ARCHIVES OF ORAL BIOLOGY 59 (2014) 1085–1093

Available online at www.sciencedirect.com

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Effect of generation 4.0 polyamidoamine dendrimer on the mineralization of demineralized dentinal tubules *in vitro*

Ru Jia [a,b], *Yi Lu* [a], *Chang-wei Yang* [c], *Xiao Luo* [b], *Ying Han* [a,*]

[a] *Department of Prosthodontics, Stomatological Hospital, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi, China*

[b] *Department of Physiology and Pathophysiology, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China*

[c] *Department of Prosthodontics, Xiamen Stomatological Hospital, Xiamen, Fujian, China*

ARTICLE INFO

Article history:
Accepted 17 June 2014

Keywords:
Dentine sensitivity
Tooth remineralization
Amides
Dendrimers

ABSTRACT

Objective: Dentine hypersensitivity is a type of clinical oral disease, which is highly prevalent worldwide. Although there are many materials to treat dentine hypersensitivity, their long-term therapeutic effects are not satisfactory. Therefore, the aim of this research was to observe and identify the biological mineralization of the generation 4.0 polyamidoamine dendrimer on the demineralized dentinal tubules at different time points.

Design: 2 mm-thick slices were obtained from the cemento-enamel junction of 36 third molar teeth that simulated the condition of sensitivity with acid etching. Slices were treated with generation 4.0 polyamidoamine dendrimer and peptide bond condensing agent, while no treatment was applied on the slices of the control group. Following immersion in artificial saliva for 2, 4, 6, and 8 weeks respectively, the mineralization condition of dentine slices was observed using the scanning electron microscope (SEM). In addition, the differences in the samples of dental slices between the 2 groups were also detected using the microhardness test.

Results: SEM results showed that the average diameter and density of the dentinal tubules in the experimental group were significantly lower than those in the control group ($P < 0.001$). The microhardness test exhibited a similar result, which suggested that the microhardness of the experimental group was significantly higher than the control group ($P < 0.001$).

Conclusion: Generation 4.0 polyamidoamine dendrimer promotes the biomineralization of demineralized dentinal tubules. Moreover, this result also suggests that the 4.0th generation polyamidoamine dendrimer has the potential value for dentine hypersensitivity treatment.

* *Corresponding author at*: Department of Prosthodontics, Stomatological Hospital, College of Stomatology, Xi'an Jiaotong University, No. 98 Xiwu Road, Xi'an 710004, Shaanxi, China. Tel.: +86 29 87218541.

E-mail addresses: jiaru123@stu.xjtu.edu.cn (R. Jia), luyi1962@mail.xjtu.edu.cn (Y. Lu), ychangwei@stu.xjtu.edu.cn (C.-w. Yang), xluo@mail.xjtu.edu.cn (X. Luo), yinghan1185@gmail.com (Y. Han).

Abbreviations: PAMAM, polyamidoamine dendrimer; G, generation; SEM, scanning electron microscope; CEJ, cemento-enamel junction; HAP, hydroxyapatite; NCP, noncollagenous protein; EDC, 1-(3-dimethyl amino propyl)-3-ethyl carbon imine hydrochloride; NHS, N-hydroxy succinimide; Mw, molecular weight; IR, infrared spectrum; NMR, nuclear magnetic resonance.

http://dx.doi.org/10.1016/j.archoralbio.2014.06.008
0003–9969/

1. Introduction

Dentine hypersensitivity is a very common oral clinical disease that makes patients experience a form of abnormal pain associated with a variety of exogenous stimuli, such as cold, heat, acid, sweet, and mechanical stimulation.[1] Nowadays, there are several principle clinical treatments for dentine hypersensitivity, such as desensitizing the nerve tissue within the tubules using potassium nitrate, irradiating Nd:YAG or other lasers, coating fluoride on the surface of the tooth, and so on.[2–5] However, these treatments have limited long-term curative efficacy since they lack the functions involved in blocking or closing the open dentinal tubules with inorganic minerals, and reducing the flow rate of liquid within the dentinal tubules.[6,7]

The biomimetic mineralization that has been developed in recent years performs the above functions. This strategy induces *in situ* remineralization of dentine by biomaterials through an 'amorphous precursor pathway' that is bioinspired from the function of non-collagenous proteins (NCPs) in the biomineralization process.[8,9] Based on such findings, the key point of biomimetic mineralization is to seek the proteins which will be the regulating template for the mineralization of inorganic crystals.[10,11] Since it is too difficult to extract or purify the natural proteins efficiently, it is essential to design and construct an organic macromolecular structure that can mimic the self-assembly behaviour and the morphology of the natural proteins.

