For Pony
Acknowledgements

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# Tables of Contents

Dedication.................................................................................................................. ii

Acknowledgements................................................................................................... iii

List of Figures........................................................................................................... viii

List of Abbreviations................................................................................................. xvii

Abstract..................................................................................................................... xix

## Chapter 1  Introduction ......................................................................................... 1

1.1  Problem Statement............................................................................................ 1

1.2  Hypothesis......................................................................................................... 3

1.3  Specific Aims...................................................................................................... 3

1.4  Significance........................................................................................................ 4

1.5  Dissertation Overview...................................................................................... 5

1.6  References......................................................................................................... 7

## Chapter 2  Literature Review ........................................................................... 10

2.1  Introduction....................................................................................................... 10

2.2  Cell Sources for Tissue Engineering............................................................... 12

2.3  Nanofibrous Scaffold Fabrication................................................................... 13

    2.3.1  Electrospinning......................................................................................... 13

    2.3.2  Molecular Self-Assembly......................................................................... 14

    2.3.3  Thermally Induced Phase Separation..................................................... 15

2.4  Surface Modification of Nanofibrous Scaffolds........................................... 16
2.5 Effect of Nanofibrous Scaffold on Cellular Behaviour and Tissue Development

2.5.1 Attachment and Proliferation

2.5.2 Differentiation and Tissue Formation

2.6 Conclusions

2.7 References

Chapter 3 Osteogenic Differentiation of Mouse Embryonic Stem Cells on Nanofibers

3.1 Introduction

3.2 Materials and Methods

3.2.1 Materials

3.2.2 Thin Matrix Preparation for Cell Culture

3.2.3 Mouse Embryonic Stem Cell Culture and Seeding

3.2.4 Scanning Electron Microscopy

3.2.5 Immunofluorescence and Alizarin Red S Staining

3.2.6 Western Blotting Analysis

3.2.7 PCR and Real Time PCR

3.2.8 Statistical Analysis

3.3 Results

3.4 Discussion

3.5 Conclusions

3.6 References

Chapter 4 Osteogenic Differentiation of Mouse Embryonic Stem Cells on Nanofibrous Scaffolds

4.1 Introduction

4.2 Materials and Methods
4.2.1 2-D Thin Matrix and Film Preparation for Cell Culture 67
4.2.2 3-D Scaffold Preparation for Cell Culture 68
4.2.3 D3 Culture and Seeding 69
4.2.4 PCR and Real Time PCR 70
4.2.5 Immunofluorescence and Histological Staining 71
4.2.6 Western Blotting Analysis 72
4.2.7 Mineral Quantification 73
4.2.8 Collagen Quantification 73
4.2.9 Statistical Analysis 74
4.3 Results 74
4.4 Discussion 77
4.5 Conclusions 80
4.6 References 90

Chapter 5 Osteogenic Differentiation of Human Embryonic Stem Cells on Nanofibrous Scaffolds 94
5.1 Introduction 94
5.2 Methods and Materials 95
5.2.1 2D Thin Matrix and Film Preparation for Cell Culture 96
5.2.2 Fabrication of NF-PLLA and SW-PLLA Scaffolds 97
5.2.3 Human Embryonic Stem Cell Culture 98
5.2.4 Human Mesenchymal Stem Cell and Mesenchymal-like Embryonic Stem Cell Derived Culture 99
5.2.5 Scanning Electron Microscopy 100
5.2.6 Real Time PCR 100
5.2.7 Immunofluorescence and Histological Staining 102
5.2.8 Mineral Quantification 102
List of Figures

Figure

2.1 SEM micrographs of a PLLA nanofibrous matrix prepared from 2.5% (wt/v) PLLA/THF solution at a phase separation temperature of 8°C. (A) 500x; (B) 20,000x. From Ma and Zhang [63], Copyright © John Wiley & Sons. Reprinted by permission of John Wiley & Sons.

