Novel nano-calcium phosphate generation to improve cell activity in bone restructuring

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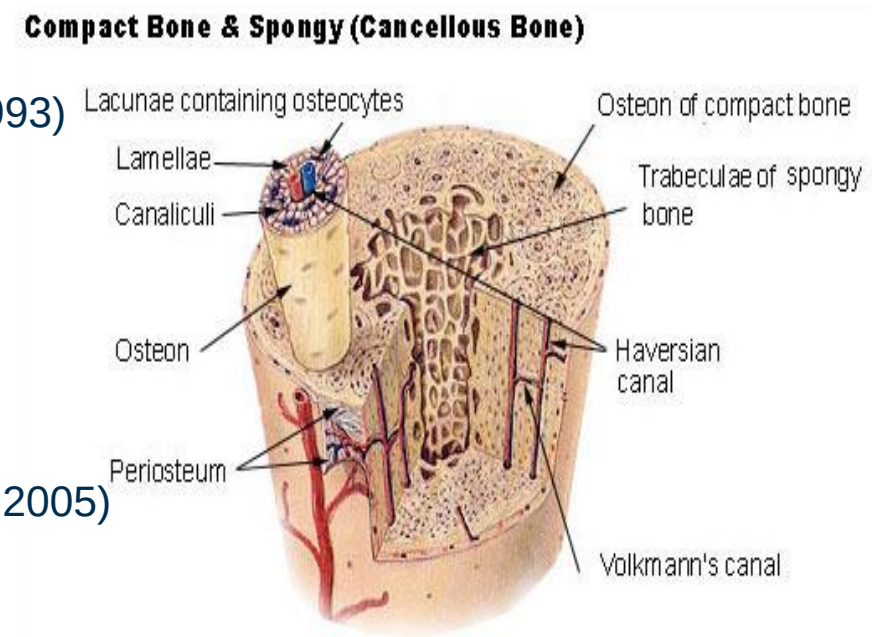
Introduction

- Replacement and repair of bone - around for centuries (Chevalier, 2009)
- Four types of biomaterials (Hench, 1993)
  - Biotoxic
  - Bioinert
  - Bioactive
  - Bioresorbable
- Bone is dynamic living tissue (Shea, 2005)
  - Nano-, micro- and macro structure



- Most abundant inorganic mineral in bone – hydroxyapatite[1]
- Good candidate for synthetic bone[1]
- Osteoblasts – depositing new bone[1]
- Osteoclasts – bone resorption[1]

[1](Ravaglioli, 1992; Best, 2008; Martin,1998)

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Problem statements

- Bioceramics are not resorbed by osteoclasts as intended
- Osteoblasts are not recruited to deposit new bone
- Most bioceramics still macro-structured while natural occurring crystals are nano-structured
- Bioceramics' surface feature for biomineralisation – unclear
- Fundamentals must be explored to facilitate breakthroughs for clinical biointeractive materials with novel physical, chemical & biological functions

Aims and objectives

- To generate novel electrospun calcium phosphate scaffolds
  - To determine the optimum ratio between hydroxyapatite and β-tricalcium phosphate for scaffold generation
  - To develop a working method for scaffold generation *via* electrospinning

- To determine in *in vitro* the response of human osteoblasts and osteoclast-like cells towards the electrospun scaffolds

Research design

Electrospinning (method development)

- Electrospinning of HA & TCP into nano-fiber scaffolds
- New structures as well as coating existing CSIR samples
- Characterization of electrospun ceramics with SEM, XRD, ATR-FTIR

In vitro analysis

- Two cell lines (human osteoblast and osteoclast-like cells)
- Cell viability, cell toxicity, cell morphology, cell attachment to scaffolds
- Signaling pathways analysis (gene ontology, protein profiles)
- Relationship between osteoclasts and osteoblasts
- Resorption of bioceramic

