Combining casein phosphopeptide-amorphous calcium phosphate with fluoride: synergistic remineralization potential of artificially demineralized enamel or not?

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Abstract. Recaldent is a product of casein phosphopeptide-amorphous calcium phosphate (CPP—ACP). The remineralizing potential of CPP—ACP per se, or when combined with 0.22% Fl gel on artificially demineralized enamel using laser florescence, is investigated. Mesial surfaces of 15 sound human molars are tested using a He–Cd laser beam at 441.5 nm with 18-mW power as an excitation source on a suitable setup based on a Spex 750-M monochromator provided with a photomultiplier tube (PMT) for detection of collected autofluorescence from sound enamel. Mesial surfaces are subjected to demineralization for ten days. The spectra from demineralized enamel are measured. Teeth are divided into three groups according to the remineralizing regimen: group 1 Recaldent per se, group 2 Recaldent combined with fluoride gel and ACP, and group 3 artificial saliva as a positive control. After following these protocols for three weeks, the spectra from the remineralized enamel are measured. The spectra of enamel autofluorescence are recorded and normalized to peak intensity at about 540 nm to compare spectra from sound, demineralized, and remineralized enamel surfaces. A slight red shift occurred in spectra from demineralized enamel, while a blue shift may occur in remineralized enamel. Group 2 shows the highest remineralizing potential. Combining fluoride and ACP with CPP-ACP can give a synergistic effect on enamel remineralization. [DOI: 10.1117/1.3210780]

Keywords: casein phosphopeptide-amorphous calcium phosphate; fluoride; artificial demineralization; remineralization.

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1 Introduction

Caries is a chronic, slowly progressing disease, with symptoms not detected at the onset of the disease but generally much later. Its initiation is associated with demineralization (calcium and phosphate loss) of subsurface tooth enamel, resulting in the formation of a subsurface lesion. It is, therefore, very important to detect caries in its early stage, when the lesion can be reversed clinically by using, for instance, different fluoride supplements.[1,2] Visual inspections, examination with a probe, and radiography have been methods used to detect caries. However, these methods have different diagnostic possibilities, depending on how they are used and who uses them.

Optical methods have always played a main role in the study of biological phenomena. The optical properties of tissues are important and informative, and the spectroscopic aspects are preeminent for lesion localization and determination. Mineral loss could be registered by several optical methods, as scattering off teeth[3,4] or quantitative laser-induced fluorescence (QLF).[5–7] A recent method for caries detection and quantification is laser-induced fluorescence. The fluorescence spectroscopy-based devices for detecting teeth condition are promising diagnostic tools with high reproducibility of results.[8–12] It may be an alternative to the probe or x-ray examination. Promising results have been demonstrated using fluorescence spectroscopy with excitation wavelengths in the violet and blue spectral region. Alfano and Yao[13] were among the first who used fluorescence to differentiate between sound and carious tooth structure using excitation wavelengths between 400 and 700 nm. Additional work by Sundström et al.[14] revealed differences between sound and carious tooth structure using excitation wavelengths of 337 to 633 nm. This led to the establishment of a quantitative relationship between gross scattering of fluorescent light and mineral loss,[15] from which the QLF technique was developed.[8]

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Most of the investigations in the field of caries research have been related with QLF,[8,10,16,17] which detects the fluorescence radiance decrease (DF %). However, one significant disadvantage of the QLF method is its inability to distinguish a demineralized area from initial enamel caries.[16] This disadvantage is avoided in laser-induced autofluorescence spectroscopic studies, which obtain information not only about intensity changes, but also about changes in the shape of the spectra as well. This information could be very useful in detecting the type of disorder. In addition, the easy and nondestructive fluorescence method for quantification of early caries improves the opportunities of clinical research and diagnostics.

