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Applications of Near-IR CW and Ultrafast Pulsed Lasers and Photo-active Biominerals in Reconstructive and Restorative Surgery of Hard Tissues

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ABSTRACT

The emergence of ultrashort pulsed near-IR lasers has opened novel opportunities not only for investigating the physics of interaction of such lasers with photo-active biomaterials for reconstructive and restorative bone and dental tissue engineering, but also for analysing the light-matter interaction in the CW laser regime for reconstructive surgical applications. The clinically-relevant examples discussed are for enamel restoration, osteoporotic bone-mass augmentation, and reconstructing damaged bone after trauma. Here, we explain the process of energy absorption in both the CW and ultrashort-pulsed regimes using the heat transfer process which impacts on the mineralisation of tissue. The aspect of thermal management by controlling the repetition rates of ultrashort pulses in near-IR lasers is also explained using sub-ablation and ablation models which are particularly relevant for dental tissue surgical reconstruction. On the other hand, for bone applications both the CW and ultrashort pulsed laser become highly relevant, depending on the type of clinical case for reconstructive surgery. The presentation will also explain the cell/human stem-cell biological characterisations of the laser-treated biomineral scaffolds *in vitro* for testing the toxicity, cell viability, and proliferation analyses. We will conclude by explaining the need for regulatory process, safety procedures needed prior to animal and first-in-human studies.

Keywords: Ultrashort pulsed lasers, bone and dental tissues, restorative and reconstructive surgery, heat transfer, tissue ablation, biominerals

1. INTRODUCTION AND CONTEXT

The ageing population (50+ years) has been increasing alongside the global population which is stretching the resources for improving health of the age group. Moreover, recent COVID-19 pandemic and the geopolitical tension have also curtailed the supply chain for healthcare products, which has led to consensus that within EU and other parts of the world the healthcare products and materials need to be a part of a resilient supply chain. In this context, the presentation focusses on procuring naturally occurring calcium phosphate biominerals and chitosan as primary source for bone and dental enamel tissue engineering. We also explained in our earlier articles [1-5], the importance of hydroxyapatite and fluorapatite minerals for the restoration of acid-eroded enamel and microbial resistant minerals.

The cell biology of bone mineral formation during osteogenesis explains that the amorphous calcium phosphate mineral forms like a gel in a neutral pH environment and then progressively transforms into a crystalline hydroxyapatite (HAp) phase in the presence of electrochemically active surface of collagen; the most abundant protein in a bone structure. The needle shape HAp crystal grows along the c-axis of hexagonal lattice (a = 9.423 Å and c = 6.875 Å), supported by the anisotropic assemblies of collagen fibres [1]. The amorphous gel and remineralisation mechanism are controlled by the osteoblast/osteoclast cellular activities in the presence of growth factors [6]. During bone formation, the mesenchymal stem-cells in the presence of bone morphogenic proteins (BMP) and vascular endothelial growth factor (VEGF), respectively, can be expressed to selectively create lineages of osteoblast/osteoclast and endothelial cells for angiogenesis [6,7]. The combination of biomechanically stable bone scaffolds for supporting osteogenesis and angiogenesis via chemotaxis are essential for bone formation. The absence of one these factors lead to compromised healing, missed bone union or non-union, demanding a costly revision surgery [8]. Based on the necessity of controlling the biology of bone formation, we designed a rheological mixture of biomineral (tricalcium phosphate) mixed with high-molecular weight chitosan and type-I collagen. In nature, the chitosan is derived from chitin, present either in the exoskeleton of shellfish or molluscs or from mushrooms [9]. The viscosity of admixture of mineral, type-I collagen with chitosan was controlled by mixing either the deionised water or medical grade ethanol, both of which lower the rheological viscosity of admixture for scaffold engineering with good control of porosity during fabrication steps (admixture gel casting followed by freeze drying).