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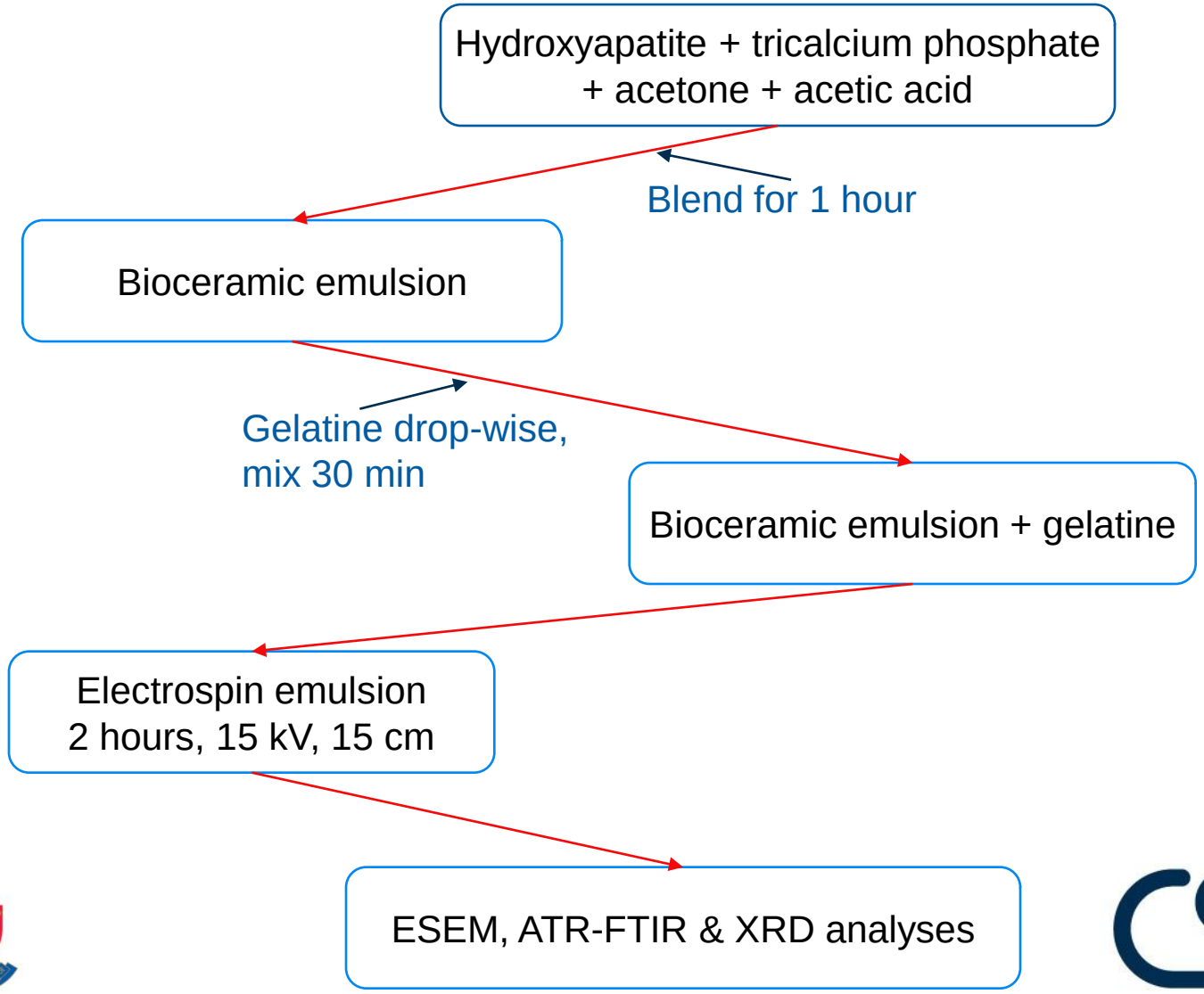
Materials & Methods



Results (electrospinning)

