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Analysis of Lipids in Deciduous Teeth by Matrix-Assisted Laser Desorption/ Ionization Mass Spectrometry (MALDI MS)

Yujin Lee [1,†], Eunji Seo[1,†], Tae-Min Park[1], Kwang-Hak Bae[2], and Sangwon Cha[1,*]

[1]*Department of Chemistry, Hankuk University of Foreign Studies, Yongin, Kyunggi-Do 17035, Korea*
[2]*Oral Health Science Research Center, Apple tree Dental Hospital, Goyang, Kyunggi-Do 10381, Korea*

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Abstract : Recently, deciduous teeth have been proposed as a promising biomatrix for estimating internal and external chemical exposures of an individual from prenatal periods to early childhood. Therefore, detection of organic chemicals in teeth has received increasing attention. Organic materials in tooth matrix are mostly collagen type proteins, but lipids and other small organic chemicals are also present in the tooth matrix. In this study, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) was employed to obtain lipid fingerprints from deciduous teeth. Phospholipids and triacylglcerols (TAGs) from deciduous teeth were successfully detected by MALDI MS with 2,5-dihydroxybenzoic acid (DHB) or gold nanoparticle (AuNP) as a matrix.

Keywords : deciduous teeth, MALDI, phospholipid, triacylglycerol, gold nanoparticle

1. Introduction

Exposomics tries to uncover the relationships between chemicals accumulated in human body and environmental exposures such as internal inflammation and external tobacco smoke.[1] Recently, deciduous teeth have been proposed as a promising biomatrix for exposomics analysis with following reasons.[2] First, single deciduous tooth contains both prenatal and postnatal exposure information. Second, due to specific growth patterns of dentine in deciduous teeth, chemicals accumulated in prenatal and postnatal periods are spatially separated by the neonatal line which is formed at birth. Third, exposure chemicals can remain stable in the mineralized dental tissues. Therefore, there are increasing needs for analyzing metals and organic chemicals present in deciduous teeth. Many studies demonstrated various methods including laser ablation inductively coupled plasma mass spectrometry (LA ICP MS) and X-ray fluorescence (XRF) for detecting metals *in situ* or *ex situ* from teeth.[3, 4] However, there have been very few studies on organic chemical analysis of teeth.[5,6]

Analysis of organic chemicals in teeth is challenging because amounts of organic chemicals in teeth are substantially lower than those of inorganic materials and also because organic chemicals are entrapped in the highly mineralized tissues and therefore mild extraction of organic chemicals is not effective. For example, inorganic materials comprises about 96 percent of the enamel, the hardest and most highly mineralized tooth microstructure. Dentine, a tooth microstructure located beneath the enamel, consists of about 70 wt% inorganic materials, 20 wt% organic materials, and 10 wt% water.[7]

In this study, lipid fingerprints were obtained from a lipid extract of powdered deciduous teeth by employing matrix-assisted laser desorption/ionization (MALDI) MS. About 90 wt% of dentine organic materials are collagen-type proteins and the rest consists of non-collagen proteins and lipids.[8] In case of rat teeth, lipids have been known to comprise 0.3 percent of total dentine weight.[9] For dental pulp which contains more lipids than any other tooth microstructures, major lipid species were determined to be phospholipids (~55%), free cholesterol (~25%), and triacylglycerols (TAGs, ~10%).[10] Our results showed that various phospholipids and TAGs were successfully detected from powdered deciduous teeth by MALDI MS with 2,5-dihydroxybenzoic acid (DHB) and gold nanoparticle (AuNP) matrices.

†These authors contributed equally.
*Reprint requests to Sangwon Cha
E-mail: swcha@hufs.ac.kr

Experimental

Materials

Methanol (MeOH), chloroform, and acetonitrile (ACN) were purchased from Fisher scientific (Fairlawn, NJ, USA). Trifluoroacetic acid (TFA) and organic MALDI matrices including DHB and trihydroxyacetophenone (THAP) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Aqueous graphene oxide (GO) solution and aqueous citrate-capped gold nanoparticle solution (AuNPs, 12 nm in diameter, 0.05 mg/mL) were purchased from UniNanoTech Co. (Yongin, Korea) and CNVision Co. (Seoul, Korea), respectively. Organic and inorganic MALDI matrix solutions were prepared as follow: DHB matrix solution (20 mg/mL) was prepared either with MeOH or with 0.1% (v/v) TFA plus 1 mM NaCl in water/ACN (7:3, v/v). THAP solution was prepared in 0.1% TFA in water/MeOH (2:8, v/v). AuNP solution (0.05 mg/mL) from CNVision Co. was directly used without any further preparation and aqueous GO solution from UniNanoTech Co. was diluted to 0.05 mg/mL with 10 mM $NaNO_3$ in water.