2.2 SEM micrographs of PLLA nanofibrous scaffolds prepared from 10% (wt/v) PLLA/THF solutions at a phase separation temperature of -20°C. (A) 100x; (B) 2000x. From Liu et al.[66], Copyright © American Scientific Publishers. Reprinted by permission of American Scientific Publishers.

2.3 SEM of MC-4 cells after 24 hours of culture on (A) nanofibrous matrices and (B) flat (solid) films. From Hu et al.[95], Copyright © 2008 by Elsevier.

2.4 Von Kossa’s silver nitrate staining of histological sections after 6 weeks of MC3T3-E1 osteoblast cultured on (A) nanofibrous scaffolds and (B) solid-walled scaffold. Scale bars are 500 μm. * denotes the PLLA scaffold, # a scaffold pore. Arrows denote mineralization. From Chen et al.[65], Copyright © 2006 by Elsevier.

3.1 SEM micrographs of (A) nanofibrous matrix, Scale bar = 10μm;
(B) solid films, Scale bar=10μm; (C) D3 cells after 12 hrs under differentiation conditions on nanofibrous matrix (Nano), flat (solid) films (Solid), gelatin coated tissue culture plastic (Control), Scale bar =5μm.

3.2 Expression of neuronal, mesodermal, and early osteogenic markers and integrins after 12 days of culture under osteogenic conditions: (A) PCR of RNAs isolated from cells grown on nanofibrous matrix (N), solid films (S) and gelatin coated tissue culture plastic (C); (B) Quantitative PCR of brachyury of RNAs isolated from cells grown expression on nanofibrous matrix (Nano), solid films (Solid) and gelatin coated tissue culture plastic (Control) * denotes p-value <0.05; (C) Quantitative PCR of nestin of RNAs isolated from cells grown expression on nanofibrous matrix (Nano), solid films (Solid) and gelatin coated tissue culture plastic (Control) * denotes p-value <0.05.

3.3 Effects of integrin blocking on mesodermal and osteogenic differentiation after 12 days of differentiation culture. (A) PCR of α2 integrin RNAs expression over time on nanofibrous matrix (N) and flat (solid) films (S); (B) quantitative PCR of brachyury RNAs isolated from cells grown on nanofibrous matrix (Nano), on nanofibrous matrix with control IGG isotype (IGG nano), on nanofibrous matrix with CD49b antibody (α2 blocking nano), on flat (solid) films (Solid), on flat (solid) films with control IGG isotype (IGG solid), and on flat (solid)
films with CD49b antibody (α2 blocking solid) ** denotes p-value <0.01; (C) quantitative PCR of Runx2 RNAs isolated from cells grown on nanofibrous matrix (Nano), on nanofibrous matrix with control IGG isotype (IGG nano), on nanofibrous matrix with CD49b antibody (α2 blocking nano), on solid-walled matrix (Solid), on flat (solid) films with control IGG isotype (IGG solid), and on flat (solid) films with CD49b antibody (α2 blocking Solid) ** denotes p-value <0.01; (D) quantitative PCR of brachyury RNAs isolated from cells grown on nanofibrous matrix (Nano), on nanofibrous matrix with control IGG isotype (IGG nano), on nanofibrous matrix with CD49e antibody (α5 blocking nano), on flat (solid) films (Solid), on flat (solid) films with control IGG isotype (IGG solid), and on solid-walled matrix with CD49e antibody (α5 blocking solid) * denotes p-value <0.05; (E) quantitative PCR of Runx2 RNAs isolated from cells grown on nanofibrous matrix (Nano), on nanofibrous matrix with control IGG isotype (IGG nano), on nanofibrous matrix with CD49e antibody (α5 blocking nano), on flat (solid) matrix (Solid), on flat (solid) films with control IGG isotype (IGG solid), and on flat (solid) films with CD49e antibody (α5 blocking solid) * denotes p-value <0.05.