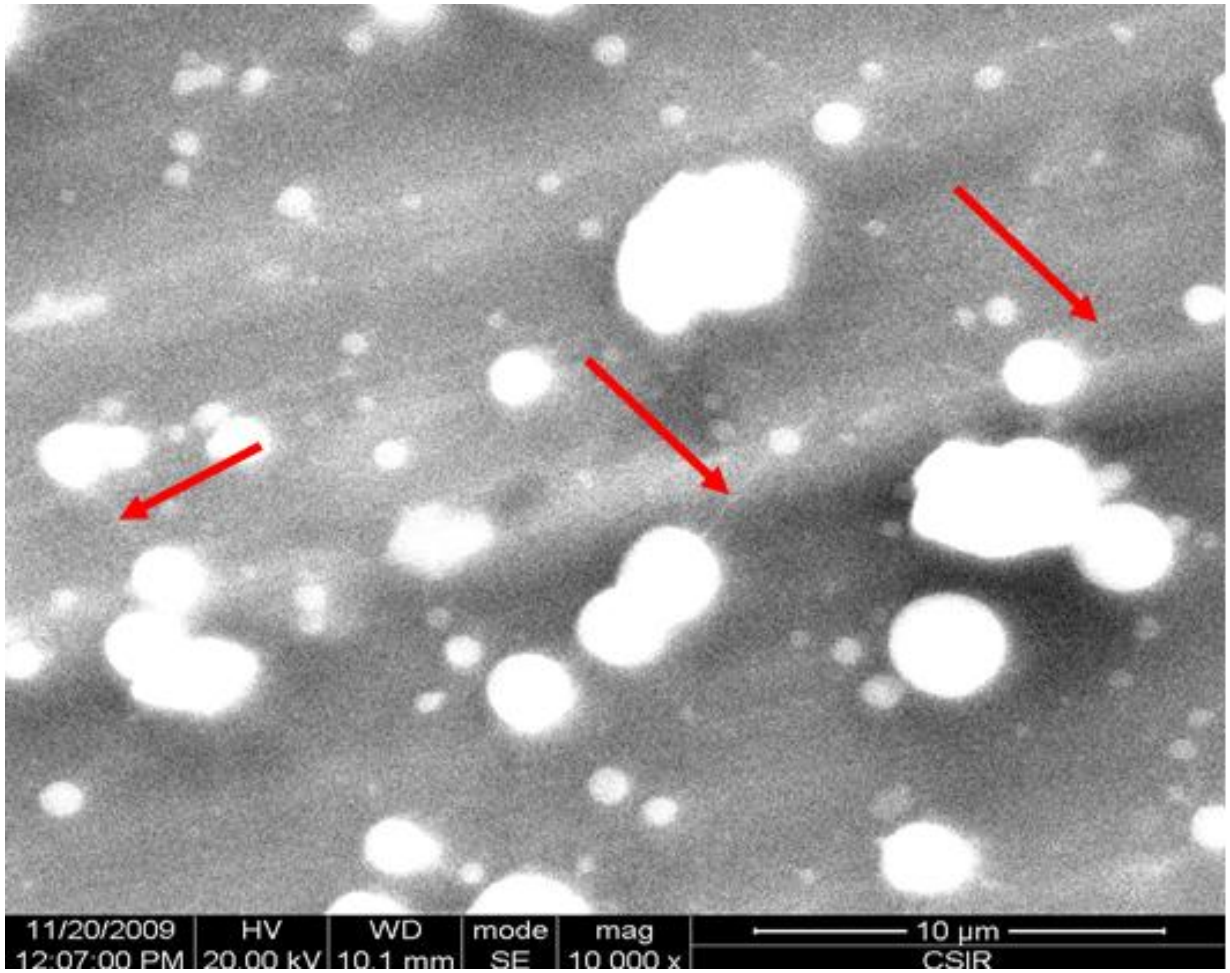


Figure 1: ESEM analysis of the electrospun mats. 10 000 x magnification. The fibers (red arrows) are visible between the beads.