Polyamidoamine dendrimer (PAMAM) is a new kind of hyperbranched macromolecular compound, which has well-defined spherical structures that symmetrically spread outwards from the central nucleus.[12] With a similar monodispersity to proteins, PAMAM is always referred to as "artificial proteins" by some researchers, and has been widely studied and applied in pharmacy as a carrier of drugs or genes in recent years.[13] According to the theory of biomineralization, the mineral process is usually modulated by a series of NCPs. PAMAM is characterized by the presence of internal cavities, and a large number of reactive end groups. These structures allow PAMAM to be used as biomimetic macromolecules, which are rigid enough to fulfil the dual function of natural NCPs in the biomineralization process, *i.e.*, sequestration of mineral ions and subsequent templating capability.[14]

Generation 4.0 polyamidoamine dendrimer (G4.0 PAMAM) is suitable to be the template for biomineralization due to the rigid biomimetic "worm-like" macromolecules and the ability of G4.0 PAMAM to mimic the assembly process of proteins. G4.0 PAMAM also acts as an analogue of non-collagenous proteins in the biomineralization field with the same biological function.[15] However, the effect of G4.0 PAMAM on occluding the dentinal tubules *in vitro* is not well-known.[16] In order to confirm the role of G4.0 PAMAM in the biomineralization of dentine, we examined the effect of G4.0 PAMAM on closing the open dentinal tubules using the scanning electron microscope (SEM) and the statistical method of micro-hardness testing. This work may provide the foundation for innovative therapeutic strategies to improve dentine hypersensitivity using PAMAM.

2. Materials and methods

2.1. Sample selection

The teeth used in this study were provided and prepared by the Department of Oral and Maxillofacial Surgery, Stomatological Hospital, College of Medicine, Xi'an Jiaotong University, China. 36 freshly extracted human third molar teeth were collected for surgical reasons at random, after informed consent had been obtained for use in this study.

2.2. Sample preparation

All the teeth were cleaned thoroughly and stored in the Ringer's solution at 4 °C for no more than a month prior to use. We set up the model of dentine hypersensitivity according to the method of Mordan.[17] The external soft tissue and debris were removed using a scaler. The 36 teeth were then cut perpendicular to the long axis of the tooth in a buccal–lingual direction, above the cemento-enamel junction (CEJ), by means of a low-speed water-cooled diamond saw (Isomet® low speed saw, Buehler, Lake Bluff, IL, USA) into 36 dentine discs, each with a thickness of 2.0 mm approximately.

After preparation of the teeth, both sides of each dentine disc were sanded with 600, 700, 800, 1000, and 1200 grit silicon carbide paper, sequentially for 30 s, under constant water irrigation to create a standard smear layer. The smear layer was subsequently removed by washing 3 times in the ultrasonic cleaners for 10 min each. The dentine discs were then rinsed 3 times with mass deionized water and dried at a low temperature soon afterwards. An appropriate quantity of 37% phosphoric acid solution was applied with a small brush to the occlusal surface evenly, and left for 10 s to remove the residual smear layer as well as to open the dentinal tubules.[17] The specimens then were rinsed 3 times with mass deionized water and blow-dried gently. Each dentine disc was numbered at random and cut to 2 similar halves along the central line by a low-speed water-cooled diamond saw. One half of the disc was marked as A and the other half was marked as B. The A category of 36 dentine discs formed the control group; while the B category constituted the treatment group. Each of the specimens was placed in a 0.5 ml centrifuge tube that had been already numbered for further use.

At the beginning of the treatment, the dentine specimens of the B group were immersed in the mixture of the 4.0th polyamidoamine dendrimer (G4.0 PAMAM), the peptide bond condensing agent (1-(3-dimethyl amino propyl)-3-ethyl carbon imine hydrochloride, EDC and N-hydroxy succinimide, NHS), for 30 min, while the specimens of the A group (control) were immersed in deionized water for 30 min at the same time. G4.0 PAMAM, applied in this research, was synthesized in our laboratory by a divergent growth method with ethylenediamine as the core. With a molecular weight (Mw) of 14,196, G4.0 PAMAM contains 64 primary amino groups on the periphery theoretically. The structure of PAMAM was characterized by infrared spectrum (IR), nuclear magnetic resonance (NMR), and terminal analysis (data not shown). And its terminal group in this study was $-NH_2$ as its original production that had not been modified. In the second step, all