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Interaction of Bio-Minerals and Gels with Ultrafast Lasers for Hard Tissue Surface Engineering

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ABSTRACT

Acid-induced enamel erosion leading to dentine hypersensitivity is a growing problem for both the young and ageing population worldwide. Dentinal hypersensitivity is rising for the ageing population because of additional enamel wear. Both conditions adversely affect lifestyle and potentially can harm the systemic health of the patients. Since the loss of enamel is an irreversible process in vivo, the only way for restoring it is via exogenous means. In this work a novel route for enamel restoration using an acid-resistant, calcium phosphate based biomineral and ultrafast (femto-second, fs) pulsed lasers in the near IR wavelength is demonstrated. A mixture of calcium phosphate (in the form of mineral brushite) powder with a bio-mineral gel was used to coat acid eroded bovine enamel surfaces. After forming the mineral layers, the samples were irradiated with two different 100-200 fs pulsed lasers, one at 800 nm with 1 kHz repetition rate, and the other at 1044nm with 1 GHz repetition rate. The laser-irradiated samples were characterized using X-ray diffraction, SEM, and Raman microscopy. It was found that irradiation with 1 GHz transformed brushite crystals to β-calcium pyrophosphate while the bio-mineral gel coating was melted and formed a homogeneous film on the enamel surface.

Keywords: Brushite, phase transformation, dentine hypersensitivity, enamel restoration, femtosecond lasers.

1. INTRODUCTION

Tooth hypersensitivity has gained attention in oral health in recent years as it is a growing problem affecting both the young and ageing population worldwide. Surveys concerning the prevalence and distribution of the disease suggest that almost 10-15% of the population suffers from tooth hypersensitivity [1]. This percentage is expected to dramatically increase in the coming years as advances in dental care result in more people retaining their natural teeth for longer (and consequently providing more opportunity for enamel erosion). Although researchers started investigating this condition almost one century ago (e.g. [2]) the exact mechanisms of tooth hypersensitivity are not clear yet. On the other hand, it is certain that the aetiology of the disease is linked to exposure of the dentine tubule system (lesion localisation) which is the result of enamel loss. According to the hydrodynamic theory, when dentine is stimulated with a hot or cold liquid, fluid flow in the exposed tubules triggers a mechanoreceptor response of the fibre nerves causing pain to the patients [3]. The quality of life of those affected by dentinal hypersensitivity is markedly compromised as long term pain relief is yet to be achieved.

Motivated by the need for an effective treatment with longevity, in this work we propose a novel route for treating tooth hypersensitivity with an exogenous re-mineralization strategy. In the proposed method developed below, we target directly the aetiology of the disease, since with the combined use of acid resistant calcium phosphate based gels and femtosecond lasers we are aiming to restore enamel. As depicted in Fig. 1 the proposed strategy can be described in three steps; **a**) cleaning and drying of the tooth surface; **b**) use of a microfluidic based delivery system to apply the calcium phosphate based gel on tooth and the formation of a thin film (<30 μm); **c**) irradiation of the film with a femtosecond pulsed laser which densifies the coating and yields bonding with the underlying tooth surface; thereby the blocking the exposed tubules causing the sensitivity. The use of femtosecond lasers is crucial in this strategy, since heat accumulation is not a major challenge unlike with conventional CW lasers.

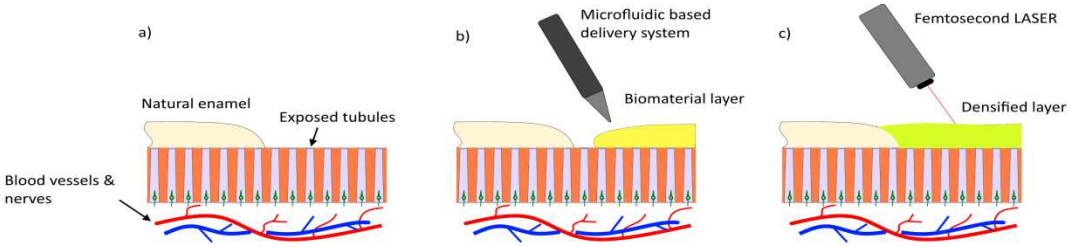

Figure 1: Proposed method for enamel restoration (magnified schematic cross section of exposed dentin tubules).

The aim of the present work is to carry out experiments and establish the form of the interaction of our biomaterial with a femtosecond laser. To achieve this, our biomaterial was applied to acid eroded enamel surfaces, prepared using bovine incisors, in order to form thin (20-50 μm) compact layers. After forming the mineral layers, each sample was irradiated with two different pulsed lasers (one at 800 nm with 1 kHz repetition rate and the other at 1044 nm with 1 GHz repetition rate) with pulse duration in the region of 100-200 fs. Similar irradiation experiments were also carried out on pressed pellets of the calcium phosphate crystals. The laser-irradiated samples were characterized using X-ray diffraction, SEM and Raman microscopy.

