An *in vitro* study of bone cells grown on an electrospun scaffold for bone repair and reconstruction

Emerging Researcher Symposium

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October 2012

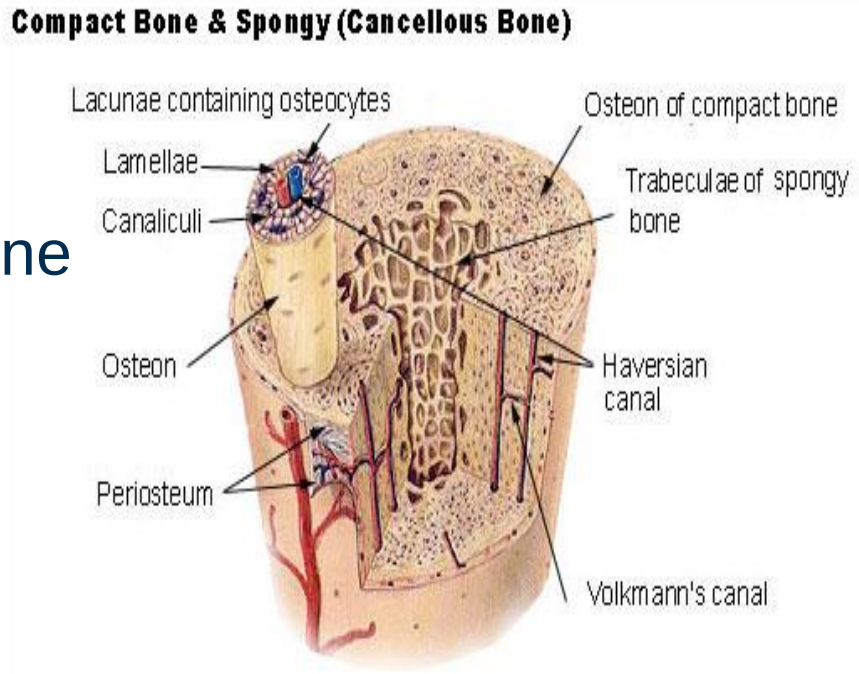
our future through science

Outline of talk

- Introduction
- Problem statements
- Aim and objectives
- Materials & methods
- Results
- Conclusion
- Acknowledgements

Introduction

- Bone is dynamic living tissue
  - Nano-, micro- and macro structure
- Four types of biomaterials
- Hydroxyapatite (HA)
- Osteoblasts – depositing new bone
- Osteoclasts – bone resorption



Chevalier et al. *(2009), J Eur Ceram Soc, 29 (7), 1245-1255*

Hench et al. *(2010), J R Soc Interface, 7, S379-S391*

Shea et al. *(2005), Adv Drug Deliv Rev, 57 (7), 945-957*

Image courtesy of: http://en.wikipedia.org/wiki/File:Illu_compact_spongy_bone.jpg

