For Pony

Acknowledgements

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Tables of Contents

List of Figures

Figure

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)

PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(L-lactic acid)
PVA	Poly(vinyl alcohol)
SW	Solid-walled
TGF-β1	Human transforming growth factor-beta1
TIPS	Thermally induced phase separation
TUJ1	Neuronal Class III β-Tubulin

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 tumorgencity 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. $\alpha 2$ and $\alpha 5$ 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.