2. SAMPLE PREPARATION AND LASER IRRADIATION

2.1 Synthesis of the calcium phosphate minerals

For the proposed treatment brushite powders could be considered to be more suitable than the more conventional hydroxyapatite. The platelet-like forms of brushite powders would provide large occlusion area while potentially would minimize heat accumulation. Brushite is a mineral that is chemically compatible with the natural enamel and is known to be acid-resistant at pH lower than 5.5 (common oral pH). In order to improve the laser radiation absorption properties of the phosphate minerals, Er^{+3} ion dopants were used for enhancing radiation absorption in the 800-1044 nm.

For the synthesis of brushite 200 mL of a 0.1 M $Ca(NO_3)_2{\cdot}4H_2O$ aqueous solution were heated until 37℃. After reaching that temperature 0.1850 g of $Er(NO_3)_3{\cdot}5H_2O$ and 0.1660 g $Al(NO_3)_3{\cdot}9H_2O$ are added to the solution. The resulting mixture was continuously stirred during the incorporation of a 0.1 M $(NH_4)_3PO_4$ solution drop by drop and the addition of 0.033 g CaF_2. The final mixture was left under continuous stirring at 37℃ for 2 h. The solution was allowed to settle 1 h to allow precipitation while covering the top of the beaker to exclude CO_2 ingress into the mineral solution. After that the brushite crystals were collected on a filter paper (Whatman grade 44 with pores of 1μm) and dried for 24 h at 70 ˚C.

2.2 Synthesis of the biomineral gel

In order to form thin compact layers with good adhesion on the enamel surface, the brushite crystals were mixed with a biomineral based gel. For the synthesis of the gel 2 mL of acetic acid was added to 98 mL of distilled water and it was heated to 37℃. Then 10 mL of orthosilicate solution were added into the solution and the mixture left to equilibrate under continuous stirring. To improve the laser absorption properties of the gel, 0.5 g of $Er(NO_3)_3{\cdot}5H_2O$ were added. Usually gelification was complete after three days at room temperature.

2.3 Preparation of the bovine enamel blocks

Bovine tooth sections were prepared in a manner aimed at mimicking natural enamel loss. Rectangular sections (6 mm long, 4 mm wide and 1 mm thick) were prepared using bovine incisors, which were collected from the local abattoir, cleaned and sterilized by γ-ray irradiation. After sectioning, the blocks were polished on each side by hand for 2 min, using a 2500 grade of silicon carbide paper. At the centre of each section a lesion (2 mm wide, 6 mm long and 30 μm deep) was then etched with a 1% citric acid solution.

2.4 Coating of the enamel samples and pellet preparation

To coat the enamel blocks, the synthetic brushite powder was mixed with the biomineral gel in a 1:10 weight ratio resulting into a shear thinning suspension (max viscosity 1000 Pa·s). Utilizing a medical cementum spatula the lesions on each enamel surface were filled with the biomineral brushite/gel mixture by producing a homogenous flat surface. The samples were left to dry at room temperature for 10-15 min. To test the interaction of the brushite with the lasers, two powder pellets were also pressed in a die with diameter of 13 mm. Approximately 0.25 g of brushite powder was filled inside the die before pressing with a load of 7 ton for 30 min.

2.5 LASER irradiation experiments

For irradiation of the pressed pellets and the coated bovine incisor samples, the average power for the 800 nm and 1044 nm lasers was adjusted to 0.400 W (measured at the output of the source). This resulted in corresponding pulse energies of ~400 μJ (800 nm source) and ~0.4 nJ (1044 nm source). The spot diameter was 30 μm while the scanning velocity was 100 μm/s for the pellets and 250 μm/s for the enamel samples.

3. RESULTS

X-ray powder diffraction techniques were used to characterise the crystalline phases formed before and after laser irradiation. The pellets were analysed on an X'Pert MPD, Philips using monochromatic CuKa radiation of 0.154098 nm. For each measurement, the step size was 0.0634° and the 2θ scanning range was from 5 to 70˚ over a period of 35 min. Besides X-ray diffraction, Raman spectroscopic measurements were also carried out to confirm the structural changes before and after laser irradiation.

For investigating the size and shape of the brushite crystals, the morphology of the applied coating and for identification of physical and chemical changes induced by laser irradiation, a Hitachi SU8230 scanning electron microscope (SEM) was used. Prior to SEM analysis, each sample was coated with 5 nm thick Pt metal so that the electrostatic charging during SEM analysis can be minimised and vacuum cleaned for 10 min.

