Nano-calciumphosphate scaffold generation for bone repair/replacement: elucidating the signalling response and cell cycle

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INTRODUCTION

Strong, bioinert materials have always been the focus for bone replacement and repair. This practice has since moved towards materials that can mimic living tissue and aid the healing process (i.e. be replaced by natural bone); thus materials that are bioactive as well as bioresorbable[1,2]. Currently, the most widely used bioactive bone substitute is calcium phosphate-based materials. However, these calcium phosphate-based materials (i.e. hydroxyapatite (HA) and β-tricalcium phosphate (TCP)) do not fulfil all the current requirements for bone repair and replacement due to some characteristics such as:

- Lack of collagen fibres[2-4]
- Very brittle, therefore not prone for use in load-bearing circumstances
- General bioactivity needs improvement
- Most applications are still macro-sized[3].

The ideal biomaterial for bone replacement implanted into the body will be resorbed by the osteoclasts over time completely, while osteoblastic activity deposits new mineralised bone at the site. Bone is dynamic living tissue, therefore it is important that novel bioceramics are developed that will initially function as bone replacement attending to all the requirements such as biocompatibility, structure, filler as well as load-bearing. These bioceramics will also be able to activate the cellular response to recruit osteoclasts and osteoblasts to the implant site for the resorbtion of the implant whilst fine-tuning the formation of new living bone[2-6].

AIM

The purpose of this study was to generate electrospun biphasic nanobioceramic scaffolds for *in vitro* testing ultimately contributing to bone tissue engineering.

MATERIALS AND METHODS

Preparation of electrospun fibers

Scaffold fabrication

Hydroxyapatite powder (Merck) and tricalcium phosphate powder (Fluka) were used for the production of the electrospun biphasic fibers. A range of solvents, biphasic compositions, as well as different stirring techniques were investigated. The most successful conditions were 20:80 wt% HA:TCP ratio in a 30% w/v solution. A 50:50 acetone:acetic acid solution was added to the ceramic powders while stirring vigorously for 1 hour. After 1 hour, gelatine was added drop- wise to the mixture to reach 4% of total volume. This mixture was stirred for a further 30 minutes.

The scaffolds were fabricated using the electrospinning process. The principle of this process is that an electrical voltage sufficient to overcome the surface tension of the solution will cause the droplets to elongate and eject very fine fibers. These fine fibers form non-woven mats when deposited. The electrospinning setup (**Figure 1**) consists of a 10ml syringe containing the solution, which is attached to a needle, and placed 15cm away from the grounded collector (aluminium plate) and a high voltage power supply (15kV). Old compact disks covered with aluminium foil were placed on the plate. The round glass cover slips (22mm diameter) were spaced on the covered compact disk. This technique still needs to be completely optimised to assure a greater fiber ratio to beads.