Problem statements

- Cell recruitment
- Scaffold structure
- Cell communication

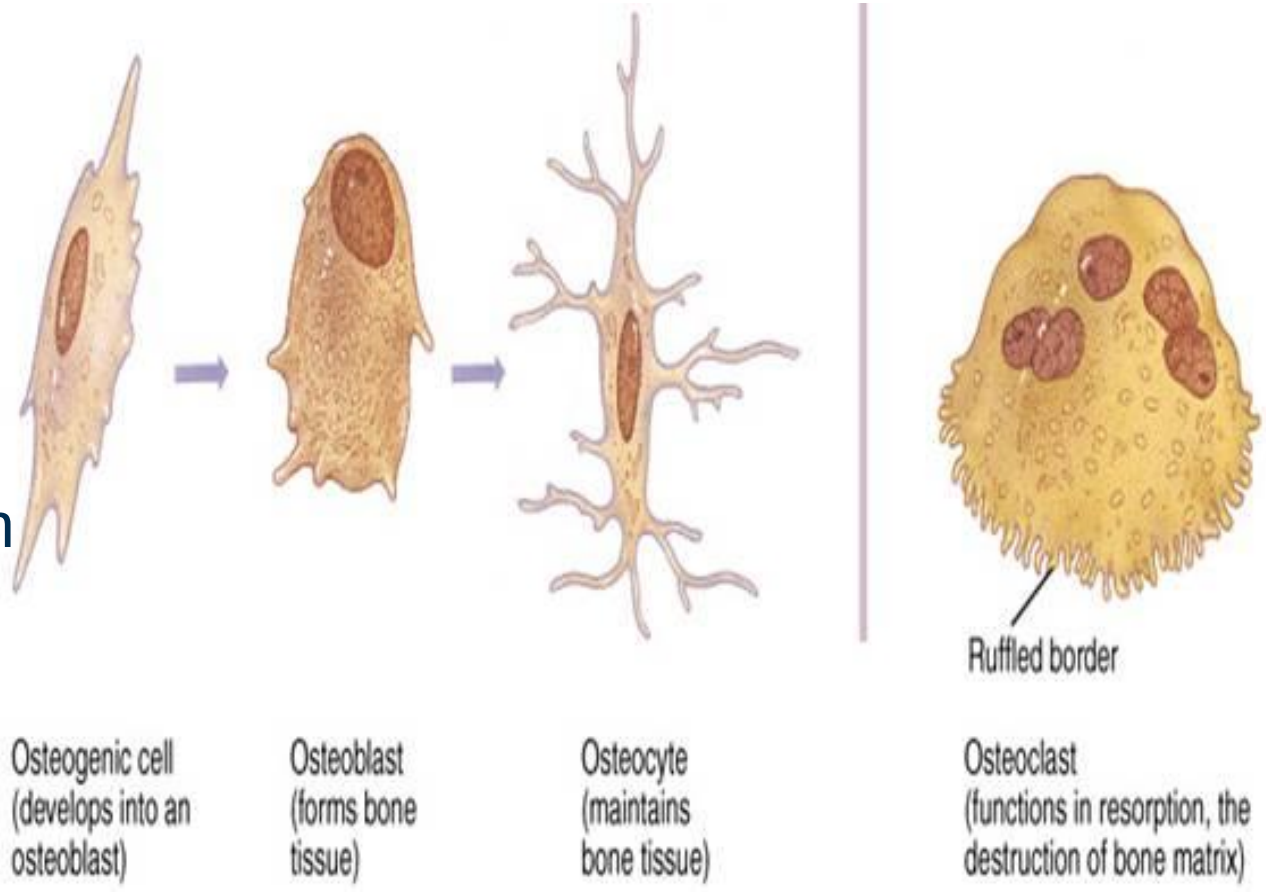


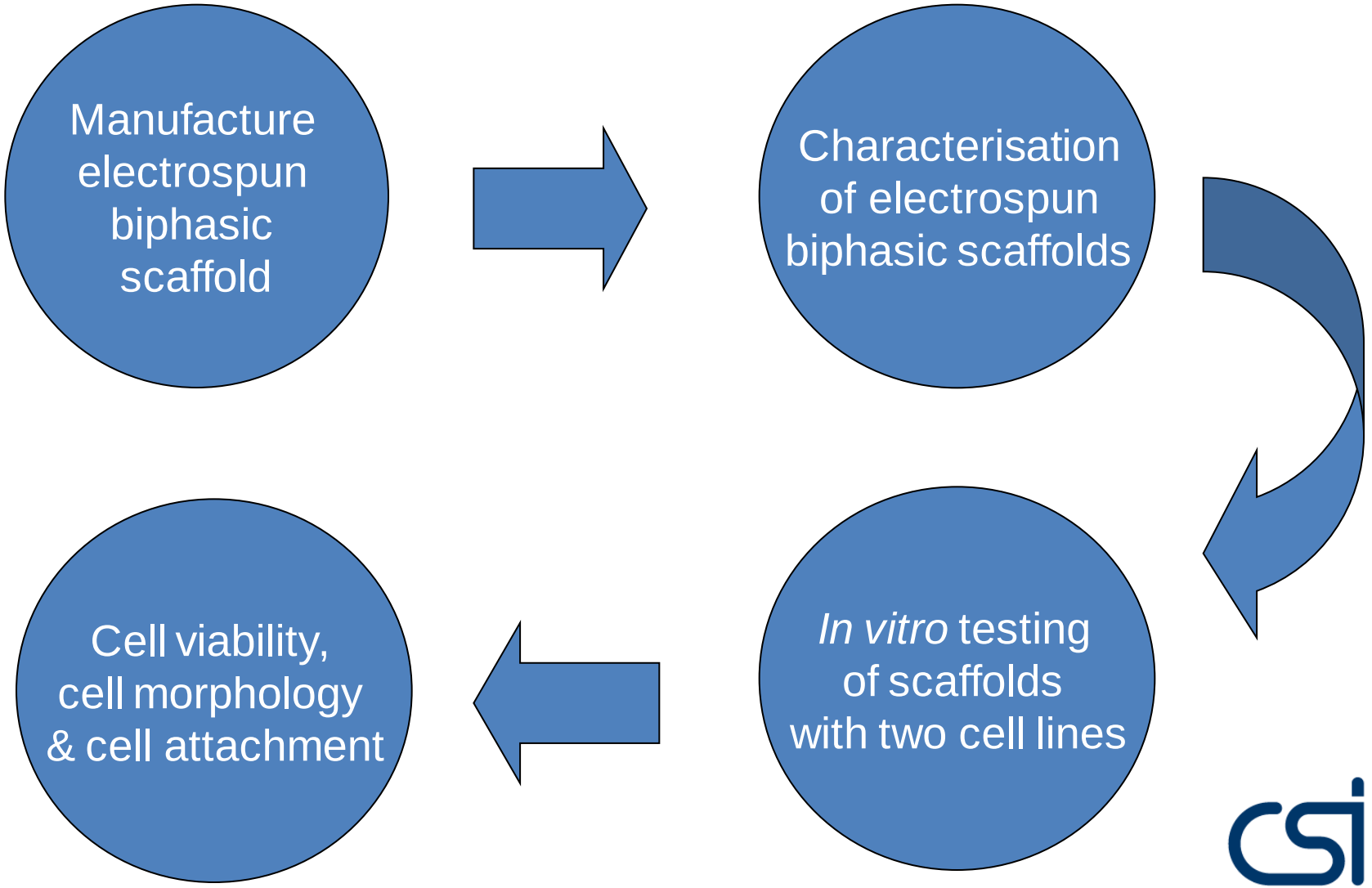
Image courtesy of:
http://spaces.imperial.edu/thomas.morrell/cha_6_tortora_bone_tissue.htm

Aims and objectives

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

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

Materials & methods



Results (SEM of scaffolds)