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Comparing the bone biology with dental tissue biology, which has a complex anatomy with a hierarchy of sub-structures as shown in see Figure 1. The *alveolar bone*, which is the main structure of jaw and contains tooth sockets, which is enveloped with *soft periodontal ligaments* (pink regions with blue stripes) as anchors for supporting the hard dental socket encased inside *cementum* (beige white) and the alveolar structure. For maintaining tissue, a *microscopic vasculature* and *nerve network* also runs inside the alveolar structure for repair/nutrition supply for cells and signal processing. The upper part of anatomy (white) is divided into *sub-gingival* and *above-gingival* regions, in which gingiva is a soft vascularised fibrous tissue and it connects with the *cementum* and the *enamel*. The enamel is avascular, and it is a highly dense mineral layer which forms over *dentine*. Both the dentine and dental enamel are mineralized tissue, with dentine being much softer than the enamel. Note that the enamel is avascular which implies that it is irreparable once damaged, and it can only be restored exogenously. As explained elsewhere [3-5], the restoration of acid-eroded and worn dental enamel rely on exogeneous mineralisation using either a cementitious polymerisation or a pulsed laser assisted bonding of exogenous mineral on damaged sites.

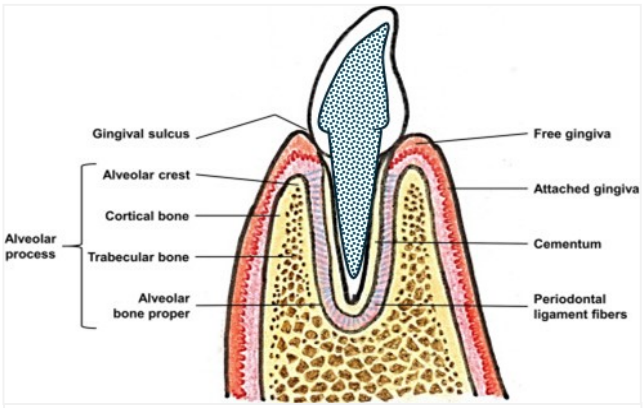

Figure-1:Anatomy of tooth reference; https://doi.org/10.1016/B978-0-08-102478-2.00001-5 [10]

2. MATERIALS, METHODS AND RESULTS

2.1) Dental Enamel Restoration: For dental enamel restoration, the base minerals were iron free Sr^{2+},$Ce^{3+,4+}$-doped fluorinated apatite (FAp) for restoring enamel with antimicrobial properties on the top surface, exposed to the oral fluid. Whereas the underlying surface with dentine was composed of Fe^{3+}-doped hydroxyapatite (Fe-HAp) [3,4]. For pulsed laser sintering, the human molars were cut and the acid-eroded with acetic acid at pH=3.5 for subsequent restorative procedure. The acid-eroded enamel cavities were also ablated at the edges for smoothening the surface and by using the ablation and heat transfer models. For the restoration of enamel, the laser irradiation experiment was carried out at two different pulse energy regimes (500 µJ and 60 µJ) using 800 nm, Ti:Sapphire femtosecond laser (100 fs, 1 kHz, 5 mm/s and 5 µm scanning line overlap) for bovine tooth preparation and sintering.

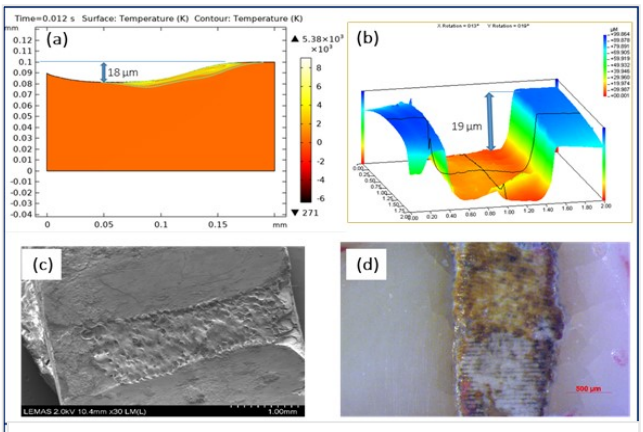

Figure 2: a) Application of FE-model for ablation control of mineral during cavity preparation on human enamel. b) Profilometric analysis of the fs-laser prepared cavity. c) Using Fe-HAp and 60µJ input energy and twin linear scanning with an 800nm 100fs 1KHz Ti-Sapphire laser, the enamel layer was rebuilt as shown in the SEM image. d) Optical image of the same area in c).

