3D Cell Culture for Enhancement of Cell Function

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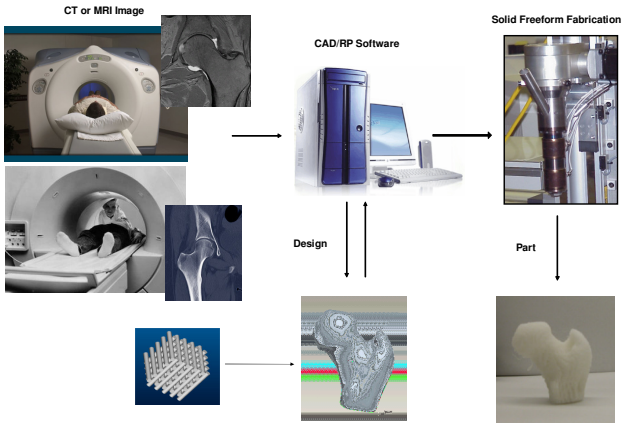
Introduction

- In the complex interplay between cells and ECM, cells cultured and arranged in conventional 2D monolayer on tissue culture polystyrene surfaces fail to reflect *in vivo* scenario with its biological subtleties.
- In 3D culture, normal fibroblast cells can move and divide more quickly, assuming the characteristic *in vivo* asymmetric shape
- 3D cultures also show promise as disease models. For example, only malignant breast cells cultured in 3D carry large numbers of the specific receptors while in 2D culture, both normal and malignant breast cells have similar, high levels of the receptors
- The appropriate environment in which to culture cells in 3D presents challenges including the selection of 1) biocompatible matrix material, 2) a suitable 3D structure fabrication method, and 3) fabrication process parameters optimally characterized for enhanced biological performance

Objective

Design and model 3D *in vitro* cell culture models to uncover patterns of biological phenomena that more closely mirror what happens in the *in vivo* microenvironment enhanced cell culture and downstream tissue engineering applications.

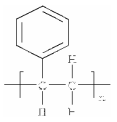
Process Development



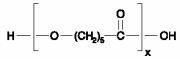
- For scaffold design, CT/MRI scan elicits patient-specific data to inform overall implant architecture
- Multiple design constraints for scaffold include biophysical, anatomical, biological, transport, and manufacturability requirements

Materials and Methods

3D InsertTM is a series of disposable 3D cell culture well inserts for use with multi-well cell culture plates. Using solid freeform fabrication technology, the scaffolds are composed of struts/filaments joined together to form a well-defined porous structure, including porosity, pore size, and surface area. Constituent materials include non-degradable or degradable polymers such as polystyrene (PS) and polycaprolactone (PCL).

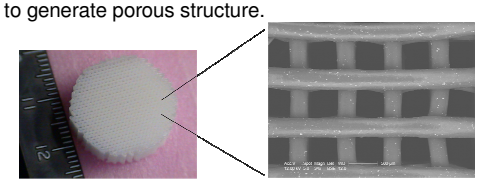


Polystyrene
- Non-biodegradable aromatic polymer.
- Transparent property allows convenient visualization under inverted microscopy
- Thermoplastic with melting temperature at about 190ºC - 260ºC.

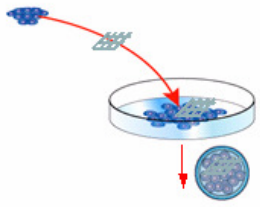


Polycaprolactone
- A semi-crystalline aliphatic polymer.
- Slower degradation rate than most biopolymers in its homo-polymeric form
- Thermoplastic with melting temperature at about 58ºC - 60ºC.

3D InsertTM Design Specifications
Fabricated cylindrical scaffolds measured at 20mm in diameter and 4-layered height, strut widths of 500µm, with pore sizes of 100um (1050), 300µm (3050), and 500µm (5050) and plasma surface treatment (ST). Each layer was filled with the designed scaffold pattern of a 0/90 degree orientation to generate porous structure.

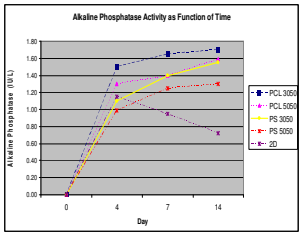


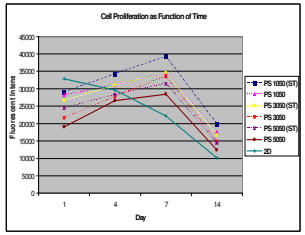
Biological Characterization Study Design
- 7F2 mouse osteoblast cells (200,000 cells / scaffold seeded directly onto top surface of 3D InsertTM with Pasteur pipet
- Control Group: 200,000 cells seeded onto 2D 12-multiwell plate
- Alamar Blue Assay (for cell proliferation) and Alkaline Phosphatase Assay (for osteogenesis) performed on 3D InsertTM at regular study time points

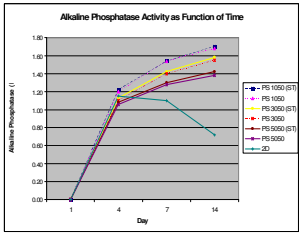


Results of Biological Characterization

3D PCL and PS versus 2D
- Cell function in both PCL and PS 3D InsertTM significantly better than 2D monolayer
- PCL material selection and smaller pore size (300µm) confers improved osteogenic response of cells







3D PS versus 2D
- Cell function in both surface treated and non-treated PS 3D InsertTM significantly better than 2D monolayer
- Plasma surface treatment and smaller pore size (100µm) shows highest rates of proliferation and cell osteogenic activity

Conclusions

- 3D cell culture systems, such as 3D InsertTM, mimic 3D in vivo scenario and confer improvements in cell expansion and differentiated function over the conventional 2D methods
- The precipitous decrease in cell proliferation and function over time in 2D scenario is attributed to over-confluence of cells on 2D surface where cells undergo contact inhibition and loss of viability. Also, 3D InsertTM have increased surface areas compared to 2D cell culture plates, allowing more cells to be cultured using the same size cell culture platform.
- Plasma surface treatment and smaller pore sizes confer improvements in cell proliferation and cell-specific function
- Results show reproducibly manufactured porous PS 3D InsertTM as preferred alternative for enhanced cell culture and PCL InsertTM as good candidate for enhanced cell culture and tissue engineering applications

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