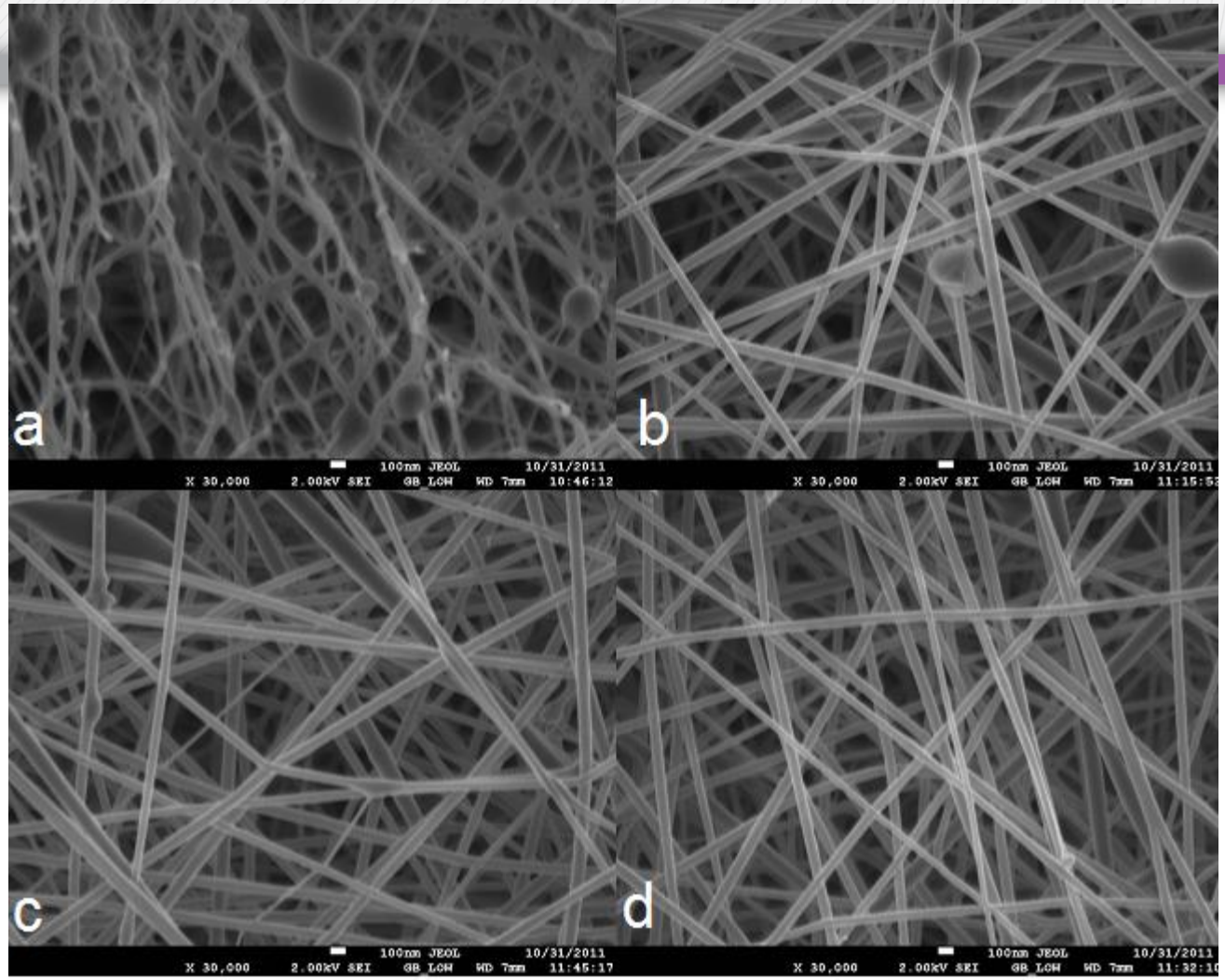


Fig. 1: Characterisation of the electrospun scaffolds by means of SEM. Images taken at 30 000x magnification of the electrospun scaffolds spun from 30% (w/v) HA:TCP (40:60) with varying gelatin concentrations. (a) 3% (b) 5% (c) 7% (d) 10% (which was used in this study).

Wepener et al. *(2012), J Mater Sci: Mater Med, DOI 10.1007/s10856-012-4751-y*

Results (Cytotoxicity assay)