Sample preparation

Deciduous teeth without dental caries were collected in a non-invasive way at local dental clinic. Donated teeth were washed with distilled water in a sonication bath for 10 min and dried. Dried teeth were pulverized by using a ball-mill grinder (Pulverisette 23, Fritsch GmbH, Idar-Oberstein, Germany) and prepared teeth powder was stored in a glass vial at room temperature.

Lipid extraction from the ground teeth powder was performed by a modified Folch method. Briefly, 1 mL of chloroform/MeOH solution (2:1, v/v) was added to 50 mg of ground teeth powder and vortexed. The whole mixture was incubated in a sonication bath at 37°C for 1hr. After incubation, 0.2 mL of distilled water was added to the mixture and vortexed. The resulting mixture was subjected to gentle centrifugation and the upper (aqueous) and the lower (chloroform) layers were collected separately. In this study, only the chloroform layer was analyzed without dilution or concentration.

MALDI MS analysis

For MALDI MS analysis, the lipid extract (1.0 μL) was first spotted onto a MALDI target plate (ASTA Inc., Suwon, Korea) followed by a 1.0 μL DHB or THAP matrix or by 2.0 μL AuNP or GO solution. The prepared sample spots were then dried under moderate vacuum (~50 Torr). MALDI MS analysis was performed with an ABI 4800 Plus MALDI-TOF/TOF analyzer (Applied Biosystems, Foster City, CA). All mass spectra were collected in the positive or negative ion reflectron mode with a 20 kV acceleration voltage. For a given sample spot, a sub-spectrum at one location was collected with 30 laser shots and then moved to another location in the spot for collecting another sub-spectrum. Sub-spectra collected from 40 different locations in the spot were averaged to generate a final mass spectrum.

Results and discussion

Phospholipids

We tested various MALDI matrices against a tooth lipid extract in order to find the best matrix for tooth phospholipid analysis. It has been shown that a DHB matrix was considered as an 'all-purpose' matrix for lipid analysis.[11] THAP showed the similar performance with DHB in lipid analysis, but THAP was better in detecting glycolipids than DHB.[11] Carbon-based matrices such as GO, on the other hand, showed the superior performance in analyzing free fatty acids and glycolipids such as cerebrosides, compared to conventional organic MALDI matrices.[11,12] Therefore, we selected DHB, THAP, and GO for optimizing detection of tooth lipids.

Among tested matrices, DHB showed the best results in the positive ion mode (data not shown). Fingerprints obtained with THAP were very similar to those with DHB, but showed the lower signal-to-noise (S/N) ratios than DHB for detected phopholipids. GO did not give any noticeable lipid signals with a tooth lipid extract. Therefore, we further optimized the solvent composition and additives for a DHB matrix. It was found that an acid additive TFA was helpful for enhancing S/N ratios and a salt additive NaCl reduced spectral complexities without losing sensitivity.

Figure 1 shows a MALDI mass spectrum of the deciduous tooth lipid extract with a DHB matrix. First, ions corresponding to lyso-phosphatidylcholine (LPC) 16:0

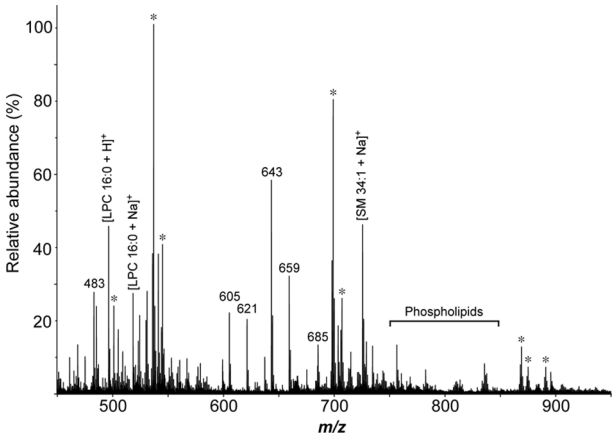

Figure 1. MALDI TOF mass spectrum (*m/z* 450-950) of a lipid extract of deciduous teeth (chloroform layer) with a DHB matrix. Signals from a DHB matrix were marked with asterisks (*). DHB matrix was prepared in 0.1% TFA and 1mM NaCl in water/ACN (7:3, v/v).

were clearly detected as $[LPC\ 16:0 + H]^+$ at *m/z* 496 and $[LPC\ 16:0 + Na]^+$ at *m/z* 518. Previous MALDI imaging MS study of teeth showed that LPC 16:0 was strongly localized at root surface.[13] Second, sphingomyelin (SM) 34:1 was clearly observed as the ion, $[SM\ 34:1 + Na]^+$ at *m/z* 725. We also observed sample-originated, intense signals at *m/z*s 605, 621, 643, and 659, but identifications for these ions have not been achieved in this study.