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Results (XRD)

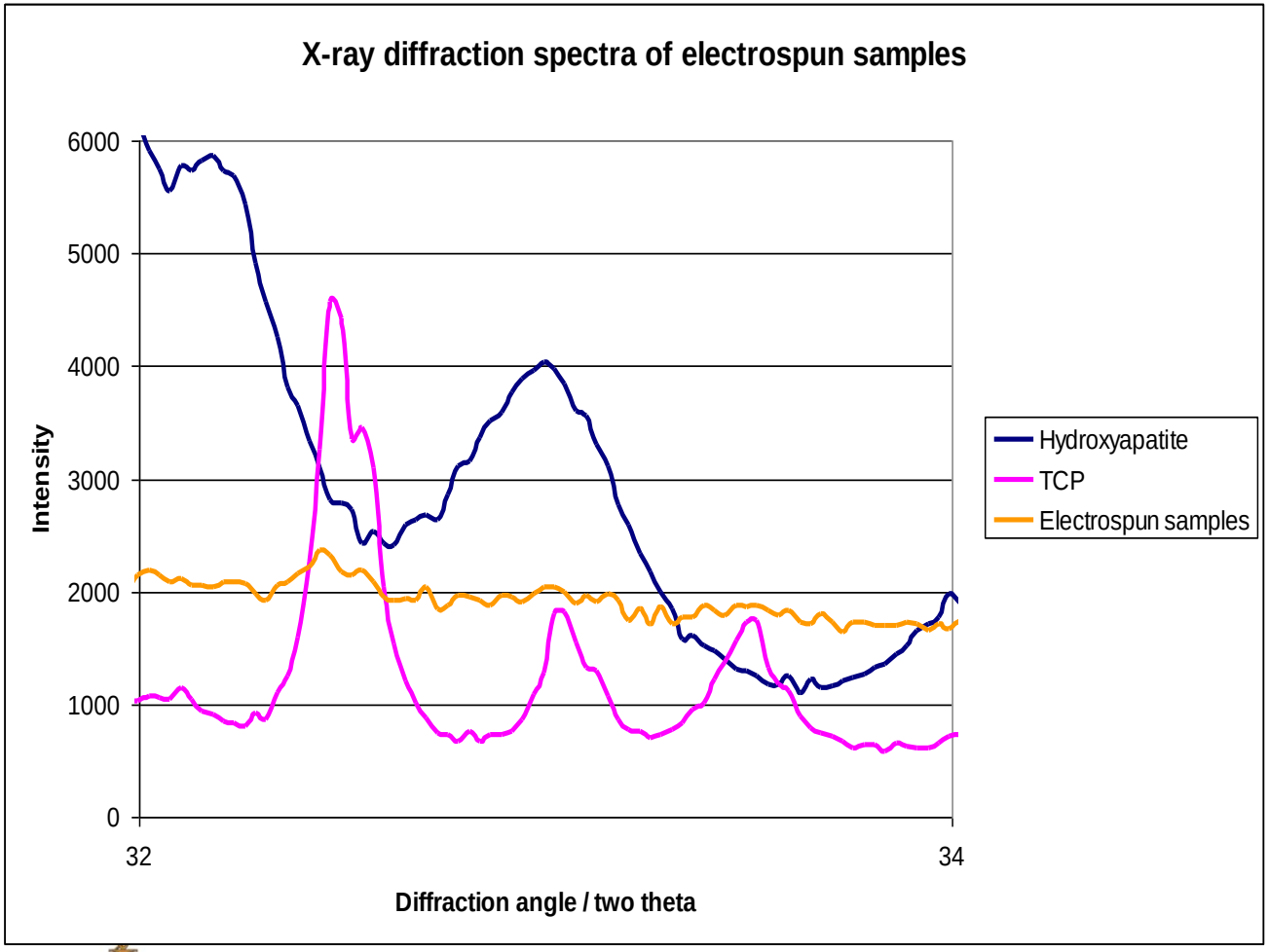

Figure 2: XRD pattern of hydroxyapatite, tricalcium phosphate and the electrospun samples.