Recently, a contemporary approach has been adopted. The noninvasive intervention of noncavitated carious lesions, by applying therapeutic agents for tissue healing was used.[18] The administration of fluoride has been proposed, and used, as a method of reducing enamel susceptibility to demineralization.[19,20] Fluoride affects the caries process by enabling the formation of high quality fluorapatite that aids remineralization and inhibits glycolysis of plaque microorganisms.[19,21–23] Early studies have demonstrated the anticaries activity of dairy products. Harper et al. studied the anticariogenicity of four cheeses and concluded that the protective effect could be attributed to the phosphoprotein casein and calcium phosphate content of the cheese.[24]

Though the concept of using casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) as a remineralizing agent was introduced in 1998,[25] using casein for caries prevention was addressed in the eighties,[26] and using ACP technology started in the early nineties.[27] The anticariogenic potential of CPP-ACP nanocomplexes in laboratory, animal, and human *in-situ* models has been well documented.[28–32] Further, CPP-ACP has also been shown to remineralize enamel with a mineral that is more resistant to acid challenge than normal enamel mineral.[33] The proposed mechanism of action of CPP-ACP is related to its localization at the tooth surface, where it buffers free calcium and phosphate ion activities, maintaining a state of supersaturation, thus preventing demineralization and facilitating remineralization.[30]

Recaldent, a mousse formula that has become commercially available, contains the bioactive agent of CPP-ACP. More recently, new products containing both fluorides and CPP-ACP have been launched in certain markets. However, few studies have addressed the effect of combining both agents on the remineralizing potential of artificially demineralized enamel. This study aimed to investigate the remineralizing potential of CCP- ACP (Recaldent) per se, or when applied in combination with 0.22% fluoride supplied in an oral care gel (Relief ACP) on artificially demineralized lesions created in enamel using the phenomenon of laser-induced autofluorescence.

2 Materials and Methods

2.1 *Materials*

Two commercial products were used in this study, as shown in Table 1, GC Tooth Mousse and Relief ACP oral care gel. Two materials were prepared in the central laboratory of Misr University of Science and Technology, as shown in Table 2. Artificial saliva was prepared according to Fusayama[34] and adjusted to pH 7 for sample storage and for the control group. An acidified gel (pH 4.8) to induce chemical demineralization was prepared according to Tam, Chan, and Yim.[35] The pH was adjusted using a pH meter. The gel was kept in the fridge and it was warmed in a hot water bath prior to use.

Table 1 Composition of commercial products used.

Material	Composition	Manufacturer
GC Tooth Mousse	Pure water, glycerol, CPP-ACP, d-sorbitol, xylitol, CMC-Na, propylene glycol, H_2O, SiO_2, TiO_2, ZnO_2, H_3PO_4, MgO_2, guar gum, sodium saccharin, ethyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate, and propyl *p*-hydroxybenzoate	GC International, Itabashi- Ku, Tokyo, Japan,
Relief ACP	0.375 % amorphous calcium phosphate (ACP), 0.22% NaFl, 5% KNO_3	Discuss Dental

2.2 *Teeth Selection and Preparation*

15 freshly extracted sound human molars were selected for this study. Teeth were stored at 8 °C in distilled water containing 0.5% sodium azide to inhibit microbial growth until their use. The mesial surfaces of teeth were examined under a stereomicroscope to verify the absence of any surface defects, cracks, or white spot lesions. Teeth were initially tested to measure spectra from sound enamel. Teeth were painted with nail varnish to protect them from the effect of the demineralizing gel, except the mesial surfaces, and then immersed in a demineralizing gel for ten days; the gel was changed daily. Mesial surfaces were then retested to measure spectra from demineralized enamel. Teeth were then divided into three groups of five molars each, according to the remineralizing regimen used. In group 1, mesial surfaces were brushed with tooth mousse per se for 3 min and then immersed in artificial saliva. In group 2, the surfaces were successively brushed with Relief oral care gel containing fluoride and ACP, then tooth mousse for 3 min each and immersed in artificial saliva. Group 3 did not receive any remineralizing regimen, but was stored in artificial saliva as a positive control group to simulate oral environmental conditions. This protocol was followed and the artificial saliva was changed daily for three weeks, then teeth were retested to measure spectra from remineralized enamel in the three groups. The spectra of enamel autofluorescence was recorded and normalized to peak intensity at about 540 nm to compare between spectra from sound, demineralized, and remineralized surfaces.

