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Spectroscopic examination of enamel staining by coffee indicates dentin erosion by sequestration of elements

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ABSTRACT

The mechanism of coffee eliciting erosion on teeth is unclear as few studies have investigated the direct effect of coffee on enamel and dentin structures. The present study identified how coffee, the most popular beverage worldwide, induces staining and erosion on teeth. We show the grade of erosion of molars and incisors in Sprague Dawley rats from two different age groups, young (four weeks) and old (six months). We quantified the concentration of metals contained in coffee by mass spectrometry (ICP-MS). To determine elemental content in enamel (i.e. superficial) and dentin (i.e. substructure), we used Laser-induced Breakdown Spectroscopy (LIBS) and X-ray fluorescence (XRF) spectroscopy, respectively. For LIBS, a significant decrease of Ca, P, and Na was observed in the young coffee group relative to age-matched controls, whereas a significant increase in Mn, Fe, and K was observed. In the old coffee group, a significant increase of Mg, Fe, and K was observed along with a decrease of Mg, Ca, P, Na, Sr and Zn. For XRF, a significant decrease of the Ca/P ratio in the coffee group was observed. Spectroscopy results were correlated with scanning electron microscopy (SEM) and histological analysis. The SEM analysis showed pores and open spaces between young and old coffee groups, respectively. Thinning of enamel layers, loss of continuity in the enamel-dentin-junction, and wide spaces in dentin tubules with coffee use was found histologically. Coffee induces decalcification of teeth that corresponds to erosion, exposing the dentin structure by reducing enamel. Coffee immersion demonstrated an intrinsic staining in dentin by metal deposition.

1. Introduction

Teeth are susceptible to staining by drinks such as coffee, one of the most popular beverages consumed worldwide. There are studies describing the advantages that coffee offers, for instance, preventing periodontal disease [1] and caries [2,3]. However, the pigmentation of teeth by coffee and other drinks could cause major alterations to dental structure, i.e. enamel and dentin. For example, coffee changes the opalescent color of teeth, and it could cause damage to the underlying dental structure [3,4]. Despite this general assumption, no study to-date has investigated the mechanism of coffee altering the structure of teeth. Coffee has a high risk of eliciting erosion [5–7], but the mechanism is not fully understood. Currently, a lack of evidence exists concerning the structural changes induced by coffee staining on teeth. In the present study, our aim was to determine the structural changes induced by coffee on teeth and determine how teeth are affected by coffee pigmentation.

Dentin and enamel are two mineralized tissues with an important function in the external protection of teeth. Dentin shares similar properties to bone in matrix composition and possesses anisotropic qualities, providing resistance to mechanical stress [8]. Although harder than bone, comprising a 65% inorganic and 35% organic matrix, dentin has the capacity to prevent fractures due to its elasticity and flexibility overlying the inelastic enamel [9,10]. Enamel is followed by the primary and secondary dentin and the dentin-enamel junction (EDJ) [9]. The EDJ provides stability to the tooth structure enamel,

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which is the principal component of the tooth and often considered to be the hardest tissue in vertebrata body [11]. Its hardness is attributed to a high level of hydroxyapatite which is deposited over the organic matrix, rich in proteins, principally collagen. Enamel contains the organic matrix and water, 1% and 4% composition, respectively, but 95% of enamel derives from mineral compounds [12]. The main elements forming the structure of hydroxyapatite are calcium (Ca), phosphorus (P) and other elements in minor amounts [13]. Enamel is the outermost layer of teeth, therefore vulnerable to damage. The staining of enamel could lead to demineralization causing erosion [14,15]. We have postulated that coffee deposition could lead to subtle erosive effects on teeth by altering enamel composition. Erosion is a progressive localized loss in the main elemental components of enamel by the effect of acid or chelation without bacterial action [15–17]. Decalcification is the first step in enamel destruction; therefore, variation in the level of Ca and P detected by spectroscopy and/or microscopy techniques could serve as markers for erosion of teeth [15].

Analytical sample processing and analyses are techniques used to investigate elements in biological specimens [18–21]. Spectroscopic analyses are popular techniques because of their capacity for specific elemental distinction in tissues and materials of interest [13,17,22–31]. In Table 1, we have summarized recent spectroscopy techniques used to determine elements in teeth enamel and dentin [13,22,23,31,32]. For example, Laser-induced Breakdown Spectroscopy (LIBS) possess high sensitivity and spatial resolution in real time allowing one to identify and sort out the nature of solids, such as teeth [13,28,30]. The LIBS technique has the potential to recognize changes in the composition of enamel in healthy teeth, carious teeth and teeth treated by bleaching. The main advantages of LIBS include easy sample preparation and relatively no sample alteration or destruction [13,26–31]. Here, we have used LIBS to measure the surface (e.g. in the enamel) erosion effect induced by coffee. We subsequently utilized a second spectroscopic technique, X-ray Fluorescence Spectroscopy (XRF), to assess dentin elemental components, due to the higher penetration, i.e. the XRF technique analyzes a sample deeper than LIBS (hence the ability to assess dentin). With XRF it is easy to identify the elemental content by depth of penetration [25,30,32]. The XRF technique provides excellent analyses of underlying tissues such as dentin. Both LIBS and XRF in combination provide an exact measure of surface (enamel) and depth (dentin) of teeth during biological changes, such as the effect of coffee. Although there are studies using spectroscopy in dental research, little evidence exists about alterations induced by coffee staining to the enamel structure and the mechanism of coffee pigmentation to dental tissues.

