Fabrication of Multi-layered Bone Scaffolds using Femtosecond Pulsed Lasers

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Clinical Need

Healing of fractured bone is a complicated process therefore an ideal bone scaffold should have:

a) exceptional osteogenic potential,
b) load-bearing properties,
c) promote angiogenesis for circulation of nutrients,
d) antibacterial resistance to avoid infections that lead to failure of the surgery.

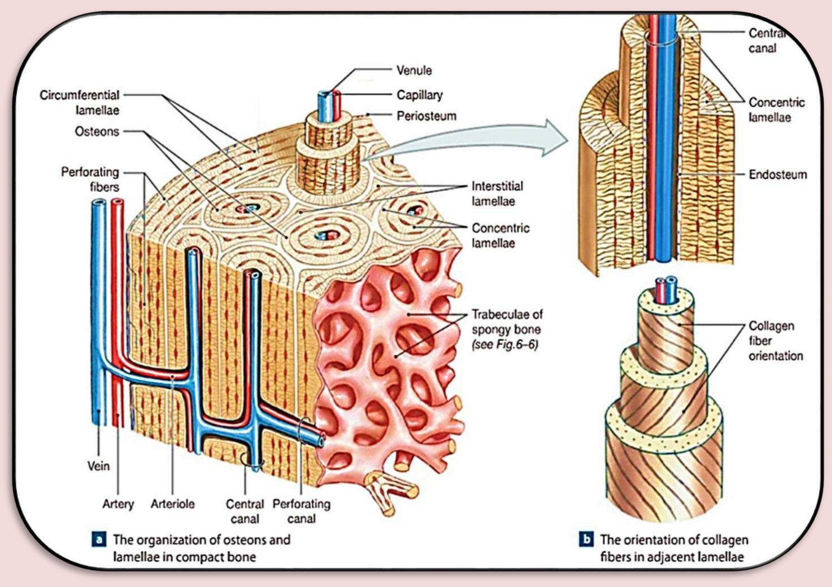

Fig.1. Complex Concentric Structure of Bone

The structure of natural bone is complex and difficult to mimic (Fig.1). Current bone scaffolds do not possess the correct structure and environment to encourage mineralization and vascularization collectively.

Aim

To fabricate **multi-layered** bone scaffold which has the potential to promote **bone mineralization**, **intrinsic vascularisation** and to provide **antibacterial properties**.

Materials

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. ß-calcium pyrophosphate) and is fabricated through freeze drying in order to achieve a highly porous honeycomb structure (Fig.2.) that will promote osteoblast growth and proliferation.