The finite element (FE) model was developed for optimizing the laser operating conditions to avoid excessive tissue removal (Fig. 2a). The rectangular cavity (1x3mm^2) with a depth of 20µm was prepared on the flat enamel surface by operating the laser at a high pulse energy regime (500µJ, Fig. 2b). Subsequently, the cavity was filled with a mineral slurry containing 1:4 ratio of Fe-HAp and chitosan. The laser sintering experiment was carried out at the low pulse energy regime (60µJ) using different scanning strategies (single and dual scan) to ensure uniformity of sintering across the depth (Fig. 2c). The study also be extended to the in-situ evaluation of restored enamel slabs positioned onto a dental appliance in the mouths of volunteers for ascertaining the statistical efficaciousness of the procedure and comparing the acid-resistant properties with natural enamel as control (Fig. 2d). The surface morphology, chemical compositions, mechanical and interfacial properties of the restored enamel surface were evaluated, and its formation mechanisms were analysed. The in-situ evaluation study confirmed that the enamel restored with the dual scan strategy was stable in the oral environment. The main conclusions from the investigation on enamel restoration are that the ablation-controlled process helps in the preparation of the acid eroded cavity for improving the bonding with exogenous minerals via fs-pulsed laser sintering. For mineral sintering onto and around the natural enamel surface, the underlying layer was reconstructed with Fe-HAp using 60µJ of pulse energy. However, for long-term patient benefit it is better to reconstruct the mineral layers as two layers – Fe-HAp as the adhesive bonded with the dentine surface and the overlaying layer as microbial resistant FAp. In summary, the availability of fs-pulsed laser offers a unique opportunity for reconstructive enamel surgery.

2.2 Reconstructive Bone Surgery: In this section our main goal is to explain an important application of novel photo-active biomineral composite with chitosan and type-1 collagen for bone reconstruction/augmentation surgery using both the CW and fs-pulsed 976 nm and 1040nm laser (120fs, 200MHz), respectively. In both types of lasers, it was critical to have a fibre guided delivery at the "point-of-surgical-procedure" which is more precise than an open-beam free-space optics option. For demonstrating bone adhesive using biomineral (Fe-HAp) was mixed with collagen type-I (75:25) ratio and mixed 3 volume% of chitosan stock solution (molecular weight 10kilo Dalton+ [9]).

There is a wealth of literature on the use of polymethyl methyl acralate (PMMA) and glass-ionomer [11] cements, which are used for bone and dental applications, respectively. The PMMA, which works in the wet environment, when used in situ releases exothermic heat which is potentially necrotic. Also, the implanted PMMA does not resorb which means the new bone formation is ruled out as observed in the Masquelet critical defect repair surgery [12]. The glass-ionomeric cement only works in dry condition which is why it is not suitable for bone applications. Recently, in a review article we compared the available bone adhesive/cements for clinical use [11], and one of the methods uses bio-inspired adhesive with a calcium phosphate mineral and phosphoserine [13], which is based on chemical reaction in situ as in PMMA and is claimed to be mechanically robust after adhesion. In the past, light-curable methyl acrylate bone adhesive was also reported [14] however as the polymer does not resorb, it induces immune response and leads to further complications leading to revision surgery for removing the implanted material.

In our approach, we have selected constituent which are biocompatible and osteoconductive as verified in our cell biological analysis. The use of pulsed and CW lasers was investigated using both the soft porcine tissue models for gathering evidence of necrosis and on hard cortical bovine bone samples. In this presentation, we demonstrate the use of optimal power for bonding bovine jaw bones (BJB) using a mineral-based bone adhesive. The presentation explains the heat management based on photo-activation for preventing for preventing collateral damaged of soft-tissues. More specifically, we demonstrated in vitro physical evidence using the soft porcine tissue for analysing the threshold laser power of a Gaussian beam required for tissue necrosis in the wet condition (see Figure 3). In Figure 3, The beam diameters at the focal point were 4.5mm, 6.6mm, 8.4mm and 13.7mm from the working distances of 5, 10, 13 and 20 cm, respectively. The optimum working laser irradiance was 8.83Wcm^{-2} delivered at a working distance of 10cm, which was found to meet the necessary power density needed for drying and promoting photo-active adhesion. Based on soft tissue interaction with the 976nm CW laser, we designed the program of bone adhesion using the laser parameters optimised for the soft tissue for preventing collateral damage.