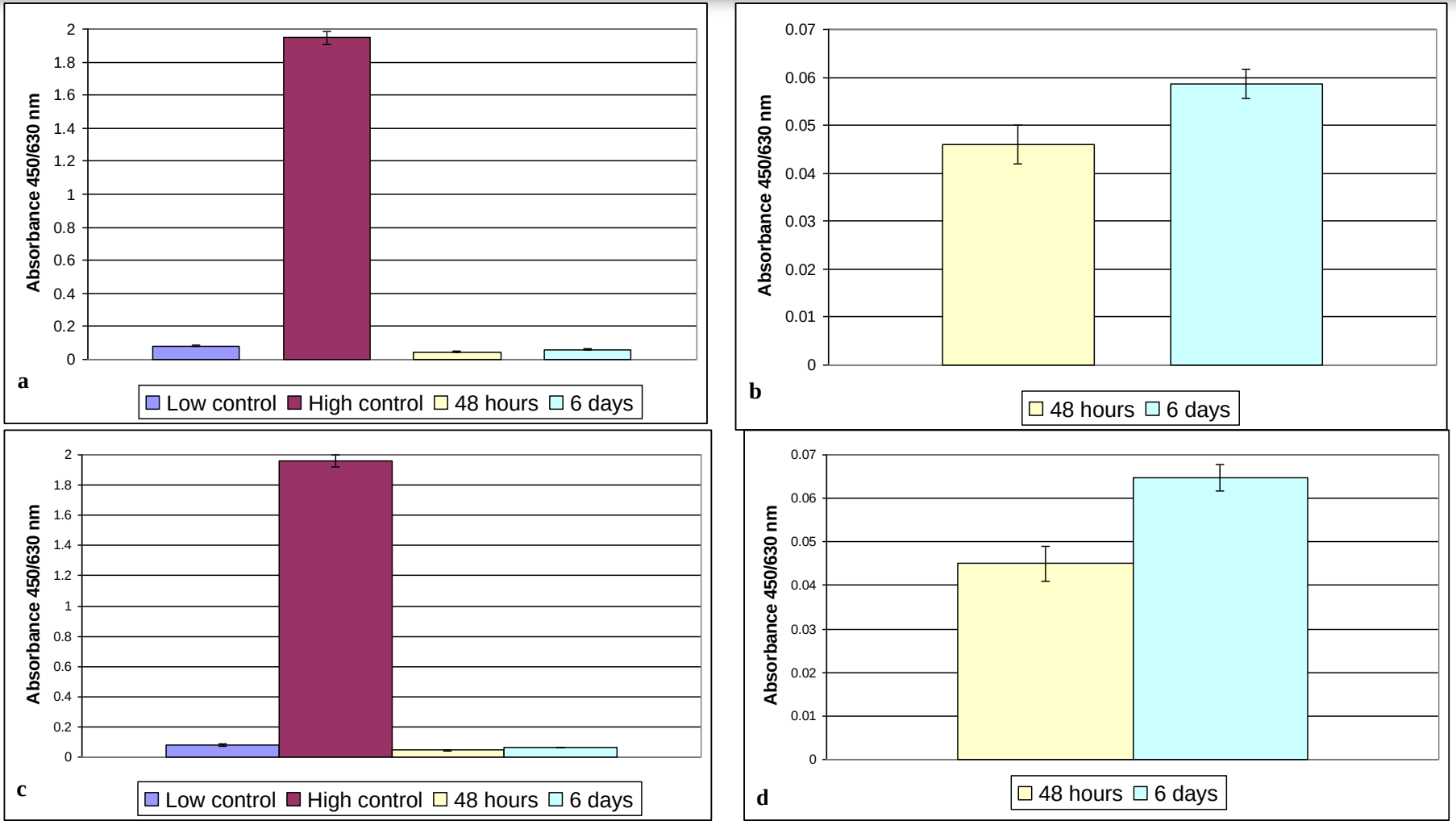


Fig. 2: Analysis of LDH release in osteoclast-like cells (a & b) and osteoblast cells (c & d) grown on electrospun scaffolds after 48 hours and 6 days.

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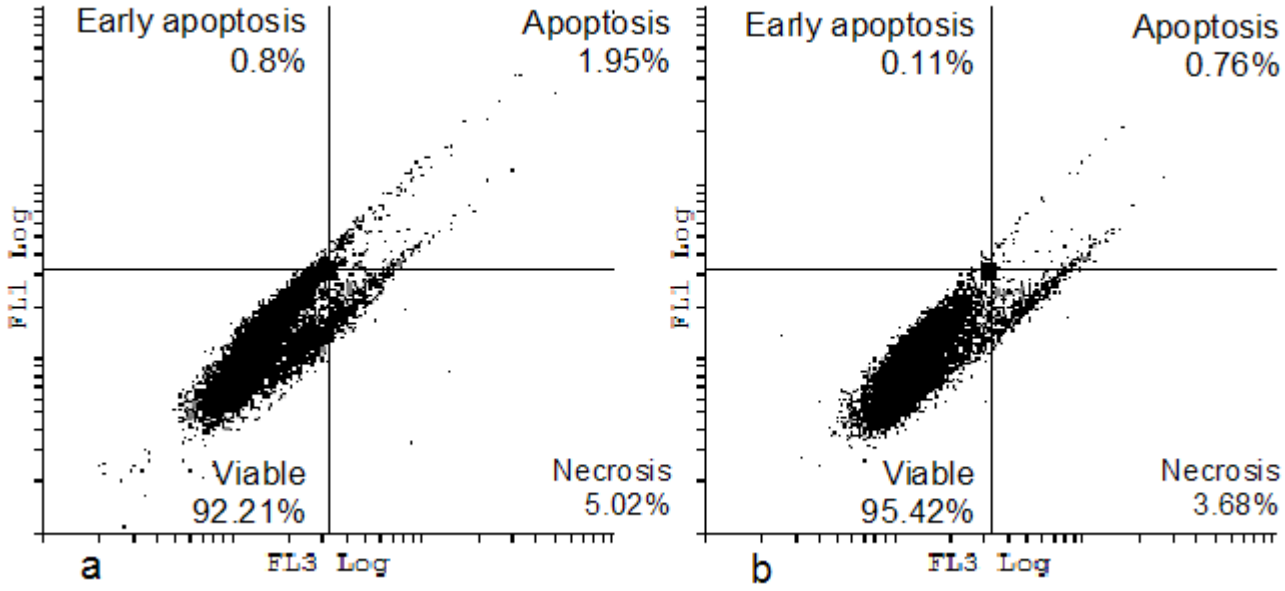
Results (Mitochondrial membrane potential)