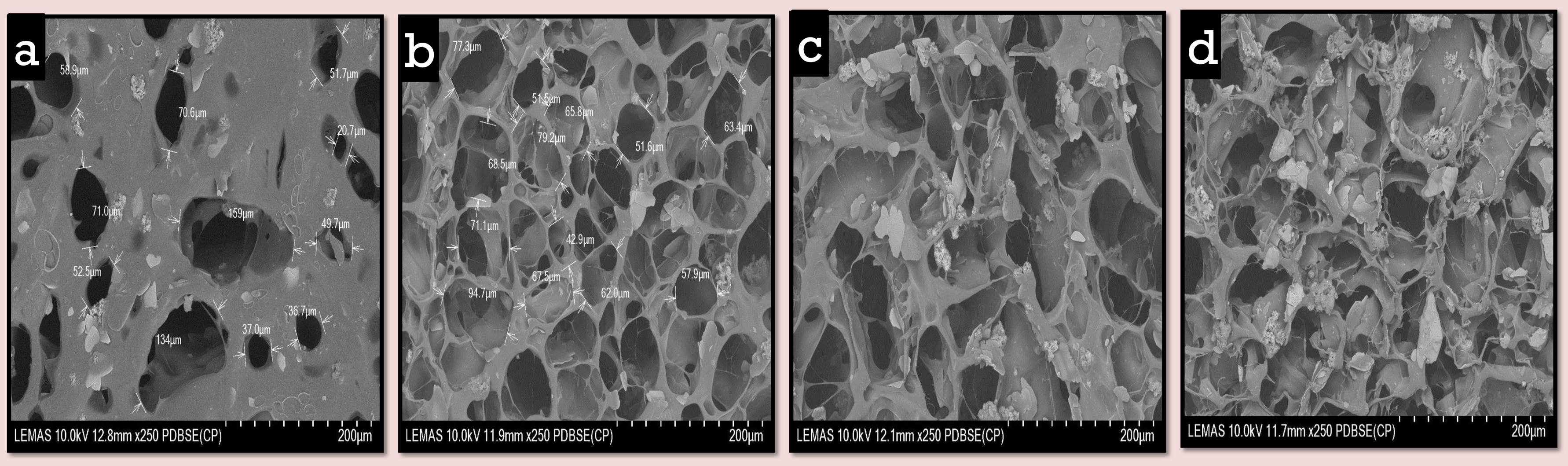

Fig.2. Comparison of Hitachi SU8230 SEM images of Type-1 freeze dried sample containing calcium phosphate minerals, (a) 20 wt%, (b) 30 wt%, (c) 40 wt%, and (d) 50 wt%.

The Type-2 samples are membrane geometries, fabricated through casting of chitosan solutions loaded with CeO_2 and Ce_2O_3 nanoparticles (NPs) which are known for their excellent antibacterial and angiogenetic properties (Fig.3) already observed in systems for in vitro and also in vivo models [2]. The membranes were laser micropatterned to enhance angiogenesis by allowing guided cell growth throughout the scaffold.