2.3 *Laser Autofluorescence Test*

2.3.1 *Excitation source*

Mesial surfaces, while still damp, were initially exposed to radiation from an Omnichrome He–Cd laser beam at 441.5 nm with 18-mW power. The experimental scheme is shown in Fig. 1.

Table 2 Composition of prepared materials.

Prepared material	Composition	Manufacturer
Artificial saliva	0.4 g NaCl, 0.4 g KCl, 0.6 g $CaCl_2$, 0.6 g NaH_2PO_4, 4 g urea, 4 g Mucin, 0.0016 g Na_2S, 0.0016 g $Mg_2P_2O_7$+1 L distilled water	Central lab MUST University
Acidified gel	Carboxy methyl-cellulose 5 *g*, 1 L deionized water, and 3 ml of lactic acid	

2.3.2 *Detection system*

The fluorescence spectra were registered using a suitable setup based on a SPEX 750-M monochromator with data scan SPEX DS 1010, provided with a photomultiplier tube (PMT) for detection of collected autofluorescence from sound, demineralized, and remineralized enamel. A personal computer was used to control the system and to store and display the data. The spectra were stored using the specialized software Spectramax 1.1, and were analyzed and graphically represented by the computer program.

2.4 *Postmeasurement Calculations*

Spectra from sound surfaces were averaged and normalized to peak intensity, that is, the intensity of the first (main) peak at 540 nm was set at 1.0. The spectra from demineralized surfaces were normalized to 1.0 for exactly the same wavelength as the respective sound enamel surface of the same tooth. This was done for the spectra from remineralized surfaces as well. The relations between the spectra from the secondary peaks to the main normalized peaks were calculated. Then the three investigated groups—Recaldent per se, Recaldent combined with fluoride, and artificial saliva as a positive control—were plotted separately to compare spectral changes.

3 Results

In this study, the fluorescence spectra from sound, demineralized, and remineralized enamel surfaces were recorded and averaged. Figure 2 shows representative fluorescence spectra from sound enamel surfaces. The spectrum of sound enamel showed an intense broad peak with a maximum at 540 nm, one secondary maximum at 630 nm, and another secondary maximum at 670 nm.

Figure 3 shows representative fluorescence spectra from demineralized enamel surfaces. These spectra showed an apparent decrease in the main peak, while there were no apparent changes in the secondary peaks. Figure 4 shows representative fluorescence spectra from remineralized enamel surfaces. These spectra showed an apparent increase in the

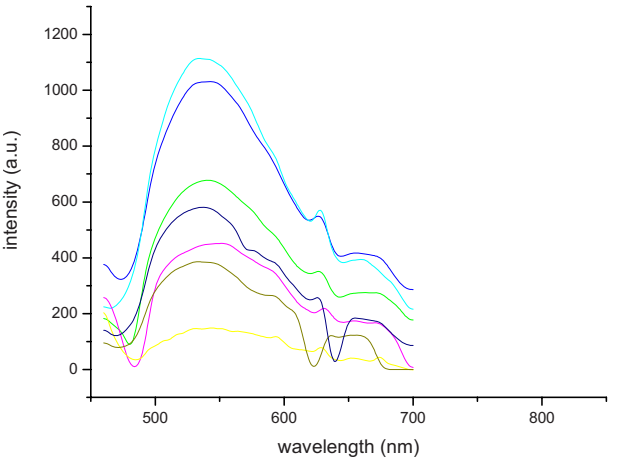

Fig. 2 Spectra of sound enamel surfaces from some samples.