Hydroxyapatite disks

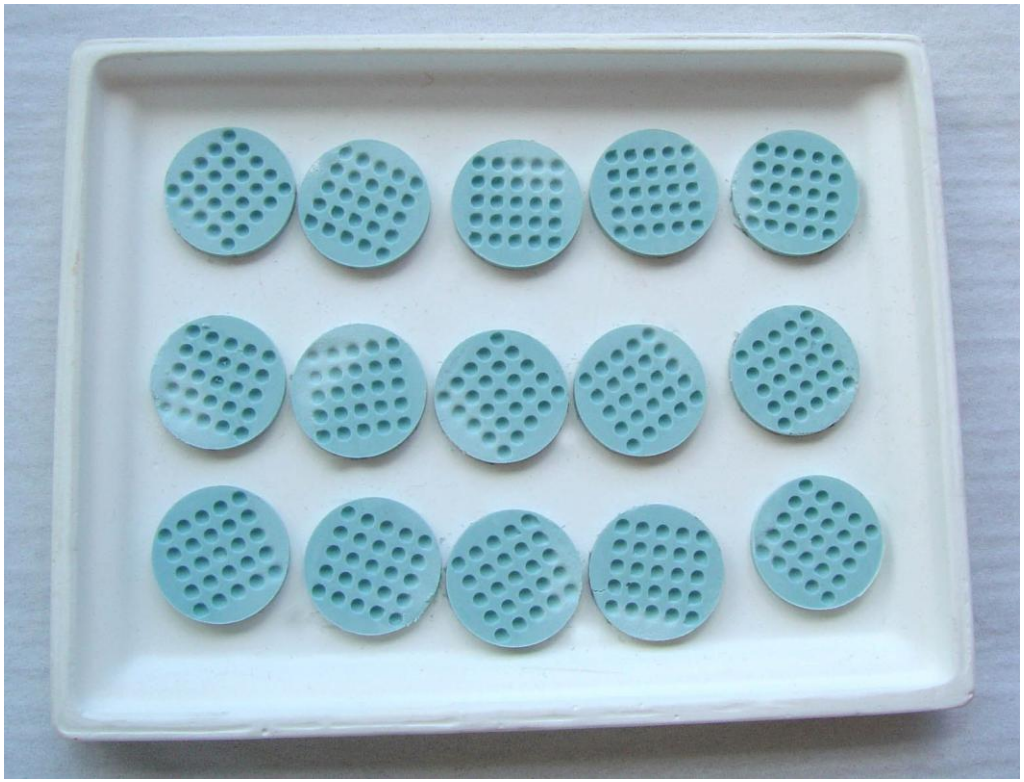

Figure 3: Dimpled HA disks before sintering.

Figure 4: HA disk after sintering.

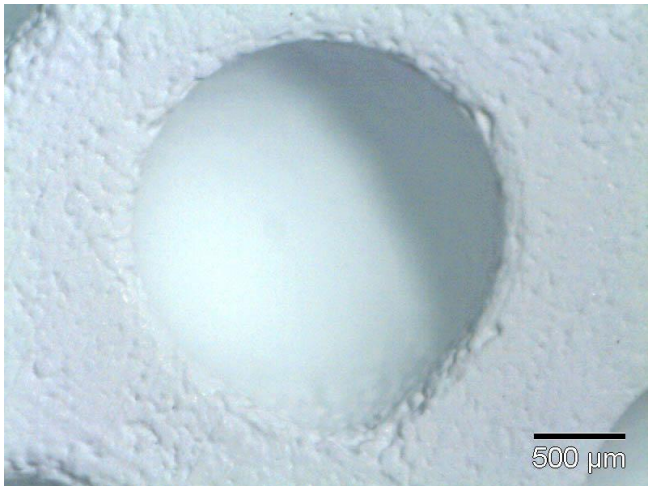

Figure 5: Higher magnification of one dimple on the HA disk.

