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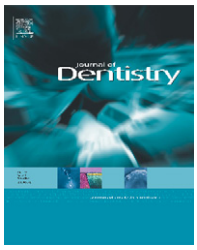

Enamel diffusion modulated by Er:YAG laser (Part 2). Organic matrix

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SUMMARY

Organic matrix (OM) has been hypothesized as a key player in the laser-induced retardation of enamel diffusion (LRED).

Objectives: Therefore, this study was aimed to quantify the contribution of OM in LRED.

Methods: Four groups of enamel sections ($n = 10$) were assigned to 'normal', 'laser treated', 'OM extracted' and 'laser + OM extraction' groups for measurement of diffusion coefficient (DC) using fluorescence recovery after photobleaching (FRAP) and fluorophores transport study (FTS). Er:YAG laser treatment and OM extraction were performed on respective groups. Sections were characterized with stereomicroscopy and polarized light microscopy. Treatment effects were statistically assessed with a factorial ANOVA.

Results: DC measured by FRAP and FTS coupled with confocal microscopy revealed the significant effect of OM ($p = 0.001$) and laser treatment ($p < 0.01$). After OM extraction, the laser effect on diffusion decreased about 34–75%, confirming the significant role of OM in LRED.

Conclusion: Both FRAP and FTS may be promising tools to quantify enamel DC.

1. Introduction

In the past, the role of the organic matrix (OM) in caries prevention was assumed to be negligible since it constituted only a tiny fraction of enamel. But later, it was postulated that OM might play a significant role in protecting enamel demineralization,[1] facilitating mineral re-deposition and controlling pathways of diffusion.[2,3]

In the past few decades, the laser-induced caries prevention has been repetitively demonstrated. The laser treatment may reduce enamel solubility through change of crystalinity[4] and reduction of carbonate content in hydroxyapatite crystals.[5] However, the effect of laser on enamel diffusion remains controversial with reports of both reduced permeability[6–8] and increased permeability.[9]

Enamel diffusion channels are occupied by a macromolecular network of organic material,[10] which may play a role in controlling enamel diffusion (ED). It was postulated that laser-induced retardation of enamel demineralization might be related to the transformation of organic matter.[6] The contribution of OM in laser-treated enamel has been demonstrated to be at least 25% and 57% in inhibiting mineral loss and lesion progression, respectively.[8] However, its effect on enamel diffusion has never been quantified. Traditional methods of measuring enamel diffusion are time-consuming and suffered from inaccuracies and/or irreproducibility.[9,10] In our previous study, FRAP has been applied successfully to quantify site-specific enamel diffusion. The fluorophore transport study (FTS) is another fluorescence technique coupled with confocal laser scanning microscopy (CLSM) to

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quantify time-dependent diffusion through the intact natural surface of tooth without active intervention or disequilibriation. The purpose of this study is to employ these two fluorescence techniques, FRAP and FTS, coupled with CLSM to quantify the role of OM in Er:YAG laser-induced retardation of enamel diffusion.

2. Materials and methods

2.1. Samples Preparation

Twenty sound human posterior teeth collected under the guidelines approved by the Institutional Review Board of the National University of Singapore, (NUS-IRB 04-106E), were stored in a 0.1% thymol solution (Kanto Chemicals Co., Inc. Tokyo, Japan) and selected under a stereomicroscope (SZ4045TR, Olympus, Japan). They were cut disto-mesially into two halves by an alloy grinder (DEMCO, Bonsall, Califf, USA) and sliced into 200 ± 10 μm-thick sections with a Silverstone-Taylor hard-tissue microtome (series 1000 Deluxe, Sci Fab, CO, USA). The flowchart of the procedure is shown in Fig. 1.

2.2. OM extraction

The OM extraction was carried out as described by Hsu et al.,[8] which consisted of a protein extraction (with NaOCl solution) sandwiched between two lipid extractions (with chloroform/methanol solvent). But for enamel sections used for the FRAP study, the procedure was simplified with the first chloroform/methanol treatment (two 4-h intervals) followed by a NaOCl treatment (two 1-h periods) before three successive chloroform/methanol treatment (1 h each).

2.3. Laser treatment

Sections were treated with Er:YAG laser 2.94 μm (Fotona Fidelis®, M002-1A, Slovenia), using a very short pulse of 50 mJ, 5 Hz for 5 s with water mist (~10 ml/min) on cut enamel surface for FRAP ($n = 10$) and 80 mJ, 5Hz for 5 s with attenuator of 10% transmission (Dielectric mirror, Einst, USA) on the natural surface of FTS samples ($n = 10$). The spot size was ~500 μm in diameter and the energy density were 26.45 J/cm^2 and 4.08 J/cm^2 for FRAP and FTS, respectively.

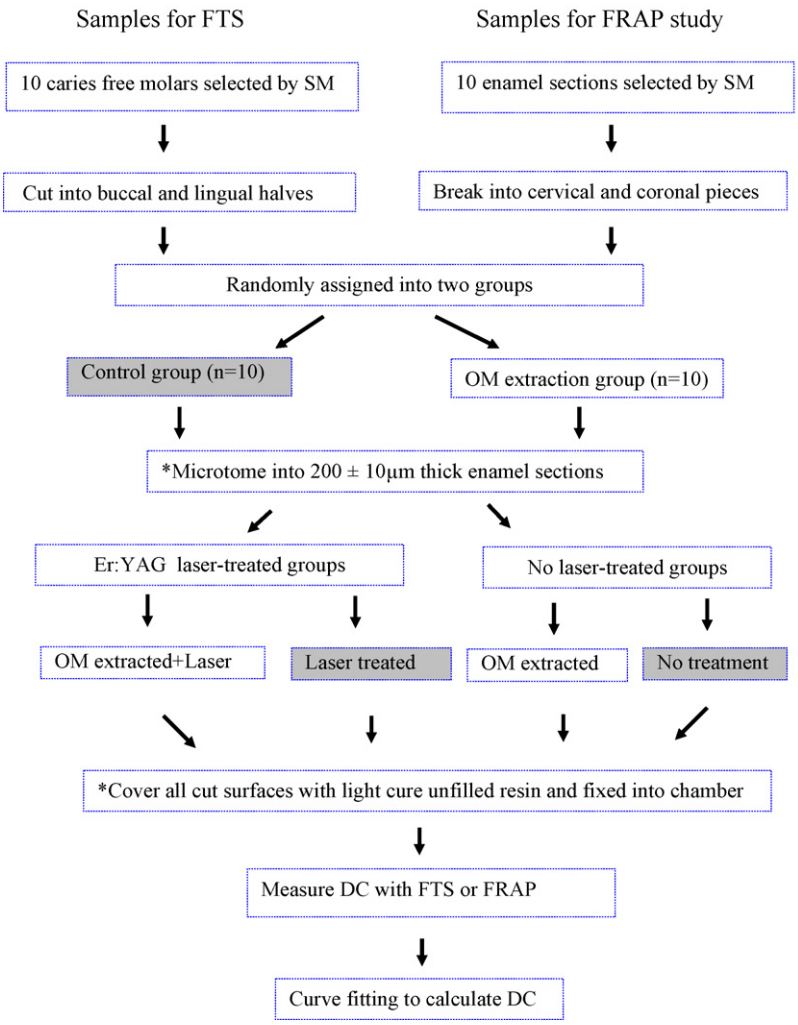

Fig. 1 – Flow chart of the sample allocation and preparation procedures. The steps mark by asterisk are only for the FTS. The OM + groups are showed in shaded box.