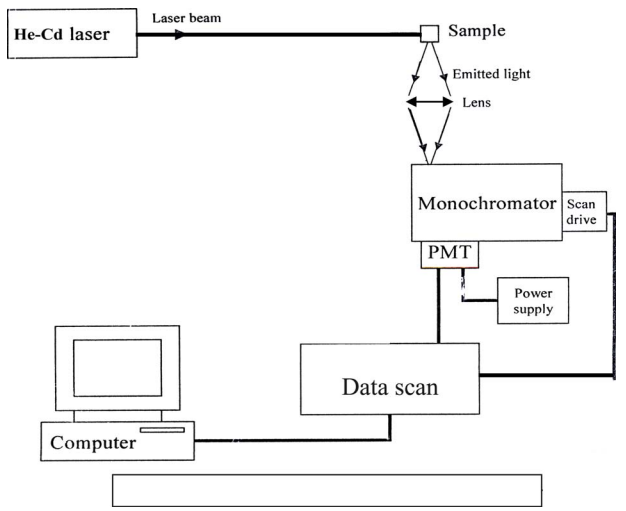

Fig. 1 The schematic setup of the optical experiment.

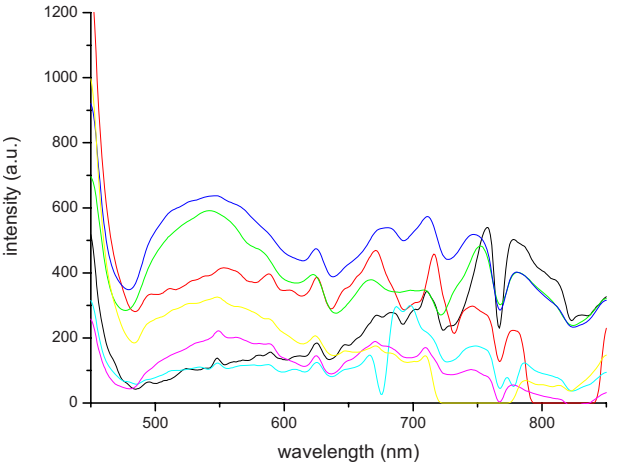

Fig. 3 Spectra of demineralized enamel surfaces from some samples.

main peak. However, this increase in peak intensity did not return the intensity like that of sound enamel surfaces.

Figures 5–7 show the spectra of the normalized intensity from sound, demineralized, and remineralized enamel surfaces with either Recaldent per se, Recaldent with fluoride gel, or artificial saliva, respectively. A slight red shift occurred in spectra from demineralized enamel, while a blue shift may occur in remineralized enamel. Group 2 showed the highest remineralizing potential compared to the other groups.

4 Discussion

Data comparing fluorescence properties of sound, demineralized, and remineralized enamel surfaces are scarce. Under the conditions of this study, the specimens were prepared to collect spectral data from mesial surfaces in the three conditions investigated, either sound, demineralized, or remineralized. It is known that dehydration causes changes in optical properties of enamel and hence may influence fluorescence emission properties.[36] In this present study, spectral data were collected when the specimens were still damp immediately after removing them from distilled water to prevent sample dehydration. This precaution was not taken in previous studies of the fluorescence of dental hard tissues.[13,14]

The helium–cadmium laser was used for testing teeth specimens in this study. The He–Cd laser is one of a class of gas lasers using helium in conjunction with a metal that vaporizes at a relatively low temperature. The typical He–Cd laser can produce a high quality beam at 442 nm (violet-blue) and/or 325 nm (UV) depending on the optics. Typical power output is in the 10 to 100 s of mW range. Its wavelengths are highly desirable for some forms of spectroscopy and nondestructive testing. In addition, it is safe, can focus on smaller spots, and is more efficient in the blue range.