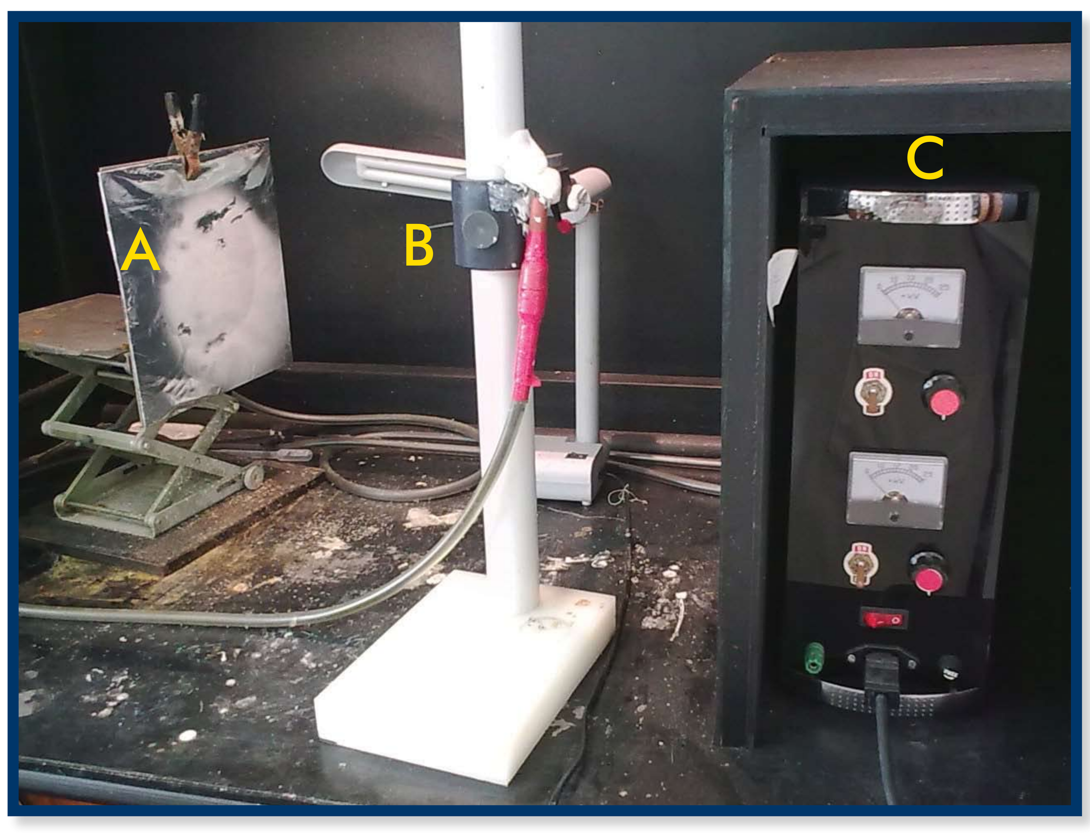

Figure 1: The electrospinning set-up with the collector plate (A), location of syringe (B) and high voltage unit (C).

Morphology analysis

An environmental scanning electron microscope (ESEM) was used to study the morphology of the electrospun mats. Electrospun samples were also characterised by X-ray diffraction (XRD) and attentuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR).

RESULTS AND DISCUSSION

Figure 2 shows the beads of the electrospun mats. Fibers are located in between the beads. During optimisation of the electrospinning process, it might be possible to increase the fibers and lower the occurrence of beads (**Figure 2**).

Figure 3 shows the XRD pattern for pure hydroxyapatite (blue graph), pure tricalcium phosphate (pink graph) and the electrospun samples (orange graph). After XRD analysis of the electrospun samples, only a small TCP peak was visible and no HA peak. ATR-FTIR revealed that HA is not detected in the sample at lower HA:TCP ratios. HA was however detectable in samples with 90% HA and 10% TCP (results not shown).