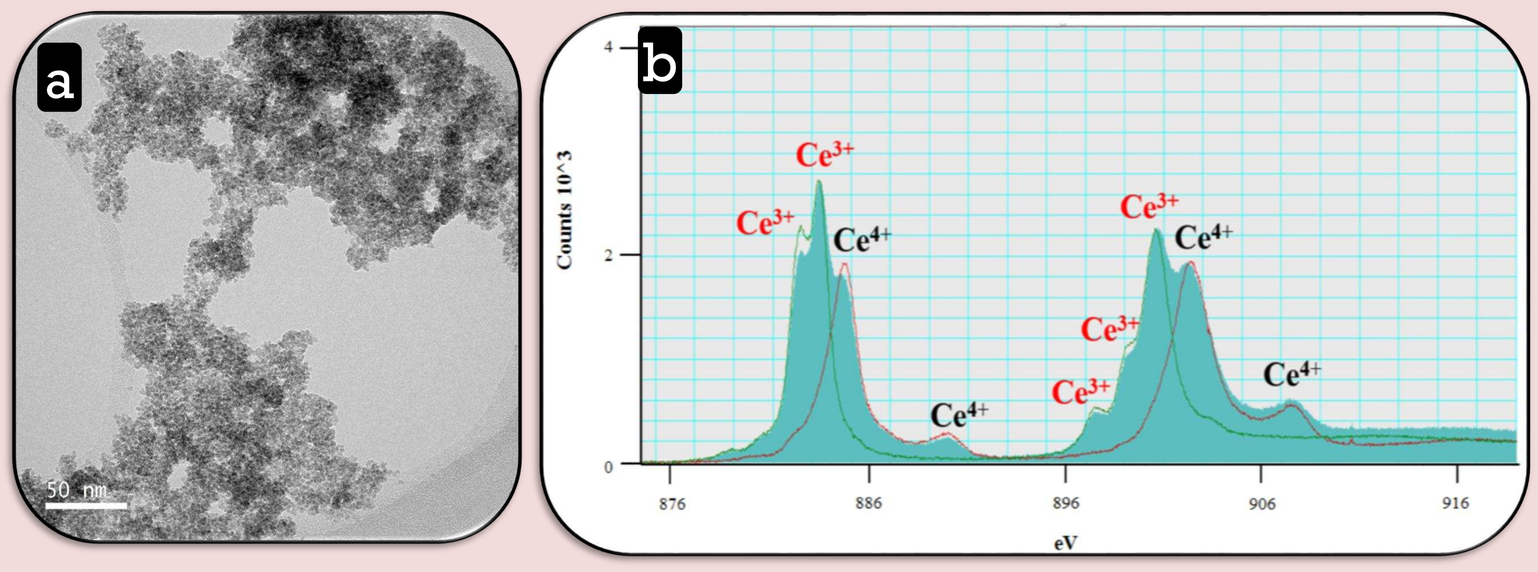

Fig.3. (a) TEM images of cerium oxide nanoparticle; (b) EELS analysis of Type-A nanoparticles depicting the presence of Ce^{3+} and Ce^{4+} oxidation states.

The scaffold can be fabricated through a **numbering up** approach where several layers of Type-1 and Type-2 samples are piled up together in order to mimic the natural structure of cancellous bone.

Femtosecond Pulsed Laser

A Coherent Libra-S-1K femtosecond laser (1 kHz repetition rate, 100 fs pulse duration, wavelength of 800 nm) was utilized to fabricate microchannel networks and patterns on the membrane surfaces depicted (Fig.4.).

- Ablation threshold of Type-2 materials; this was determined to be 5.4 J/cm^2.
- Initial laser power was set at 40 mW thus consecutive channels were formed by gradually increasing the power in steps of 20 mW per line (maximum power used was 220 mW).

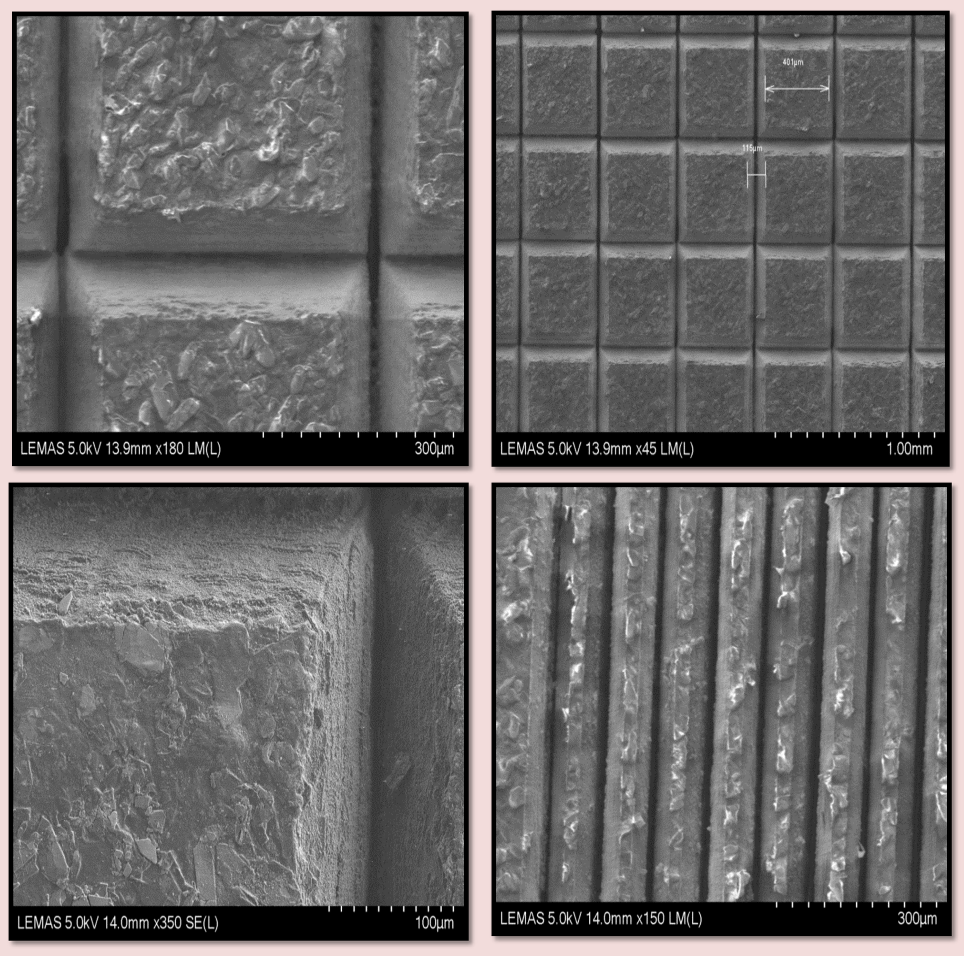

Fig.4. Hitachi SU8230 SEM images of consistent micro-networks created with the aid of a femtosecond laser (1kHz) upon chitosan membrane containing 1g of mineral.