This study investigated the remineralizing potential of CPP—ACP (Recaldent) per se on artificially demineralized enamel lesions, or when applied in combination with 0.22% fluoride and ACP supplied in an oral care gel (Relief ACP), in comparison to samples immersed in artificial saliva as a positive control. Using a He–Cd laser beam at 441.5 nm with 18-mW power, this study showed a spectrum of sound enamel with an intense broad peak maximum at 540 nm, one

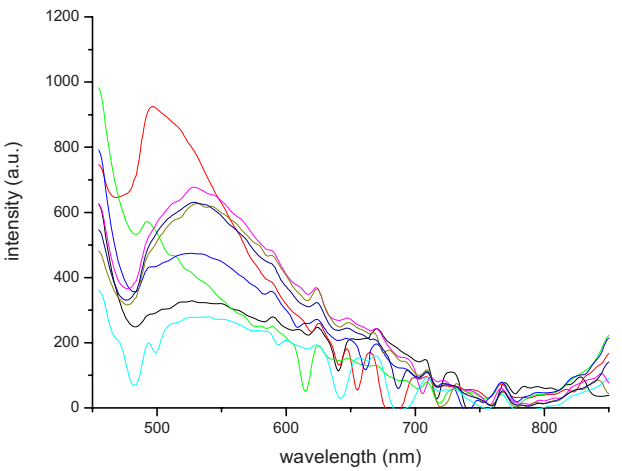

Fig. 4 Spectra of remineralized enamel surfaces from some samples.

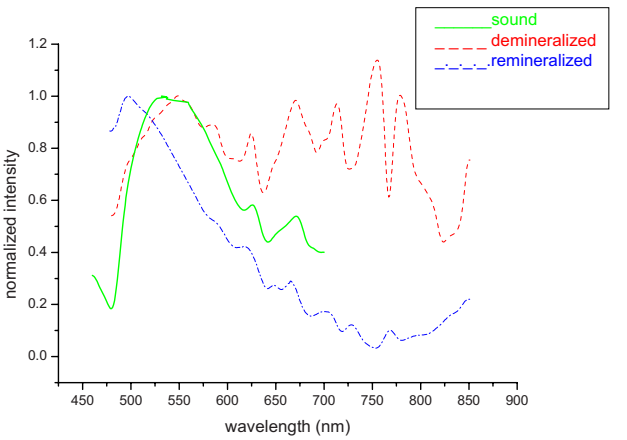

Fig. 5 Averaged normalized spectra of teeth in the conditions of sound, demineralized, and remineralized with Recaldent group.

secondary maximum at 630 nm, and another secondary maximum at 670 nm. The spectra from demineralized enamel surfaces showed an apparent decrease in the main peak, but there were no apparent changes in secondary peaks. Fluorescence spectra from remineralized enamel surfaces showed a relative increase in the main peak when compared with that of demineralized enamel, but was not equal to that of sound enamel. These results agree with the findings of Sundström et al.,[14] who concluded that illumination at 488 nm produced fluorescence with a peak at about 540 nm in enamel as well as dentin. The difference in the intensity of fluorescence between sound and carious enamel was generally greater at this wavelength than at any of the others previously tried. Buchalla[37] found that light brown spot lesions revealed three independent peaks at 624, 650, and 690 nm. The results also came in accordance with Borisova et al. in 2004 and 2006,[38,39] who stated that the reasons for the fluorescence signal decrease in demineralized teeth are not quite clarified, but the possible explanation lies in the changes in the light-scattering properties as a result of destruction of the enamel layer. This might be explained by the fact that when the tooth is irradiated by

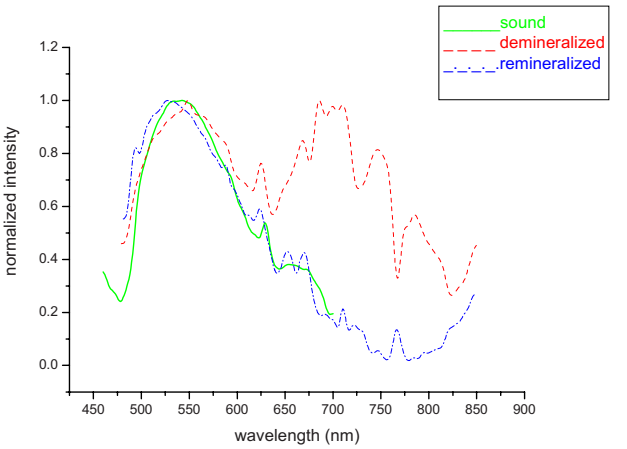

Fig. 6 Averaged normalized spectra of teeth in the conditions of sound, demineralized, and remineralized with Recaldent and fluoride gel group.