3.1 Characterisation of brushite before and after laser irradiation

Figure 2a shows the diffraction pattern of the non-irradiated material which is compared with the reference pattern of brushite (JCPDS-01-074-6549). As can be seen all the major peaks are identified (for 2θ; 11.56, 21.14, 23.48, 29.38, 35.54, 39.83, 45.39, 47.90) and consequently the synthesized calcium phosphate may be considered to be 98% brushite (with some monetite). Irradiating the material with the 1 kHz laser did not affected the crystal structure of the brushite and this is obvious if we compare Fig. 2a with Fig. 2b. The position and relative intensity of the peaks is exactly the same in the two patterns with the only exception being the peak at 2θ = 11.56 [brushite (0 2 0) plane]. This difference may be attributed to morphological changes on the surface of the pellet caused by the 1 kHz laser (i.e. the texture of the crystals has been altered). On the other hand irradiation with the 1 GHz laser transforms the biomaterial dramatically. The data from XRD indicate that the new dominant phase after irradiation is β-calcium pyrophosphate ($Ca_2P_2O_7$) (referenced to the pattern JCPDS-04-009-8733). In Fig 2b we also see the peak (0 2 0) peak for brushite at 2θ = 11.56° which appears to be the original preferred habit of the brushite powder. Taking into account that the penetration depth of the X-ray beam ranges from 20-120 μm (depending on the Bragg angle, 2θ) we suggest that although there is complete transformation of the material at the surface of the pellet some of the underlying mineral remains unaltered. Analysis of the cross section of the pellet with SEM supports this conclusion since there is a clear evidence that morphological changes in crystals has occurred only up to 45 μm depth (Fig. 3a) (consistent with the laser radiation absorption depth analysis).

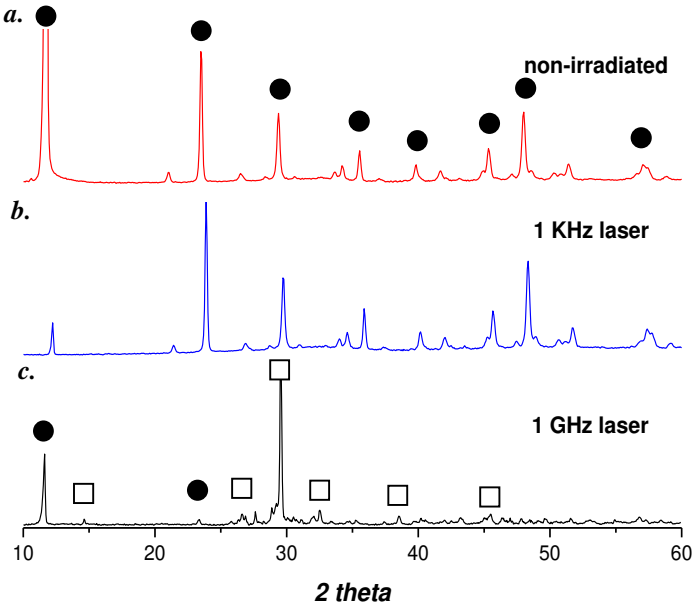

Figure 2: Powder X-ray diffraction patterns for a) non-irradiated pellet; b) pellet irradiated with 1 kHz laser; c) pellet irradiated with 1 GHz laser. (● brushite peaks; □ β-calcium pyrophosphate).

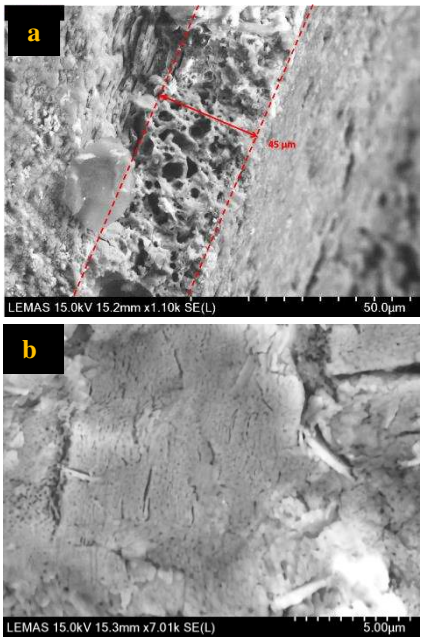

Figure 3: SEM images of the irradiated pellets with 1 GHz laser; a) cross-section; b) surface.

The transformation of brushite to β-calcium pyrophosphate was also verified by Raman spectroscopy. Figure 4 shows a comparison between an unirradiated sample and a sample irradiated with the 1 GHz laser. For the first case the most intense vibration peak is observed at the 988 cm^{-1} and this may be assigned to the v_1 PO_4^{-3} groups while the lower peaks at 575 cm^{-1} may be assigned to v_4 PO_4^{-3} groups and the peak at the 872 cm^{-1} to the symmetric stretching vibrations of POH units [4]. In the case of the irradiated sample multiple strong peaks were identified in the range of 942 to 1150 cm^{-1}. These peaks are assigned to the v^{as} PO_3 groups of the β-calcium pyrophosphate [5].

