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**Proceedings Paper:**
Alsubhe, E, Anastasiou, A, Maddi, C et al. (3 more authors) (2019) Interaction of Femtosecond Pulsed Lasers with $Fe^{2+}$ and $Fe^{3+}$ Doped Calcium Phosphates for Bone Tissue Engineering. In: Proceedings Biophotonics Congress: Optics in the Life Sciences Congress 2019 (BODA,BRAIN,NTM,OMA,OMP). Biophotonics Congress: Optics in the Life SciencesCongress 2019 (BODA,BRAIN,NTM,OMA,OMP), 14-17 Apr 2019, Tucson, AZ, United States. Optical Society of America .

https://doi.org/10.1364/BODA.2019.JT4A.36

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# Interaction of Femtosecond Pulsed Lasers with $Fe^{2+}$ and $Fe^{3+}$ Doped Calcium Phosphates for Bone Tissue Engineering

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**Abstract:** In this work, we aim to investigate the effect of $Fe^{2+}$/$Fe^{3+}$ doping on the laser sintering of calcium phosphate minerals for the fabrication of bone scaffolds. The laser-matter mechanisms and the biological response are discussed. **OCTS code:** 320.2250, 140.7090.

**Introduction:** Current trends in tissue engineering (i.e. personalised medicine and near patient manufacturing) favour the use of additive manufacturing techniques for the fabrication of bone scaffolds. Selective laser sintering (SLS) is one of the well-established and widely used methodologies. Many studies have been reported by utilizing the SLS technique for manufacturing of biomaterials; e.g. metal alloys, polymers and bioglass [1]. Of particular interest though, is the investigation of calcium phosphate (CaP) biominerals due to their chemical and structural similarity with the natural bone mineral. Recently, it was reported that doping CaP with $Fe^{2+}$ and $Fe^{3+}$ ions improves energy absorption and allows the localised sintering of the materials without inducing any thermal damage to the surroundings [2, 3]. During irradiation with a femtosecond laser, the Fe-rich phases (e.g. $Fe_2O_3$ nanoparticles) absorb the laser radiation and act as thermal antennae, dissipating energy to the vicinal mineral phase, thereby triggering the sintering and densification of the surrounding calcium phosphate crystals. Based on this established mechanism of laser radiation absorption, the present work aims to identify an optimum doping concentration of $Fe^{2+}$ and $Fe^{3+}$ ions and investigate the effect of doping on i) the mechanism of laser matter interaction leading to changes in the materials structure; and effect of such changes on ii) the cytobiological adaptability of the laser-radiation transformed materials for ultimate in vivo use.

**Materials and methods:** In this investigation we synthesised brushite ($CaHPO_4 \cdot 2H_2O$) doped with different concentrations of $Fe^{2+}$ and $Fe^{3+}$ ions (0%, 5%, 10%, 20% and 30% mol by substituting $Ca^{2+}$ ions in the phosphate structure). The laser experiments were performed in ambient atmosphere using a Ti:sapphire amplified femtosecond laser system (Coherant-Libra), which emits radiation at a wavelength of 800 nm with 1kHz repetition rate and 100 fs pulse duration. The laser energy was controlled using a polarizer and a half-wave plate. The threshold fluence ($F_{th}$) of all $Fe^{2+,3+}$-doped brushite was calculated by using $D^2$ method [4]. The pre/post irradiation $Fe^{2+,3+}$-doped brushite biomaterials were characterised using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) for investigating changes in the crystal structure and phase constitution. The in vitro biocompatibility of laser-irradiated scaffolds was characterised by evaluating the adhesion and proliferation of osteoblast (G-292) cell lines.

**Result and discussion:** During the synthesis of the minerals some of the iron is incorporated into the crystal lattice while the rest, results in the formation of secondary iron rich phases (e.g. iron phosphate - $FePO_4$). For doping concentrations up to 10 mol%, brushite is the dominant phase and its original crystal structure remains intact after $Fe^{2+}$/$Fe^{3+}$ incorporation. At 20% mol. an amorphous iron phosphate phase is formed alongside brushite crystals and for higher iron concentrations (>20%), the amorphous iron phosphate becomes the dominant phase.

Initially, laser irradiation experiments were carried out with different energy densities, in order to identify the ablation threshold ($F_{th}$) for each material. It was found that the presence of iron results in the shifting of $F_{th}$ to higher energy densities as it increases exponentially with the doping concentration (0.20 J/cm$^2$ for un-doped brushite, 0.5 J/cm$^2$ for 5% Fe, 1.5 J/cm$^2$ for 10% Fe and 5.9 J/cm$^2$ for 20% Fe).

In **Figure 1** we compare the surface topology of undoped, 5% Fe-doped and 20% Fe-doped brushite after irradiation of pelletized calcium phosphate materials with constant laser pulse energy (200 µJ), scan speed of 100 mm/s. Using the comparable energy density conditions (1.7 J/cm$^2$), deep microchannels were formed which is a clear indication of ablation. In the case of the 5% $Fe^{2+,3+}$-doped material, by using identical energy density conditions, partial melting was induced and shallow microchannels were observed. For 20% $Fe^{2+,3+}$-doping, there is no indication of ablation by forming microchannels, however such highly doped materials readily absorbed energy and cause extensive melting of the crystals. Also due to the localised temperature rise, thermal stresses were developed resulting into the cracking of the surface.