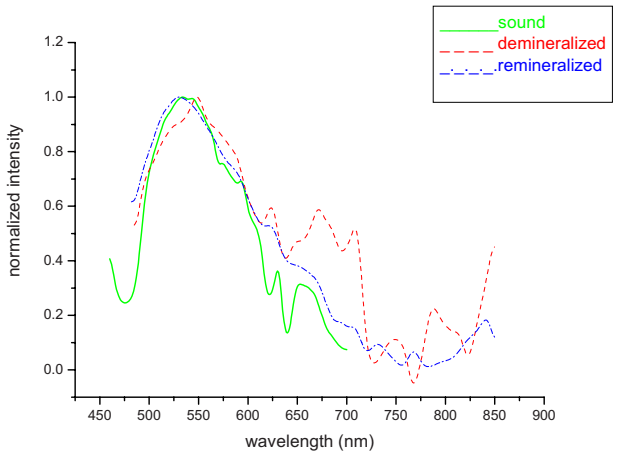

Fig. 7 Averaged normalized spectra of teeth in the conditions of sound, demineralized, and remineralized with artificial saliva group.

light, it may be absorbed by chromophores and cause fluorescence. The normal enamel layer has a prism structure[40] with waveguide properties, and if one irradiates the tooth surface, the light will penetrate deeply.[41] When the tooth surface is etched, the structure of the enamel layer is changed and its waveguide properties disappear, so that the excitation light cannot penetrate as deeply as in the case of normally structured enamel. Therefore, the fluorescent signal obtained has lower intensity compared with sound tooth fluorescence.[10]

It was logical to find a relative increase in the main peak intensity of samples subjected to remineralizing regimens, opposing the findings of demineralized samples. In many other studies,[25,28–35,42–45] the remineralizing potential of CPP ACP has been verified, although the methodology of application and assessment was different than our study. This was attributed to the fact that casein phosphopeptides (CPP) containing the sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu stabilize nanoclusters of amorphous calcium phosphate (ACP) in metastable solution.[25,30] The multiple Ser(P) residues bind to forming nanoclusters of ACP in supersaturated solutions, preventing growth of these clusters to the critical size for phase transformations. At the same time, the synergistic remineralizing potential of the fluoride with CPP-ACP has been proven in other studies.[45–47] This might be attributed to many reasons, one of which is the formation of amorphous calcium fluoride phosphate ions at the enamel surface. At the same time, the precipitate formed by the CPP method is not apatite [HAP; $Ca10(PO_4)6(OH)_2$], but a component of tooth and bone, dicalcium phosphate dihydrate (DCPD; $CaHPO_4.2H_2O$). Fluoride was found to enhance the conversion of DCPD to HAP.

The averaged spectra from the demineralized samples showed a red shift when compared to sound enamel samples, which was compatible with the results of some studies[14,35,36] that noticed a variable red shift according to the degree of demineralization. On the other hand, averaged spectra from remineralized samples showed a variable slight blue shift, which might assure reminerlization behavior due to contradicting behavior to demineralized spectra.

Although all investigated groups showed remineralizing potential as previously evidenced and explained, we argue that group 2 achieved the highest remineralizing potential. We observed that the averaged remineralized spectra of the second group approached the averaged sound spectra of the same group both in pattern and intensity. The two curves even coincided on many points. This can be attributed to both the synergistic actions of the fluoride with CPP-ACP, and the fact that Relief ACP also contains the ACP part, which increases the remineralization potential as well.

5 Conclusions

Combining fluoride and ACP with CPP-ACP can give a synergistic effect on enamel remineralization. In addition, laser autofluorescence is an accurate technique for assessment of changes in tooth enamel minerals.

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