Fluorescein diacetate stain of osteoclast-like cells

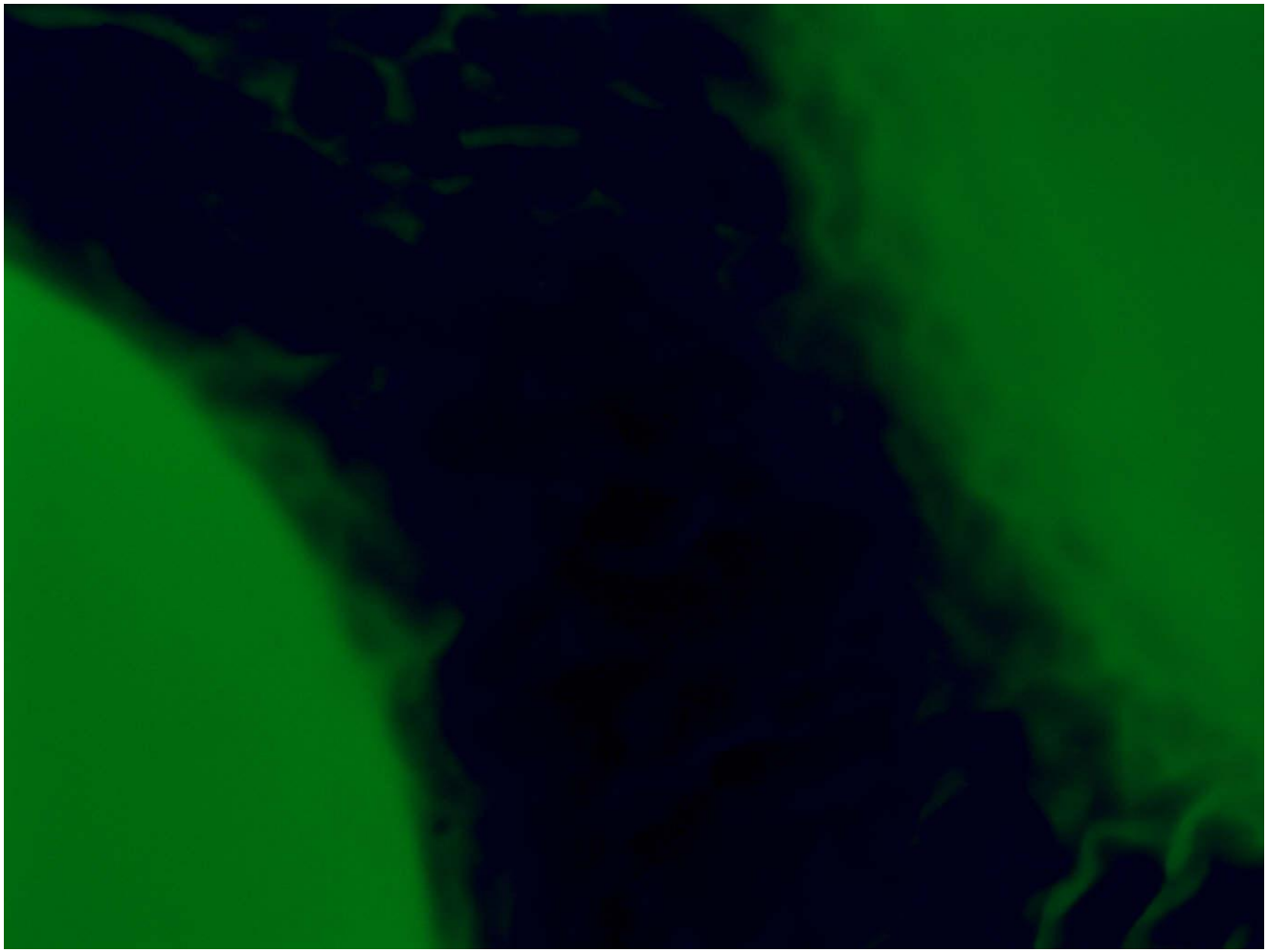

Figure 6: Two “dimples” on the HA disk are seen on either side with increased density in viable cells.