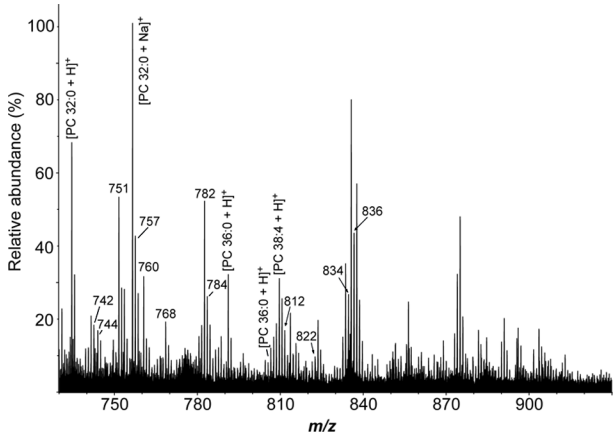

Figure 2. MALDI TOF mass spectrum (*m/z* 730-930) of a lipid extract of deciduous teeth (chloroform layer) with a DHB matrix. Solution for DHB was 0.1% TFA and 1 mM NaCl in water/ACN (7:3, v/v). Possible peak assignments are listed in Table 1.

Table 1. Mass peak assignments for phospholipids in MALDI mass spectrum of a lipid extract of deciduous teeth with DHB.

m/z[a]	Possible Ions Assigned[b]
496	$[LPC\ 16:0 + H]^+$
518	$[LPC\ 16:0 + Na]^+$
714	$[PE\ 34:3 + H]^+$
725	$[SM\ 34:1 + Na]^+$
734	$[PC\ 32:0 + H]^+$
738	$[PE\ 36:5 + H]^+$, $[PE\ 34:2 + Na]^+$
742	$[PE\ 36:3 + H]^+$
744	$[PE\ 36:2 + H]^+$
746	$[PE\ 36:1 + H]^+$
756	$[PC\ 32:0 + Na]^+$
760	$[PC\ 34:1 + H]^+$, $[PE\ 36:5 + Na]^+$
768	$[PE\ 38:4 + H]^+$, $[PE\ 36:1 + Na]^+$
782	$[PC\ 36:4 + H]^+$, $[PC\ 34:1 + Na]^+$
784	$[PC\ 36:3 + H]^+$, $[PC\ 34:0 + Na]^+$
790	$[PC\ 36:0 + H]^+$
806	$[PC\ 38:6 + H]^+$
809	$[PC\ 38:4 + H]^+$
812	$[PC\ 38:3 + H]^+$, $[PC\ 36:0 + Na]^+$
822	$[PC\ 39:5 + H]^+$, $[PC\ 37:2 + Na]^+$
834	$[PC\ 40:6 + H]^+$, $[PC\ 38:3 + Na]^+$
836	$[PC\ 40:5 + H]^+$, $[PC\ 38:2 + Na]^+$
838	$[PC\ 40:4 + H]^+$, $[PC\ 38:1 + Na]^+$

[a]Indicated *m/z*s are nominal *m/z*s. [b]Peak assignments were based on previously reported identifications[11, 12] and MALDI MS/MS experiments.

Phospholipids other than LPC and SM were detected mainly in the *m/z* region of 730 - 850. Figure 2 shows a MALDI mass spectrum of deciduous tooth lipids for this *m/z* region and tentative peak assignments are listed in Table 1. As shown in Figure 2 and Table 1, detected phospholipids are mainly phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs). Among these, PC 32:0 showed the most intense signals as the ions, $[PC\ 32:0 + H]^+$ at *m/z* 734 and $[PC\ 32:0 + Na]^+$ at *m/z* 756. We also tried to obtain lipid fingerprints in the negative ion mode. Although we observed trace signals at *m/z* 880-910 which could correspond to phosphatidylinositols (data not shown), but their intensities and S/N ratios were too low to be confirmed.