3.4 Protein adsorption to materials after exposure to differentiation media containing 20% bovine serum protein or purified bovine fibronectin (100μg/ml) for 1 hr: (A) 4-12% polyacrylamide gels stained with Coomassie blue from protein extracts from
nanofibrous matrix (N) and flat (solid) films (S) treated with media; (B) western blot of fibronectin extracted from nanofibrous matrix (Nano) and flat (solid) films (Solid) treated with media; (C) western blot of fibronectin extracted from nanofibrous matrix (Nano) and flat (solid) films (Solid) treated with purified bovine fibronectin.

3.5 Expression of osteogenic markers after 26 days of culture under osteogenic differentiation conditions: (A) PCR of RNAs isolated from cells grown on nanofibrous matrix (N), flat (solid) films (S) and gelatin coated tissue culture plastic (C); (B) quantitative PCR of bone sialoprotein RNAs isolated from cells grown on nanofibrous matrix (Nano), flat (solid) films (Solid) and gelatin coated tissue culture plastic (Control) * denotes p-value <0.05; (C) quantitative PCR of osteocalcin RNAs isolated from cells grown on nanofibrous matrix (Nano), flat (solid) films (Solid) and gelatin coated tissue culture plastic (Control) * denotes p-value <0.05; ** denotes p-value <0.01.

3.6 Mineralization characterization after 26 days of culture under osteogenic differentiation conditions (A) Calcium staining after 26 days under osteogenic differentiation conditions on nanofibrous matrix (Nano), flat (solid) films (Solid) and gelatin coated tissue culture plastic (Control); (B) Calcium staining after 26 days under osteogenic differentiation conditions on nanofibrous matrix without ESC.

3.7 Immunofluorescence localization of neuronal (TUJ1) and late
Bone differentiation (Osteocalcin) Marker expression after 26 days under osteogenic differentiation conditions on nanofibrous matrix (Nano), flat (solid) films (Solid) and gelatin coated tissue culture plastic (Control). Scale bar = 50 μm

4.1 Expression of bone differentiation markers on nanofibrous thin matrices (Nano), flat films (Solid) and gelatin-coated tissue culture plastic (Control) with various media supplementations ((D) basic differentiation media, (O) osteogenic media, and (B) BMP media) after 3 weeks of culture.

4.2 Expression of bone differentiation markers on 2D nanofibrous thin matrices (N), flat films (S) and control (C) and 3D nanofibrous (N) and solid-walled (S) scaffolds (3-D) in BMP media over 2 weeks of culture.

4.3 Expression of bone differentiation markers on nanofibrous scaffolds comparing cellular (A) type I collagen and (B) osteocalcin expression in BMP media and TBI media over 2 weeks. * denotes a p<0.05. ** denotes a p<0.01.

4.4 Expression of bone differentiation markers on nanofibrous (Nano) and solid-walled (Solid) scaffolds after 4 weeks of culture in TBI media. * denotes a p<0.05. ** denotes a p<0.01.

4.5 Calcium staining over 4 weeks of culture on nanofibrous matrices (Nano), flat films (Solid) and Control in TBI media.

4.6 (A) Histology of cellular (H&E) and calcium (Alizarin Red) staining over 4 weeks of culture on nanofibrous (Nano) and solid-walled (Solid) scaffolds in TBI media. Scale bar = 500 μm.
(B) Quantification of scaffold calcium content after 4 weeks of culture in TBI media. (C) Quantification of scaffold collagen content after 4 weeks of culture in TBI media. ** denotes a p<0.01.

4.7 Immunofluorescence of late bone differentiation (Osteocalcin-red) and neuronal (TUJ1-green) marker expression over 4 weeks of culture in TBI media on nanofibrous (Nano), solid-walled (Solid) scaffolds. Scale bar =50μm

4.8 Protein adsorption to materials after exposure to medium containing bovine serum protein or fetal bovine serum for 4 hr:  (A) MicroBCA of protein extracts from nanofibrous scaffolds (Nano) and solid-walled scaffolds (Solid) treated with media; ** denotes a p-value < 0.01. (B) western blot of fibronectin extracted from nanofibrous scaffolds (Nano) and solid-walled scaffolds (Solid) treated with differentiation media or fetal bovine serum.