Results

During the synthesis of the nanoparticles it was observed that the drying method (i.e. freeze drying or furnace drying) significantly affected the physicochemical properties, size, shape and the Ce^{4+} to Ce^{3+} ratio.

- Freeze dried NPs were successfully fabricated with particle sizes between 4nm and 8nm whereas the particle sizes of furnace dried NPs were between 15nm and 20 nm.

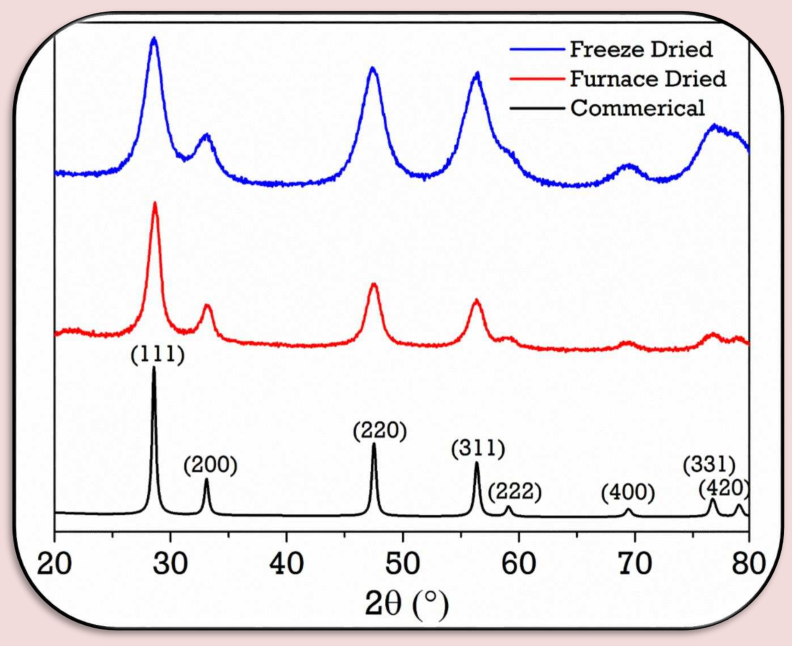

Fig.5. X-ray diffractometer spectrum of cerium oxide NPs, 2θ scanning range was 20° to 80° at a scan speed of 5s and increment of 0.03.

- For **Type-2** samples the initial rate of resorption was significantly high for all the membranes, however; as time progressed the rate began to stabilize (Fig.6).
- Membrane resorption releases NPs into the surrounding area which may then aid in the prevention of infection.
- It is essential to optimize the rate of resorption of the scaffold with the rate of bone mineralization and tissue remodeling.