Osteoclast-like cells



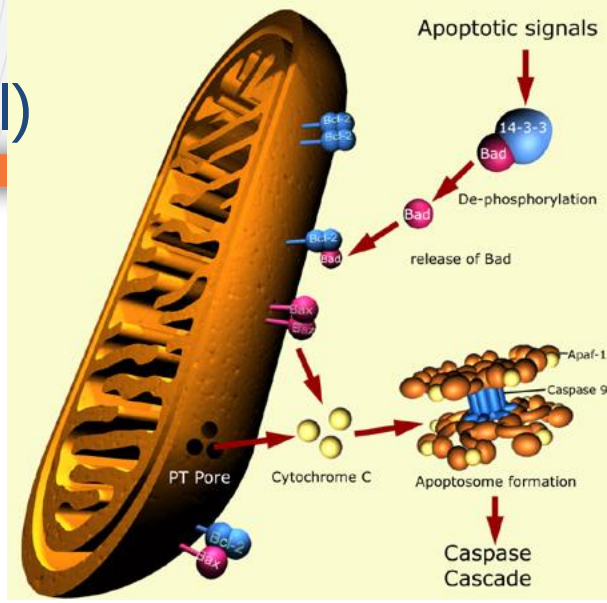


Fig. 3: Mitochondrial membrane potential measurement of osteoclast-like cells grown on (a) tissue culture plates (control) and (b) on electrospun scaffolds. Cells grown on electrospun scaffolds (b) exhibited a higher percentage of viable cells when compared to the control (a). Each figure is representative of three repetitive experiments where at least 11 000 cells were counted.

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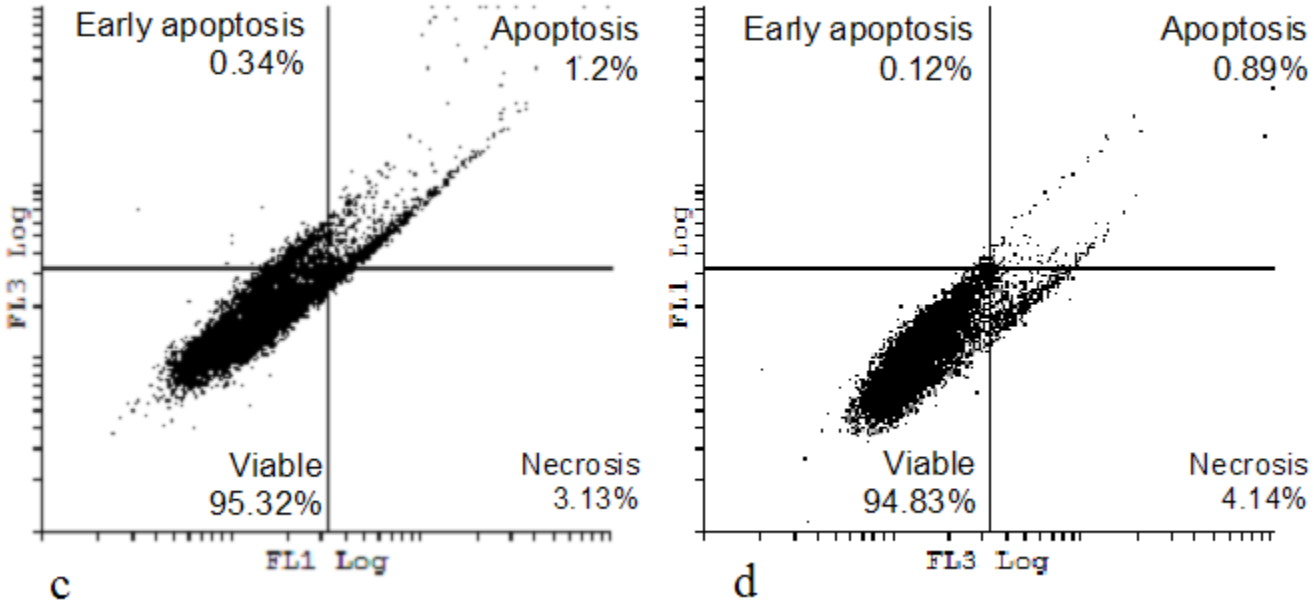
Results (Mitochondrial membrane potential)

Osteoblast cells



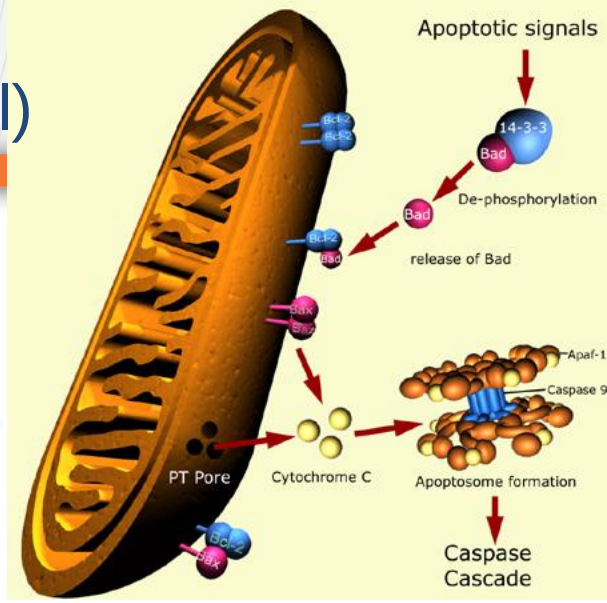
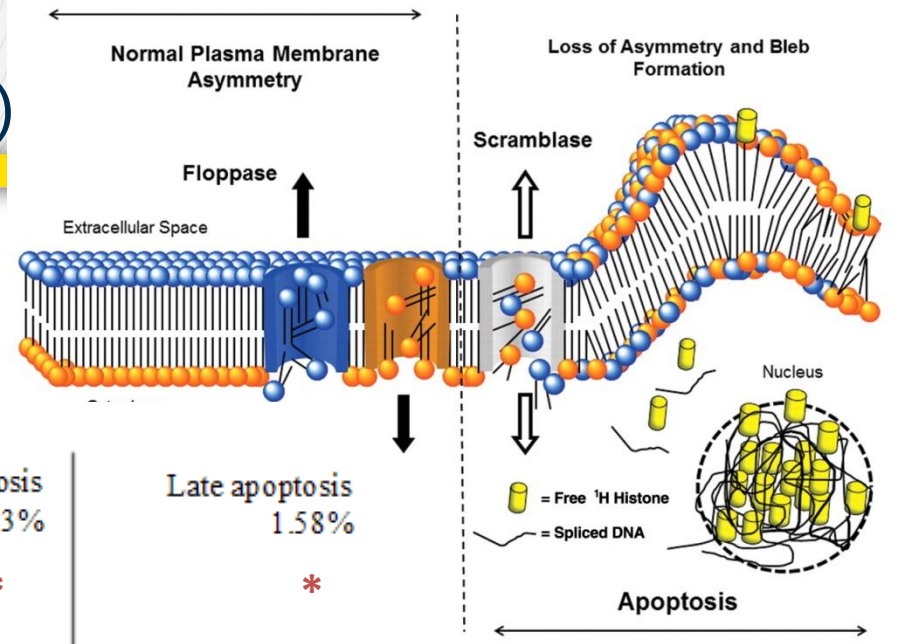