5.1 Comparison of the osteogenic potential of hESC derived osteogenic progenitor cells and hESC derived mesenchymal cells. (A) Quantitative PCR collagen type 1 and Runx2 after 2 weeks of osteogenic differentiation. Expression levels were normalized to β actin. * denotes p-value <0.05; (B) Alizarin red staining of hESC derived osteogenic progenitor cells and hESC derived mesenchymal cells after 3 weeks of osteogenic differentiation; (B) Quantitative PCR expression of osteogenic markers by hESC derived osteogenic progenitors with and without bFGF
supplementation prior to seeding on nanofibrous matrices (nano), flat films (solid) and 0.1% gelatin coated tissue culture plastic (control). Expression levels were normalized to β actin. * denotes p-value <0.05. ** denotes p-value <0.01.

5.2 SEM micrographs of hESC derived osteo progenitor cells after 48 hrs of culture under osteogenic differentiation conditions on nanofibrous matrix (Nano), flat films (Solid), gelatin coated tissue culture plastic (Control), Scale bar =20μm (Nano & Control), 50 μm (Solid).

5.3 Expression of markers of osteogenic and neuronal differentiation over time under osteogenic differentiation conditions on nanofibrous matrices (nano), flat (solid) films (solid) and 0.1% gelatin coated tissue culture plastic (control) using quantitative PCR for (A) Collagen type 1, (B) Runx2, (C) osteocalcin, and (D) TUJ1. * denotes p-value <0.05. ** denotes p-value <0.01

5.4 (A) Immunofluorescence localization of Neuronal (TUJ1) and late bone differentiation (Osteocalcin) markers after 2 weeks culture under osteogenic differentiation conditions on nanofibrous matrix (Nano), flat films (Solid) and gelatin coated tissue culture plastic (Control). Scale bar =50μm. (B) Calcium staining after 3 weeks under osteogenic differentiation conditions on nanofibrous matrix (Nano), flat films (Solid) and gelatin coated tissue culture plastic (Control).

5.5 Effects of integrin blocking on osteogenic differentiation
after 2 weeks of differentiation. (A) PCR of expression of \( \alpha_2 \) integrin on nanofibrous matrix (N), flat (solid) films (S) and 0.1% gelatin coated tissue culture plastic (C); (B) quantitative PCR of expression of Runx2 on nanofibrous matrix (Nano), on nanofibrous matrix with control IGG isotype (Nano with isotype control), on nanofibrous matrix with mouse anti-human integrin alpha 2 monoclonal antibody (\( \alpha_2 \) blocking nano), on flat films (Solid), on flat films with control IGG isotype (solid with isotype control), and on flat films with mouse anti-human integrin alpha 2 monoclonal antibody (\( \alpha_2 \) blocking solid). * denotes p-value <0.05. ** denotes p-value <0.01. (C) Quantitative PCR of expression of osteocalcin on nanofibrous matrix (Nano), on nanofibrous matrix with control IGG isotype (Nano with isotype control), on nanofibrous matrix with mouse anti-human integrin alpha 2 monoclonal antibody (\( \alpha_2 \) blocking nano), on flat films (Solid), on flat films with control IGG isotype (solid with isotype control), and on flat films with mouse anti-human integrin alpha 2 monoclonal antibody (\( \alpha_2 \) blocking solid). * denotes p-value <0.05. ** denotes p-value <0.01.

5.6 Quantitative PCR analysis of (A) Collagen type 1 (B) Runx2 (C) osteocalcin and (D) TUJ1 over time under osteogenic differentiation conditions on nanofibrous scaffolds (nano), and solid-walled scaffolds (solid). ** denotes p-value <0.01.

5.7 (A) Histology organization of cellular (H&E) and calcium
Von Kossa) staining of the scaffolds after 6 weeks of culture on nanofibrous (Nano) and solid-walled (Solid) scaffolds. Scale bar = 200μm. (B,C) Quantification of scaffold calcium (B) and collagen (C) content after 6 weeks of osteogenic culture. * denotes a p<0.05.