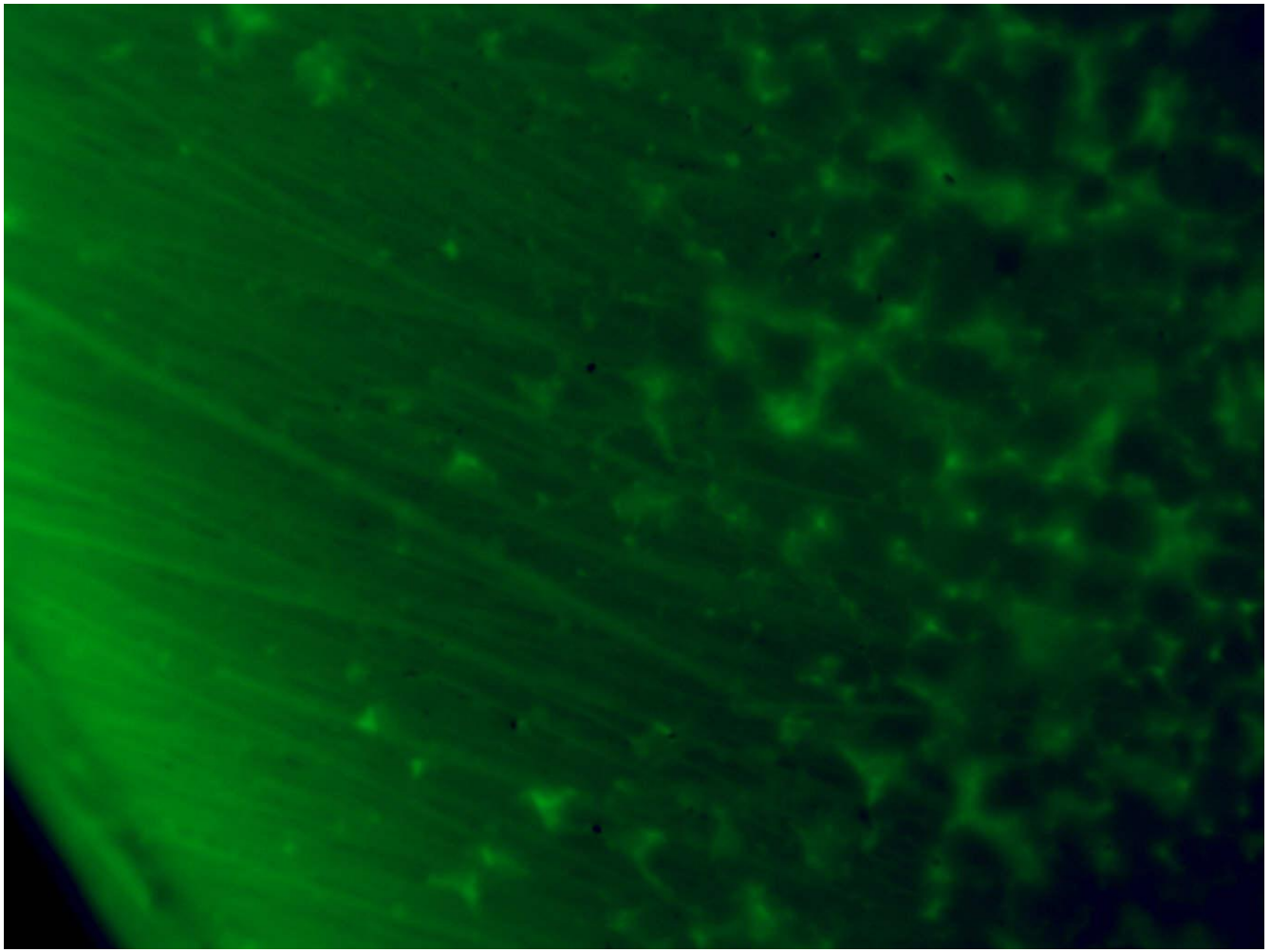

Figure 7: The edge of the HA disk

Conclusion

- Fibers were formed during electrospinning
- XRD analysis showed no other compounds present
- ATR-FTIR showed HA is not detected at lower HA:TCP ratios (results not shown)
  - HA was detected in samples with 90% HA and 10% TCP
- Electrospinning protocol needs optimisation
- Higher density of live osteoclast-like cells in dimples of HA disk

Future work

- Optimisation of electrospun method
- Exact composition of manufactured samples
- Electrospun samples and HA disks are being tested *in vitro*
- Experimental work includes:
  - Cell toxicity
  - Cell attachment
  - Cell cycle activation

- Unravelling signaling 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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