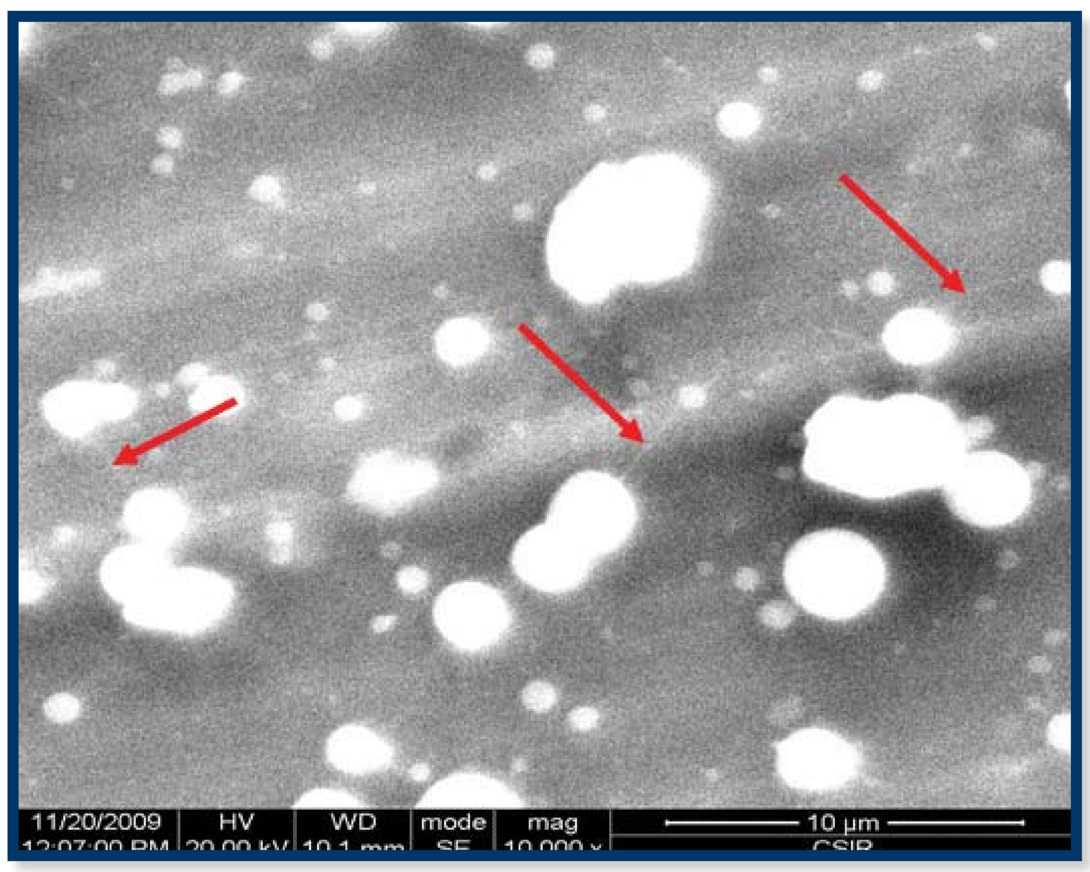

Figure 2: ESEM analysis of the electrospun mats at 10 000x magnification. The fibres
(indicated by red arrows) are visible between the beads.

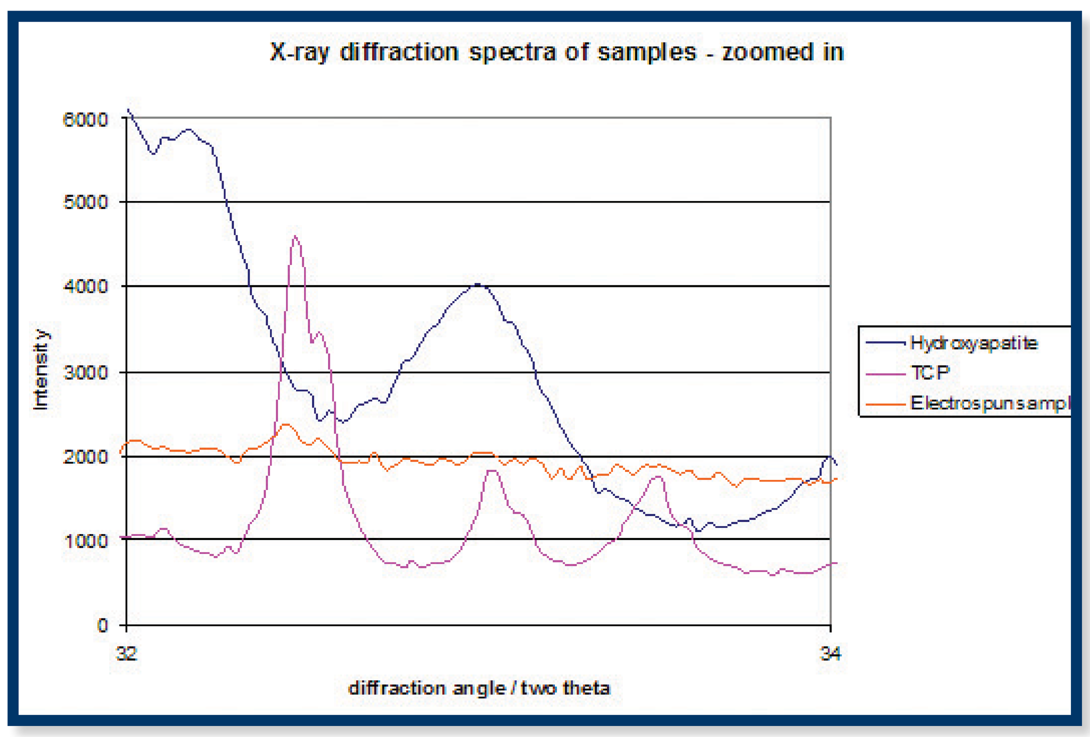

Figure 3: XRD diffraction pattern of HA, TCP and electrospun samples with 2θ from 32-34.

CURRENT AND FUTURE WORK

- The electrospinning method will need to be optimised to ensure higher production of electrospun biphasic nanofibres. The spinning conditions need to be optimised i.e. the distance between the collector plate and syringe could be increased and the electrospinning could be done at lower voltages. Many parameters can be adjusted during the solution preparation and/or electrospinning phase.
- Future work will also include an investigation into the exact composition of the samples and why it seems that HA is not part of the final product as the XRD analysis and FTIR analysis show.
- The electrospun biphasic samples as well as HA disks are currently being tested in *in vitro* cell culture studies with human monocytic cell line THP-1. These cells differentiate into osteoclast-like cells when 1,25-dihydroxy-vitamin D_3 is added to the growth media.
- Future experimental work will include cell toxicity, cell attachment and recruitment to the scaffold, as well as the scaffold degradation assays.

Improving bone graft substitutes through the combination of materials science and cell biology.

- Unravelling signalling pathways and the relationship between osteoclasts and osteoblasts when responding to an implanted biomimetic bone scaffold, will provide improved understanding of bioactive ceramics' surface features for biomineralisation.

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