5.8 Protein adsorption to scaffolds after exposure to medium containing bovine serum protein or fetal bovine serum for 4 hr: (A) Amount of adsorbed proteins (MicroBCA assay) from nanofibrous scaffolds (Nano) and solid-walled scaffolds (Solid) treated with media. ** denotes a p-value < 0.01. (B) western blot of fibronectin extracted from nanofibrous scaffolds (Nano) and solid-walled scaffolds (Solid) cultured in fetal bovine serum for 4hr.
List of Abbreviations

2D Two dimensional
3D Three-dimensional
αMEM Alpha Minimum Essential Medium
BMP-2 Bone morphogenic protein-2
DMEM Dubecco’s Modified Eagle Media
D-PBS Dulbecco’s Phosphate Buffered Saline
EBS Embryoid bodies
ECM Extracellular matrix
ESC Embryonic stem cells
FBS Fetal bovine serum
FGF2 Fibroblast growth factor 2
HBSS Hank’s buffered salt solution
hESC Human embryonic stem cells
IGF Insulin-like growth factor I
LIF Leukemia inhibitory factor
NF Nanofibrous
PCL Poly(caprolactone)
PDAC Poly(diallyldimethylammonium chloride)
PEO Poly(ethylene oxide)
PET Polyethylene terephthalate
PHB Poly(3-hydroxybutyrate)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PLLA</td>
<td>Poly(L-lactic acid)</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
</tr>
<tr>
<td>SW</td>
<td>Solid-walled</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Human transforming growth factor-beta1</td>
</tr>
<tr>
<td>TIPS</td>
<td>Thermally induced phase separation</td>
</tr>
<tr>
<td>TUJ1</td>
<td>Neuronal Class III β-Tubulin</td>
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ABSTRACT

Effects of Nanofibrous Scaffolding Architecture on Bone Tissue Development from Embryonic Stem Cells

by

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Embryonic stem cells, typically isolated from the inner cell mass of blastocysts, represent a potentially unlimited cell source for tissue engineering. However, the potential tumorigency of the undifferentiated cells and the heterogeneous cell population generated by current differentiation protocols impede the use of embryonic stem cells as a clinical cell source for tissue engineering applications. This thesis examines the effects of emulating the differentiation signals provided by the extracellular matrix during development with synthetic poly (L-lactic acid) nanofibers on the differentiation of the embryonic stem cells to osteoblasts.
First, undifferentiated mouse embryonic stem cells were seeded onto two dimensional nanofibrous thin matrices or flat (solid) films. With osteogenic supplementation the nanofibrous architecture was found to promote the osteogenic differentiation and mineralization of the mouse embryonic stem cells. α2 and α5 integrin appear to contribute to this osteogenic differentiation.

Next, the effects of biologically active factors and three dimensional culture were examined on mouse embryonic stem cells which were partially differentiated via embryoid body formation prior to seeding on the materials. The nanofibrous architecture was found to facilitate further differentiation of the cells in the absence of osteogenic stimulation, while the cells cultured on solid film required osteogenic supplements and growth factors to support osteogenic differentiation. Three dimensional culture on nanofibrous scaffolding was found to further enhance the osteogenic differentiation and mineralization more than two dimensional culture on either the nano-fibrous or solid architecture and three dimensional culture on the solid-walled scaffolding.

The osteogenic differentiation of human embryonic stem cells was examined next. In both two and three dimensional culture, the nanofibrous architecture enhanced the osteogenic differentiation and mineralization of the human embryonic stem cells compared to the solid architecture.

In summary, the nanofibrous architecture enhances the osteogenic differentiation of mouse and human embryonic stem cells compared to the more traditional solid-walled tissue engineering scaffolding architecture. This indicates that emulating size scale of the
extracellular matrix with synthetic nanofibers is advantageous in promoting osteogenic differentiation of embryonic stem cells.