Fabrication of Multi-Layered Bone Scaffolds using Femtosecond Pulsed Lasers

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Abstract: An IR femtosecond pulsed laser was used for micropatterning of biomineral containing chitosan membranes, aiming to enhance bone mineralization and angiogenesis. Pre and post irradiation materials have been characterized with XRD, SEM and spectroscopic techniques. **OCIS codes:** 310.6845, 310.6870, 320.7090

1. Introduction

Bone is a complex living tissue with significant metabolic and regenerative activities, which are disrupted when the tissue is damaged. Healing of fractured bone is a complicated process therefore; an ideal bone scaffold should have: i) exceptional osteogenic potential in order to promote new bone formation; ii) load-bearing properties; iii) appropriate microstructure for promoting angiogenesis for circulation of nutrients and iv) antibacterial resistance to avoid infections that lead to failure of the surgery. Research efforts have been focused on development of the "perfect" biomaterial, targeting to achieve all the aforementioned properties with a single material system; e.g. blocks of calcium phosphates, coated Ti-implants [1], polymers [2] etc. The aim of our research is to establish a novel approach, for the development, fabrication and implantation of bone scaffolds, based on the fundamentals of advanced manufacturing using femtosecond lasers.

In the present work we discuss the fabrication of a multi-layered scaffold which has the potential to promote both bone mineralization and the formation of intrinsic vasculature. To achieve this we developed two types of chitosan mineral loaded samples. The Type-1 sample, aims to enhance osteogenic potential, it is loaded with calcium phosphate minerals (e.g. □-calcium pyrophosphate) and is fabricated through freeze drying in order to achieve a highly porous honeycomb structure. The Type-2 samples are based on membrane geometries, fabricated through casting of chitosan solutions loaded with CeO2 and Ce2O3 nanoparticles which are known for their excellent antibacterial and angiogenetic properties already observed in systems for in vitro and also in vivo models [3]. A Coherent Libra-S-1K (100fs) femtosecond laser (1 kHz repetition rate), at a wavelength of 800 nm, was utilised to fabricate microchannel networks and patterns on the Type-2 membrane surfaces. Micropatterning and microchannels are expected to promote guided cellular growth leading eventually to the formation of vasculature. In addition, the increased capillary forces in the channels will allow for better circulation of the nutritional components which are necessary for the formation of new blood vessels. The scaffold can be constructed through a numbering up approach where several layers of Type-1 and Type-2 membranes are piled up together in order to mimic the natural structure of cancellous bone.

2. Materials & Characterizations

Cerium oxide nanoparticles were formed using a hydroxide mediated approach. A 0.3M aqueous solution of sodium hydroxide was added dropwise to a cerium nitrate solution (1M) at 25°C. The mixture was left under continuous stirring for 24h until it gets a cloudy pink colour (is due to precipitation of the NPs??). After filtration the collected nanoparticles were washed several times with distilled water and were subsequently frozen at -80°C for 24h and then placed into a freeze drier for 24 hrs. X-Ray powder diffraction (Fig.1a) and FTIR ~~have been~~ were used to verify the formation of the CeO_2 nanoparticles.

For the synthesis of both Type-1 and Type-2 materials, 6g of chitosan flakes was dissolved in a 2% v/v aqueous acetic acid solution under continuous mixing for 24h. Type-1 samples were synthesised by adding to the chitosan solution different quantities of calcium phosphate minerals (i.e. 20, 30 and 40% w/w). After freeze drying, porous membranes have been obtained with pore sizes in the range of 42μm and 94μm (fig.1b) which are appropriate for bone regeneration (the apparent porosities are shown in fig.1c). For Type-2 membranes, CeO_2 nanoparticles have been added to chitosan solution and the mixture left for ~~t~~ drying until the formation of a compact membrane.

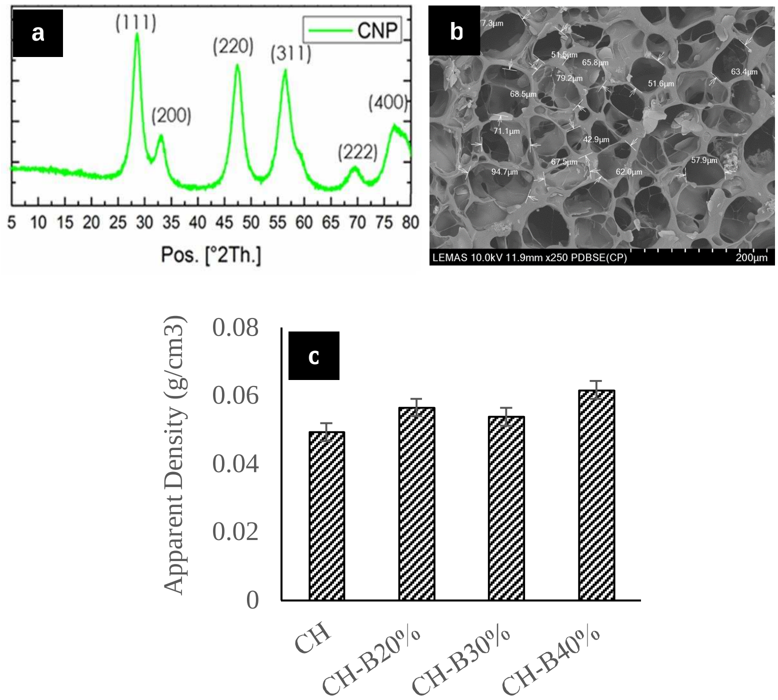

Fig. 1. (a) XRD analysis of cerium oxide nanoparticles; (b) Type-1 freeze dried sample containing 30 wt% calcium phosphate minerals; (c) Calculated apparent density of freeze dried calcium phosphate-chitosan samples

3. Femtosecond Pulse Laser & Porosity

Experiments were carried out to identify the ablation threshold of Type-2 materials for the fabrication of channels and micropatterns (report the ablation threshold). The initial average power of the laser was set at 40mW and then consecutive channels were formed by gradually increasing the power in steps of 20mW per line (maximum power used was 220mW). As it was expected, the channel width is increased by increasing the power of the laser in a range between 30µm and 140µm. Fabrication of microchannels on the membrane surfaces were successful, as can be seen in Figure 2a.

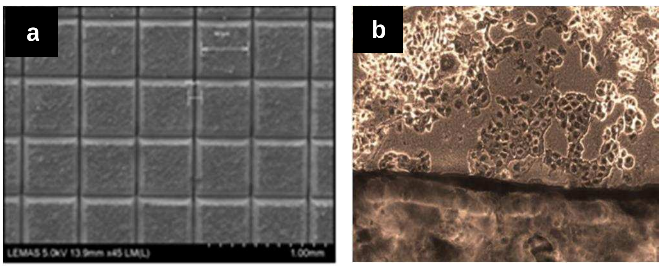

Fig. 2. Hitachi SU8230 SEM images (a) Regular and periodic microchannels fabricated with the aid of a femtosecond laser operating at 800 nm; (b) Optical microscope image of inital osteoblast contact test on chitosan-calcium phospahte membrane

Initial tests verified that both the Type 1 and Type 2 samples do~~esn’t~~ not present any toxicological risks. Osteoblast cells (cell line G292) were grown on and close to our samples as shown in fig.2b. Further experiments are still under process in order to confirm that cell growth and proliferation occurs within the micropatterns/channels.

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5. References

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