The transformation of brushite to β-calcium pyrophosphate is known to occur at temperatures higher than 750℃ a fact that proves the localized temperature incursion may be arising during irradiation with the 1 GHz laser.

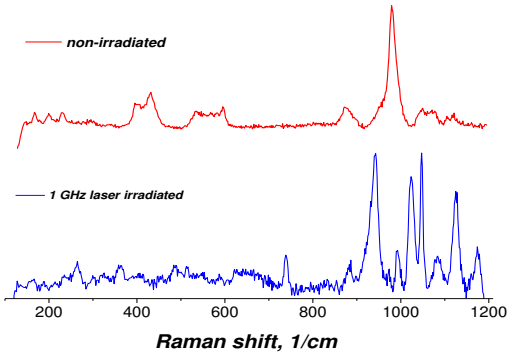

Figure 4: Comparison of Raman spectroscopy between non-irradiated and irradiated with 1 GHz laser.

3.2 Irradiation of the coated enamel samples

Figure 5 shows images of samples prior to irradiation (a), and after irradiation with the kHz source (b) and the GHz source (c). For both types of repetition rates, the laser irradiation has caused a clear modification of the original biomineral layer, which was applied to the bovine surface as a gel. In the case of the 1 kHz laser the modified surface seems to be quite porous, as a result of ablation which is the dominant process. On the other hand the results from the 1 GHz indicate the formation of reconstituted and recrystallized biomineral showing a homogeneous layer (Fig. 5c) which is the result of ultrafast transformation and melting of the biomaterial.

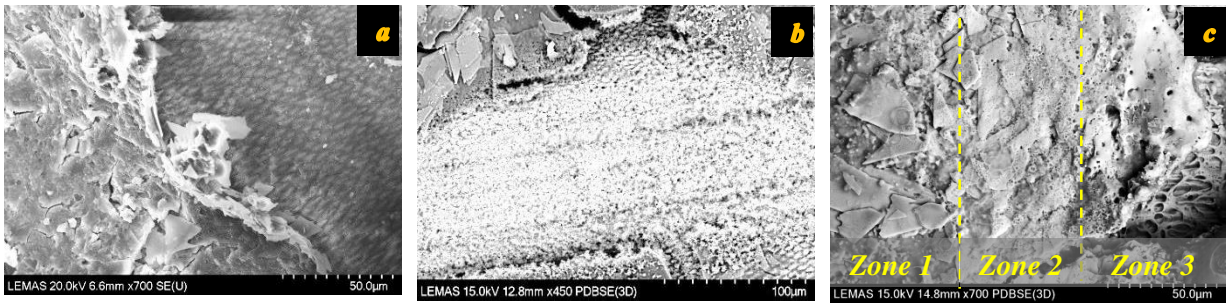

Figure 5: Comparison of the biomaterial a) before laser irradiation; b) after 1 kHz fts; c) after 1 GHz fts irradiation.

After analysing the irradiated samples there is evidence for localized melting when using 1 GHz repetition rate laser indicating that the local temperature rise may be of the order of 1350 ˚C (melting point of the Ca_2P_2O7 phase). In Fig. 5c three different zones are clearly identifiable. Moving from left to right (most probably from low to high intensity regions) we can speculate how the new layer is formed. In *zone 1* (low intensity) discrete brushite crystals are observed. In *zone 2* (higher intensity) the crystals seem to fade and bond together, while for the maximum intensity (middle of the focussed beam *zone 3*) it is not possible to distinguish individual crystals since a homogeneous surface has been formed. Elemental analysis by energy dispersive X-ray spectroscopy (*EDS*) indicates that all the chemical compounds of the biomineral gel are homogeneously distributed in the transformed layer with a relative composition (by % weight) of: O (40%), C (22%), Ca (16% and Si (8%). Although there are many studies demonstrating melting of semiconductors or metals with femtosecond lasers (e.g. [6]), to the best of our knowledge there are no reports of melting using such lasers in ceramic materials.

4. CONCLUSIONS

The following are the main conclusions of our investigation:

- Irradiation with 1 kHz laser causes change in the mineral (brushite pellets and coated enamel samples) only due to ablation.
- Irradiation with the 1 GHz laser causes local temperature to rise resulting in the transformation of brushite to β-calcium diphosphate in powder pellets while in the case of the brushite/gel coatings on enamel, melting of the synthetic biomineral was achieved.
- The local temperature rise, based on the transformations observed appears to be between 750 and 1350 ℃.
- On the coated enamel samples irradiation with the 1 GHz laser resulted in the formation of a homogeneous reconstituted mineral surface which is critical to the technique proposed for enamel restoration.

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