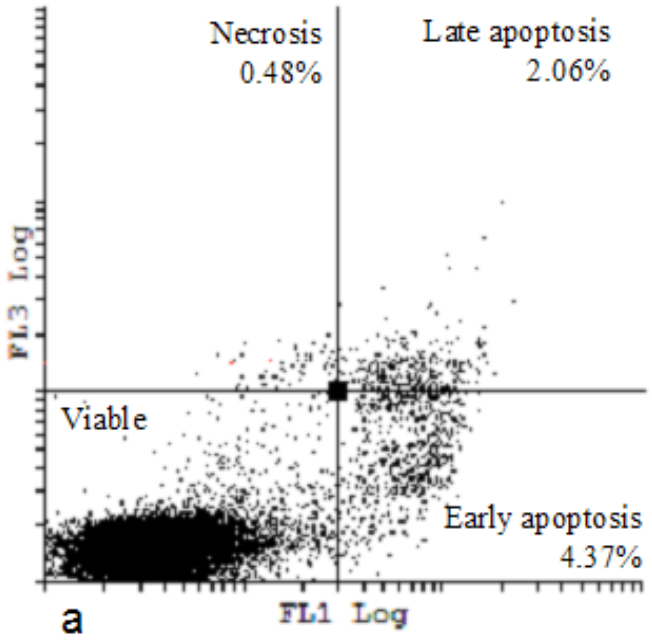
Fig. 4: Mitochondrial membrane measurement of osteoblast cells grown on (a) tissue culture plates (control) and (b) on electrospun scaffolds. Cells grown on electrospun scaffolds did not exhibit a significant decrease in viable cells and compared well to the control (a).

Wepener et al. *(2012), J Mater Sci: Mater Med, DOI 10.1007/s10856-012-4751-y*

Results (Phosphatidylserine flip)

Osteoclast-like cells





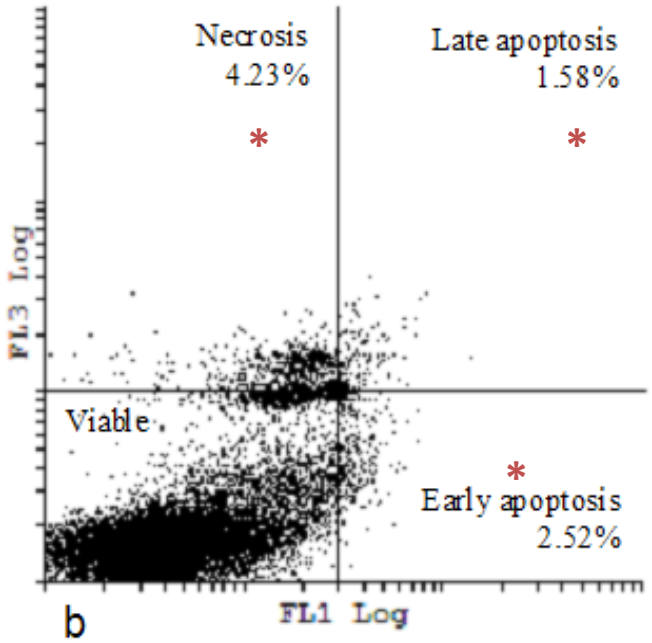


Fig. 5: Apoptosis detection through flow cytometry and annexin V-FITC of osteoclast-like cells (a) control (cells grown on cell culture plate only) and (b) cells grown on electrospun scaffolds.