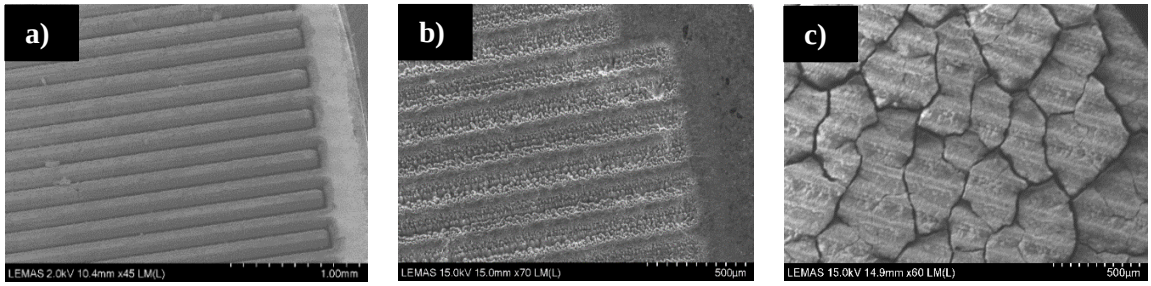


**Fig 1.** SEM images show the laser tracks and irradiation area on a) 0% Fe-brushite, b) 5 % Fe- brushite and c) 20 % Fe-brushite.

The effect of $Fe^{2+,3+}$ doping on the laser – matter interaction mechanisms is also evident after the phase analysis using XRD of the post irradiated samples. After laser irradiation of 0% Fe doped brushite, the material retained the same crystal structure. However, 5 and 10% doped brushite were transformed into a mixture of monetite ($CaHPO_4$) and brushite: while for 20% doped brushite, the monetite structure was only observed (**Fig. 2)**. The transformation of brushite into monetite normally occurs at ~200 °C during slow heating in air in an oven. Based on the comparative studies of phase transformations in laser irradiated $Fe^{2+,3+}$-doped calcium phosphate materials, it is evident that the doping concentration $Fe^{2+}/Fe^{3+}$ in the calcium phosphate controls the laser penetration depth and ablation, and also the resulting phase and phase morphologies formed at the irradiated surface.

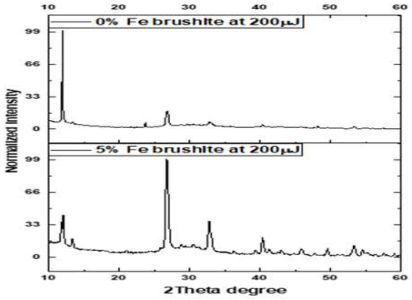


**Fig 2.** Comparison of X-ray diffraction patterns of brushite and 5% Fe doped brushite scaffolds after laser irradiation.

Osteoblast cell lines (G-292) were used to test the cell viability and proliferation on sintered $Fe^{2+}$brushite materials. For cytotoxicity experiments contact and extract (adenosine triphosphate ATP) method was followed. DNA using picogreen protocol was applied for the proliferation process. It was proven that doping with $Fe^{2+/3+}$ does not induce any toxicological risk, even for high doping up to 20% concentrations. Moreover, cells were attached and proliferated for (1, 3, and 7 days) on the $Fe^{2+/3+}$ containing minerals more extensively than in the case of undoped material (Fig. 3). The results of seeded osteoblast cell lines on the top $Fe^{2+/3+}$ -doped pyro-phosphate microchannels will also be reported by comparing with the cell proliferation data, as exemplified in **Fig. 3**.

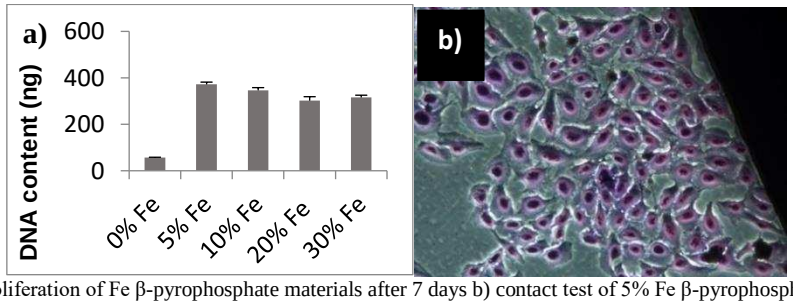


**Fig 3**: a) Proliferation of Fe β-pyrophosphate materials after 7 days b) contact test of 5% Fe β-pyrophosphate presents cells growth around sample.

**Acknowledgements**: This work is sponsored by Taibah University, Madinah, Saudi Arabia as a part of a PhD scholarship.

## References

[1] Harun, W. S. W., et al. "A review of powder additive manufacturing processes for metallic biomaterials." Powder Technology (2017).

[2] Anastasiou, A. D., et al. "Sintering of calcium phosphates with a femtosecond pulsed laser for hard tissue engineering." Materials & Design 101 (2016): 346-354.

[3] Anastasiou, A. D., et al. "Exogenous mineralization of hard tissues using photo-absorptive minerals and femto-second lasers; the case of dental enamel." Acta biomaterialia 71 (2018): 86-95.

[4] Liu, J. M. "Simple technique for measurements of pulsed Gaussian-beam spot sizes." Optics letters 7.5 (1982): 196-198.