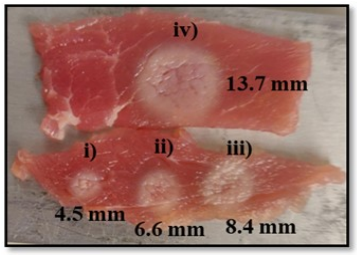

Figure 3: Characterisation of the Gaussian foot-print of a fibre-coupled Amtron Q-CW laser operating at 976nm, showing beam shape and at following different distances i) 5cm, ii) 10cm, iii) 13cm, and iv) 20cm from the laser focal point.

The analysis of laser-adhered BJBs showed the evidence of reduction in OH-absorption (3300cm^{-1}-3400 cm^{-1}) resulting from the evaporation of water, which were also verified using the soft poultry, porcine, and bovine bones in the 8W/cm^2 for a quantitative comparison (see Figure 4a and 4b). For delineating the margin of damaged soft-tissue, two supplemental peaks in the IR region were analysed and attributed to the C-H bond vibrations in the (2824-2883cm^{-1}) and (2883-2993cm^{-1}). The flexural modulus of the BJBs glued by drying in air, hot-air, and using laser, were found to be 8.11, 22.55 and 26.50MPa, respectively. The laser-bonded BJBs were also tested for cytotoxicity using XTT extract testing, and 98% average cell viability via live/dead analysis. We also investigated the application of 1040nm and 800 nm fs-pulsed laser for bone adhesive bonding. These results also show promise with substantially reduced risk of necrosis and faster surgical procedure. Power scaling in fs-laser offer much more advantage in terms of future technology development.

For clinical translation of the in vitro/in situ research lays the foundation for early-stage ethically approved trials using suitable animal model or models, which must be stage-gated for generating unambiguous statistically significant data, so that the essential regulatory process might be in a position to validate the data for individual h cases of bone and dental clinical conditions for efficaciousness of the designed procedure using a medical device.

3. CONCLUSIONS

In this presentation we have demonstrated the use fs-pulsed laser (near-IR 800 nm, 1KHz) for the preparation of enamel cavity using ablation and then refilling and bonding the cavity with a photoactive biomineral composite using the fs-laser. The experimental investigation on enamel restoration in vitro was extended for volunteer based in situ mouth appliance trials from which the oral acid-resistance data were satisfactorily compared with the natural enamel surface. For bone adhesion, the in vitro studies, using the wet soft tissue and bovine bone models, have shown that at modest CW power density of 8.83 W/cm^2, the bovine bone pieces may be

successfully joined within few minutes for enabling a process which may be translated into a suitable animal model (e.g. rabbit and sheep).

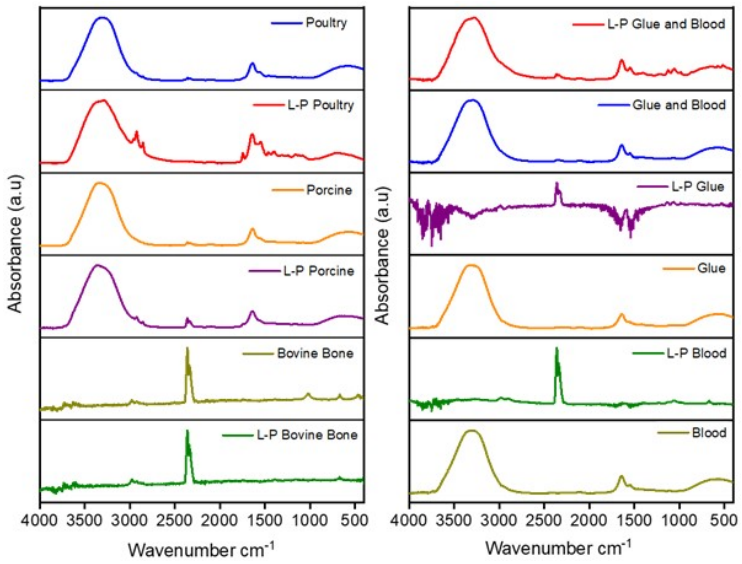

Figure 4: The molecular vibration spectroscopic analysis of the non- and laser-processed samples characterised using the attenuated total reflection (ATR) mode in the Vertex 70 FTIR spectrometer; (a) non and laser-processed (L-P) soft and hard tissues, (b) non and laser-processed (L-P) adhesive, soft and hard tissues mixed with blood.

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