In the present study, our objective was to identify the changes in the enamel surface elicited by coffee immersion through LIBS, due to the high sensitivity of detection of elements. Secondly, we complemented our LIBS surface analysis with XRF instrumentation as a standard technique to compare the spectrum of nine elements from every specimen. Additionally, we performed morphological analysis using Scanning Electron Microscopy (SEM) and histological assessment by Hematoxylin/Eosin (H&E) and Masson's Trichrome staining to report the histological changes induced by coffee. Lastly, we have identified the quantity of metals contained in coffee by mass spectroscopy. In summary, we report that coffee elicits a loss of mineral content in teeth induced by superficial staining of the enamel, resulting in structural erosion of the underlying dentin.

Table 1
Spectroscopic methods used to investigate dental tissues.

Dental tissue	Spectroscopy method	Ref.
Enamel	LIBS	Gazmeh et al. [31], Imam et al. [13].
	XRF	Gomes et al. [32], Djomeri et al. [22], Imam et al. [13].
Dentin	LIBS	Gazmeh et al. [31], Imam et al. [13].
	XRF	Djomeri et al. [22], Beres et al. [23].

2. Material and methods

This study used 24 unilateral jaws immersed in coffee or water during two weeks. The samples were dried in a freeze dryer prior to the start of the experiments using LIBS, XRF, SEM and histology techniques. Fig. 1 summarizes the experimental design and logical flow of our study.

2.1. Back-of-the-envelope calculations

In the style of E. Fermi we wanted to roughly estimate the effects of coffee on teeth by simulating the deposition of coffee over a long duration. Methodologically, we followed a protocol similar to previous immersion procedures [33]. Here, we have some back-of-the-envelope calculations to estimate drinking coffee for a long period duration. We choose a decade to simulate our coffee drinking period. We choose young (4 weeks) and old groups (6 months) because we anticipated older rats would have changes in their inorganic matrix, causing more prominent erosion compared to younger rats. We wanted to replicate the deposition of coffee which takes place over 3650 days (decade), but in an accelerated format; therefore, we calculated if an individual drinks coffee for 5 min a day, every day a year for a decade: 5 min × 365 days = 1825 min a year or 18,250 min in a decade. To simulate this type of deposition, we placed our samples in coffee groups (old and young) in liquid coffee for 18,250 min (Fig. 1a). Since there are 10,080 min in a week and 20,060 min in a two week period, we simulated a decade of deposition by placing the samples in coffee for two weeks. Roughly a fortnight of teeth in coffee should be equivalent to drinking coffee for approximately a decade or more. Each daily coffee dose consisted of approximately 30 g brewed coffee. All coffee was donated from a Starbucks coffee shop located on the University campus.

2.2. Animals

Twenty-four unilateral jaw bones from male Sprague-Dawley (SD) rats of one month or six months of age, considered as young or old group (N = 12, 250–350 g or N = 12, 500 g, respectively) were used. Animals were provided by the accredited Laboratory Animal Unit of the University of Hong Kong. The present study was approved by the animal research ethics committees of the City University of Hong Kong, the University of Hong Kong, and the Department of Health of the Hong Kong Special Administrative Region. The animals were housed under a constant 25 °C temperature and 60–70% humidity at the Laboratory Animal Research Unit of the City University of Hong Kong. Animals were housed in 12/12 h light/dark cycles with access to food and drinking water, ad libitum. Animals were acclimated to the housing environment for at least one day prior to euthanasia and surgical extraction of jaw bones. The entire bilateral jaw was extracted and used for the experiments, separated from the adjacent soft tissue and cleaned with fresh water. Teeth were conserved on jaws. Twenty-four unilateral jaws were divided into 4 groups of six samples per tube consisting of 50 ml distilled water or coffee (Fig. 1a). The control water group or the coffee groups were changed every 24 h for two weeks duration. The coffee was 30 mg/50 ml steamed coffee (coffee groups) or 50 ml distilled water. Six jaws were grouped in tubes and labeled as follows: young control, young coffee, old control, old coffee.

2.3. Coffee preparation

We used *Arabica coffee* which was changed every 24 h during the 2-week period. The pH of coffee was evaluated using Eutech-Instruments-700 pH meter in water at room temperature. We prepared 30 g of coffee

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