Wepener et al. *(2012), J Mater Sci: Mater Med, DOI 10.1007/s10856-012-4751-y*

Results (Cell cycle progression)

Osteoclast-like cells

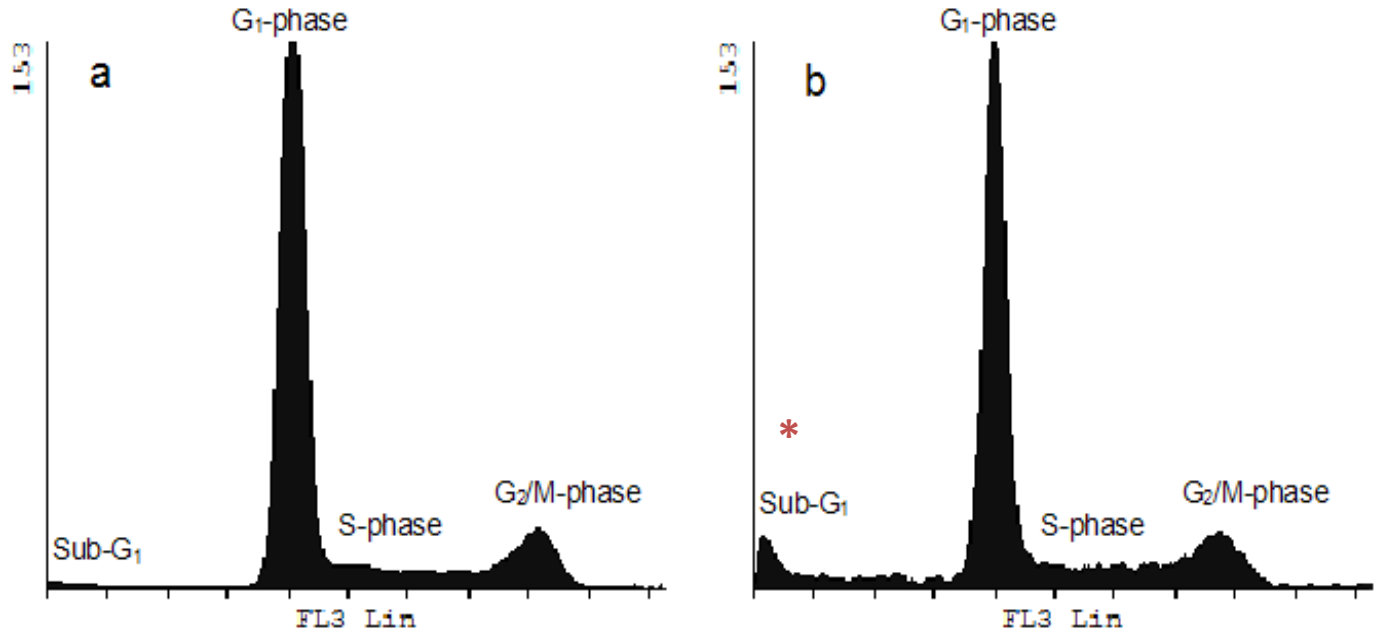


Fig. 6: Histogram representation of osteoclast-like cells grown for 6 days on (a) tissue culture plates (control) and (b) electrospun scaffold. Normal cell phase distribution is seen.

Wepener et al. *(2012), J Mater Sci: Mater Med, DOI 10.1007/s10856-012-4751-y*

Results (Cell cycle progression)

Osteoblast cells

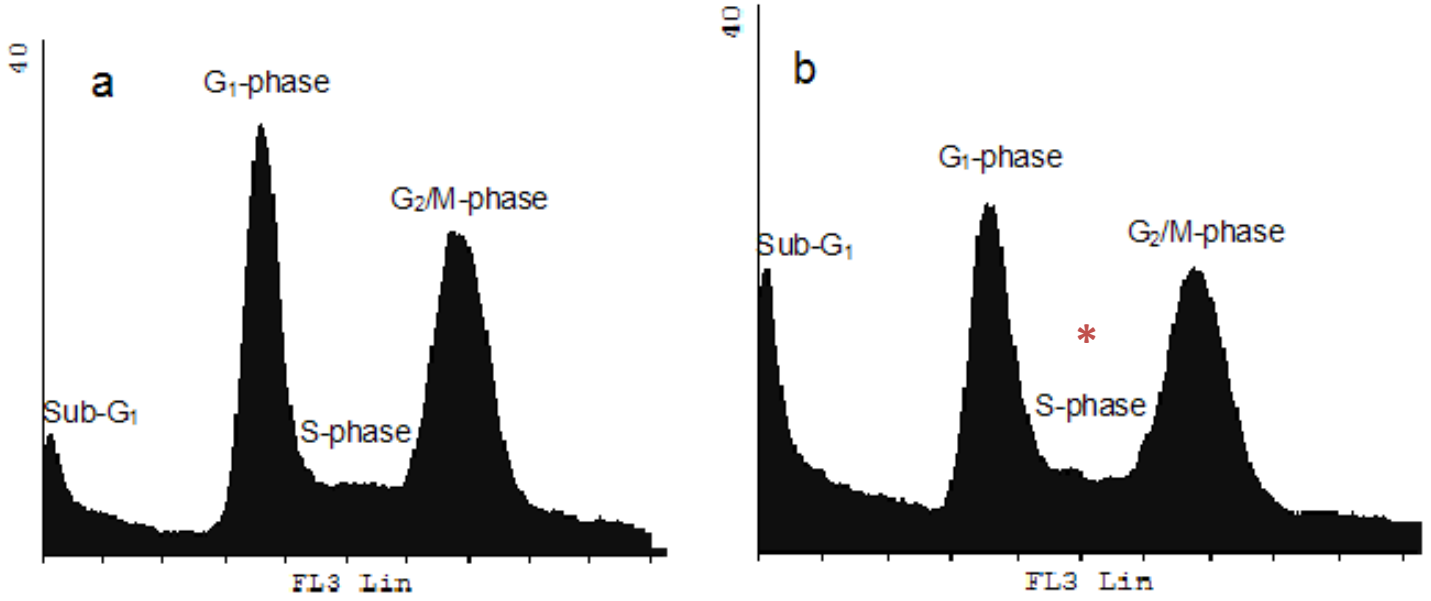


Fig. 7: Histogram representation of osteoblast cells grown for 6 days on (a) tissue culture plates (control) and (b) elestrospun scaffold. Normal distribution of the cell phases is detected.

