical fluoride applications such as toothpaste, gels, varnishes, or mouth rinse are typically used in homes, dental offices, and schools for public health dentistry. It's believed that iontophoresis should increase the uptake and penetration of F into enamel because iontophoresis devices incorporate the principle of electrophoresis; therefore, fluoride-iontophoresis is often used as a means of promoting the incorporation of more F into enamel at dental clinics in Japan. However, few studies have examined the relationship between iontophoresis and F uptake in enamel; therefore, this relationship remains controversial and unclear. A few studies reported positive effects: in animal studies, fluoride-iontophoresis significantly enhanced the effects of F [13], iontophoresis enhanced the activity of F with respect to its uptake and retention in the enamel surface *in vitro* [14], and active lesions at the incipient caries level were clinically reduced after treatments using iontophoresis [15]. In contrast, Kim et al. [16] showed that fluoride-iontophoresis was not significantly superior to the conventional application of fluoride in terms of remineralization effects, and another study reported no difference in surface microhardness between fluoride-iontophoresis and other topical fluoride applications [17]. These studies also used different approaches.

Therefore, we examined the effects of iontophoresis on the uptake of F into enamel, and determined how acid resistance was affected by the amount of F incorporated using an experimental system that was as close as possible to that used in clinical practice.

2. Materials and methods

2.1. Collection of bovine teeth

Two hundred and fifty sound teeth without cracks or white spots were extracted from bovines that had been processed for consumption. Since the bovines were sacrificed NOT for the present study, the researchers did not have to get permission by the ethical committee of Kyushu Dental University. To avoid the effects of differences in species, age, and tooth type, the mandibular central incisors of 25- to 32-month-old Japanese black cattle were collected from May 2012 to February 2013.

The whole procedure was carried out at Kyushu Dental University. Just after the teeth collection, plaque, connective tissue, and other substances had been eliminated from sample teeth using a toothbrush, scalpel, and polishing brush, and each sample was ultrasonically washed with ultrapure water three times for 5 min each time. The samples were then stored individually in a polypropylene vessel (Sanplatic, Osaka, Japan) with physiological saline at 4 °C for approximately 1 wk until use, and saline was exchanged every 2–3 d.

2.2. Fluoride treatment

A commercial 2% NaF solution (Neo-dental.com, Tokyo, Japan) was used as the fluoride solution. The sample tooth root was covered with gauze impregnated with saline 3 or 4 mm away from the cervical portion, and the root surface between the cervical portion and gauze was sufficiently dried to prevent the leakage of current. To determine the effects of iontophoresis on F uptake, the crown portion of one mandibular incisor was immersed in 5 mL of fluoride solution in a pure titanium container (immersion method), and an electric current was applied to the other mandibular incisor from the same individual by holding the gauze surrounding the root in the anode of the iontophoresis device (Pyo-cure®, Narcohm, Japan) and connecting the container with the cathode while the tooth was immersed (iontophoresis method). A microammeter was inserted in the line to confirm that an accurate current was flowing to the tooth. An electric current of 200, 400, or 500 μA was delivered to the samples, and the current-carrying and immersion times were 3, 5, or 10 min. The samples were randomly assigned to respective electric current and immersion time. After the uptake of F, samples were washed with pure water 8 times, and subsequently washed with ultrapure water 3 times.

Classification of the samples was shown in Table 1.

2.3. Decalcification

The root was cut off using a diamond disk. The sound enamel surface was covered with vinyl tape, and the rest was coated with quick cure resin (Shofu, Kyoto, Japan). After polymerizing the resin, the tape was peeled off of the sample and its area was calculated using a planimeter (Marble, Sydney, Australia). Each sample was washed with ultrapure water. This ensured that only the healthy enamel region was exposed to 0.1 M lactic acid solution (decalcification solution) for decalcification. Each sample was immersed in 25 mL of decalcification solution adjusted to pH 3.0 or 5.0, and shaken slowly at 37 °C for 5 or 15 min.

2.4. Determination of F and Ca concentrations

After decalcification, 2.5 mL of TISAB buffer was added to the decalcification solution. F levels eluted from the enamel were

Please cite this article in press as: Tanaka T, et al., Preventive effects of iontophoresis on bovine enamel decalcification through enhancing uptake and transportation of fluoride -*in vitro* study, Pediatric Dental Journal (2018), https://doi.org/10.1016/j.pdj.2018.02.001