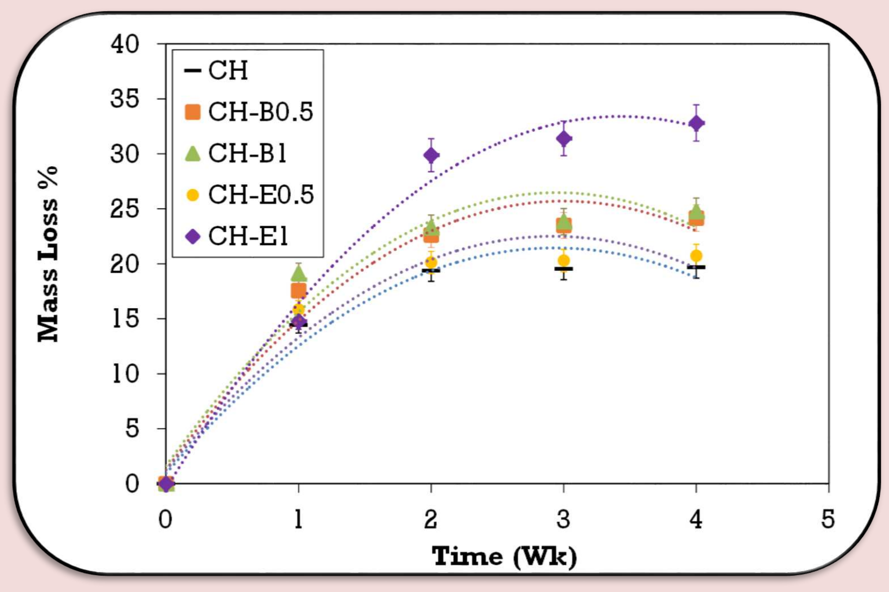

Fig.6. Mineral based membrane samples immersion in saline solution for a period of 4 weeks to test the rate of degradation.

- Co-culturing is essential to ensure osteoblast cells (mineralisation) and endothelial cells (Fig.7) (vascularisation) are able to grow on all the membranes fabricated
- Cytotoxicity testing was successful thus; determining all membranes were not toxic to cells.

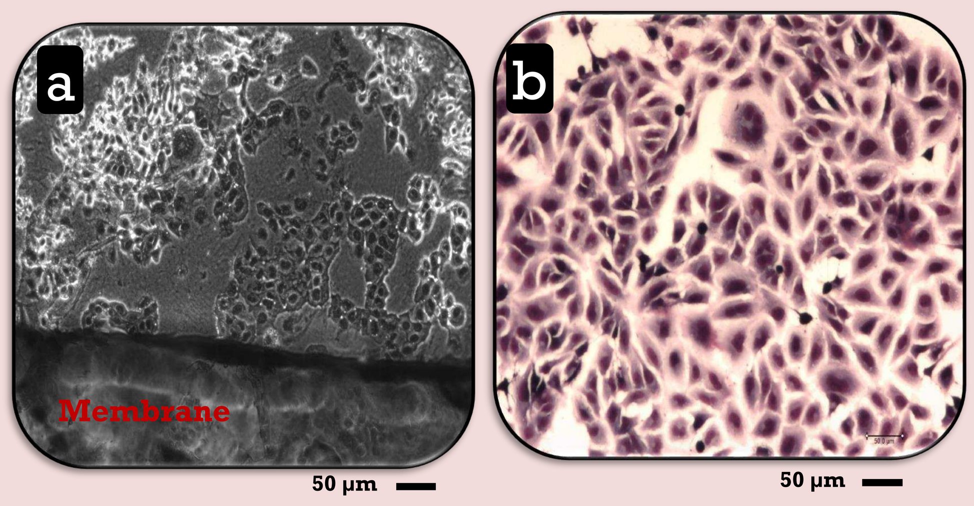

Fig.7. Microscope images of G292-osteoblast growth on membrane samples (a) chitosan film, (b) NaoH treated chitosan film.

Conclusions

- Fabrication of microchannels on the membrane surfaces were successful, as can be seen in fig.4. Microchannels were created without damaging the surrounding material. The channel width sizes ranged between 30 µm and 140 µm for laser power between 40 mW and 220 mW.
- We fabricated 2 types of mineral loaded samples, when layered together the scaffold induces mineralization, enhances angiogenesis but also has antibacterial potential.
- Human osteoblast cells were successful grown on Type-1 samples indicating the potential for bone generation and mineralisation which is essential for bone remodelling.
- Type-2 samples containing cerium oxide NPs have the potential to reduce the prevalence of bacteria during and after bone scaffold implantation. This is linked the samples ability to resorb allowing the NPs to be released at a steady rate, the NPs will aid in minimizing or preventing infection.

QR code for LinkedIn Profile

Acknowledgments

This research is supported by The University of Leeds and is funded by the Engineering and Physical Sciences Research Council (EPSRC).

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