Wepener et al. *(2012), J Mater Sci: Mater Med, DOI 10.1007/s10856-012-4751-y*

Results (Cytoskeletal & nuclear staining)

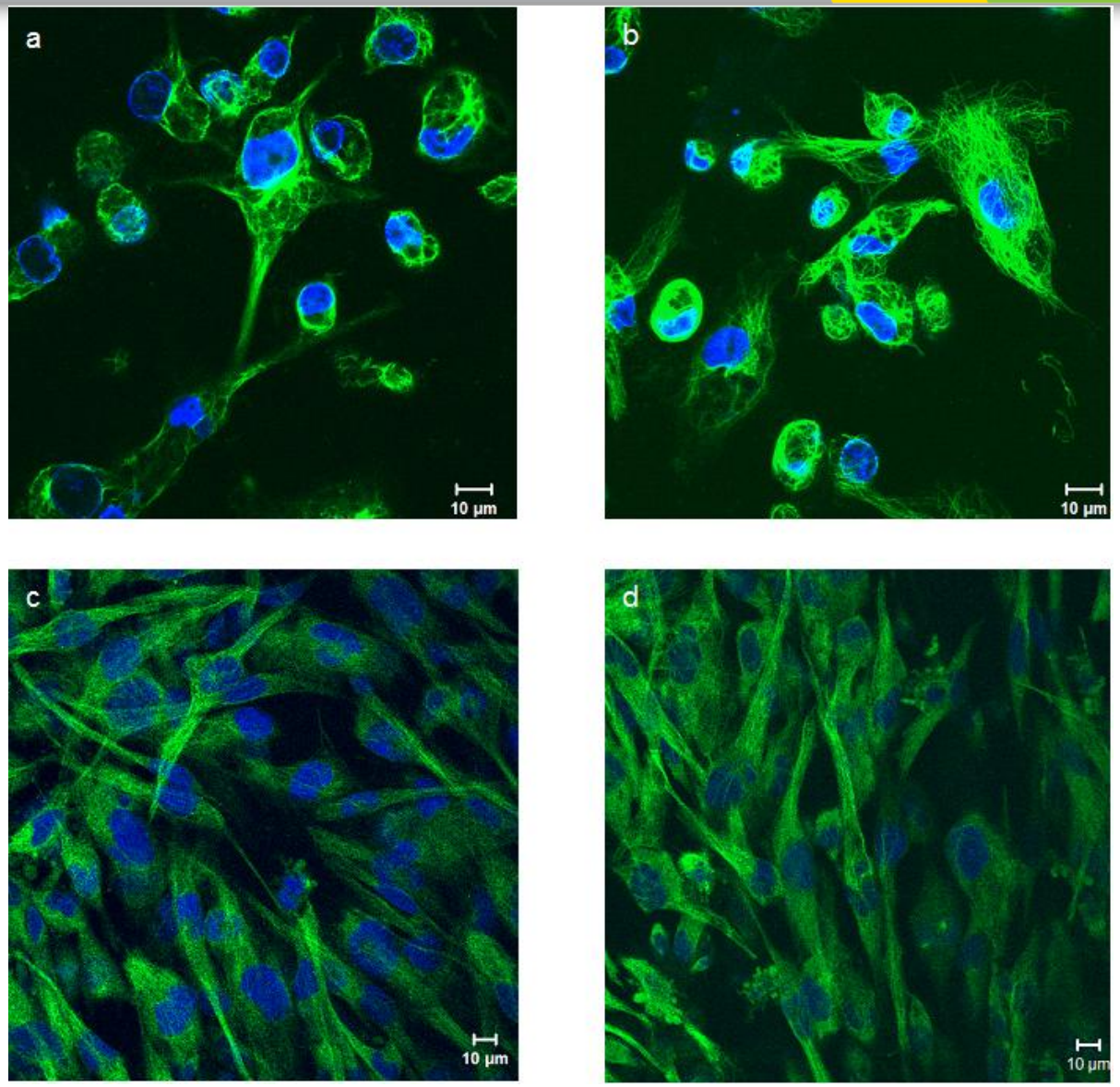


Fig. 8: Nucleus and cytoskeleton dynamics: Confocal microscopy images, staining tubulin structures of osteoclast-like cells (a & b) and osteoblast cells (c & d) grown on cell culture plate (control) (a & c) and on electrospun scaffolds (b & d).

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Conclusion

- Electrospinning successful to create biphasic scaffold
- Uniform fibers, interconnected pores – cell attachment and growth
- No cytotoxicity in both cell lines
- Cells responded well to electrospun scaffolds
- No important significant changes between cells grown on scaffolds compared to the controls
- No differences in cell morphology
- Electrospun biphasic scaffolds are biocompatible, appropriate for growth and adherence of bone cells
- Possible candidate scaffold for bone tissue engineering

Acknowledgements

- Prof A Joubert (supervisor)
- Dr W Richter (co-supervisor)
- Dr Y Lemmer
- Polymers & Composites team at CSIR
- Dr V Jacobs
- Physiology and Pharmacology departments at UP
- Biosciences at CSIR
- CSIR for funding

Thank you

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