Triacylglycerols (TAGs)

Pure TAGs could be sensitively detected by MALDI MS with DHB.[14] However, significant signal suppression of TAGs occurred when both TAGs and PCs are present in the same sample spot.[15] In order to solve this issue, TAGs were first separated from phospholipids by employing a solid phase extraction-based purification step, and purified TAGs were analyzed by conventional MALDI MS.[15] Another way to solve this problem was the use of the sodium-enriched, citrate-capped AuNPs as a MALDI matrix instead of a conventional, organic acid matrix.[16] In this study, we used the AuNP matrix for selective detection of TAGs from deciduous tooth lipids and Figure 3 shows the resulting spectrum and possible peak assignments are

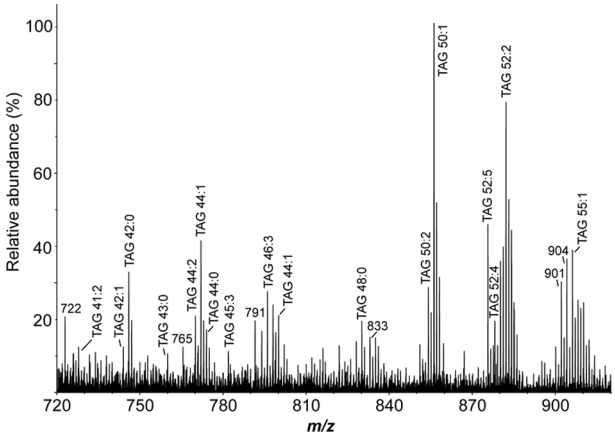

Figure 3. MALDI TOF mass spectrum of a lipid extract of deciduous teeth (chloroform layer) with AuNP as a matrix. All peaks assigned as TAG species had forms of sodiated ions, $[TAG + Na]^+$. See Table 2 for the detailed peak assignments.

Table 2. Mass peak assignments for triacylglycerols (TAGs) detected in the MALDI mass spectrum of a lipid extract of deciduous teeth with the AuNP matrix (Figure 3).

m/z [a]	Possible Ions [b]	*m/z* [a]	Possible Ions [b]
727	$[TAG\ 41:2 + Na]^+$	853	$[TAG\ 50:2 + Na]^+$
743	$[TAG\ 42:1 + Na]^+$	855	$[TAG\ 50:1 + Na]^+$
745	$[TAG\ 42:0 + Na]^+$	875	$[TAG\ 52:5 + Na]^+$
759	$[TAG\ 43:0 + Na]^+$	877	$[TAG\ 52:4 + Na]^+$
769	$[TAG\ 44:2 + Na]^+$	881	$[TAG\ 52:2 + Na]^+$
771	$[TAG\ 44:1 + Na]^+$	899	$[TAG\ 53:0 + Na]^+$
773	$[TAG\ 44:0 + Na]^+$	901	$[TAG\ 54:6 + Na]^+$
781	$[TAG\ 45:3 + Na]^+$	905	$[TAG\ 54:4 + Na]^+$
795	$[TAG\ 46:3 + Na]^+$	907	$[TAG\ 54:3 + Na]^+$
799	$[TAG\ 46:1 + Na]^+$	911	$[TAG\ 54:1 + Na]^+$
821	$[TAG\ 48:4 + Na]^+$	925	$[TAG\ 55:1 + Na]^+$
829	$[TAG\ 48:0 + Na]^+$		

[a]Indicated *m/z*s are nominal *m/z*s. [b]Peak assignments were based on previously reported identifications[16] and MALDI MS/MS experiments.

listed in Table 2.

As shown in Figure 3 and Table 2, TAG fingerprints were selectively obtained without interference from phospholipids and this lead to identification of twenty three TAGs. Among TAGs, TAG 50:1 at *m/z* 855 and TAG 52:2 at *m/z* 881 showed the most intense signals as the ions, $[TAG\ 50:1 + Na]^+$ at *m/z* 855 and $[TAG\ 52:2 + Na]^+$ at *m/z* 881.

Conclusions

In this study, tooth lipids were analyzed by the positive-ion mode MALDI MS with two matrices which have different ionization characteristics from each other. The DHB matrix with a salt additive gave the fingerprint of phospholipids including LPC, SM, PC, and PE. On the other hand, the AuNP matrix gave the TAG fingerprint almost exclusively. However, optimization of the negative-ion mode MALDI MS for tooth lipids was not achieved in this study. In addition, we performed lipid analysis only against the ground powder of the whole teeth. In near future, differential lipid analysis will be performed according to tooth structural compartments such as enamel, dentine, cementum, and dental pulp.

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