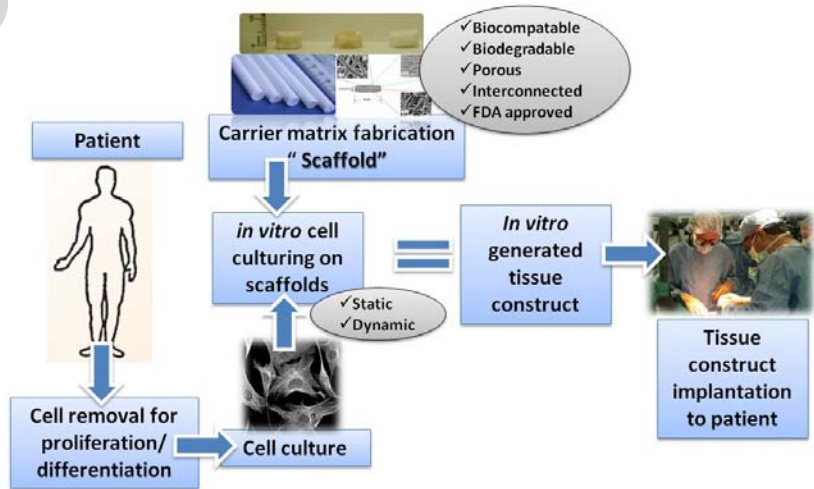


Fabrication and Application of Novel Scaffolds for Tissue Engineering

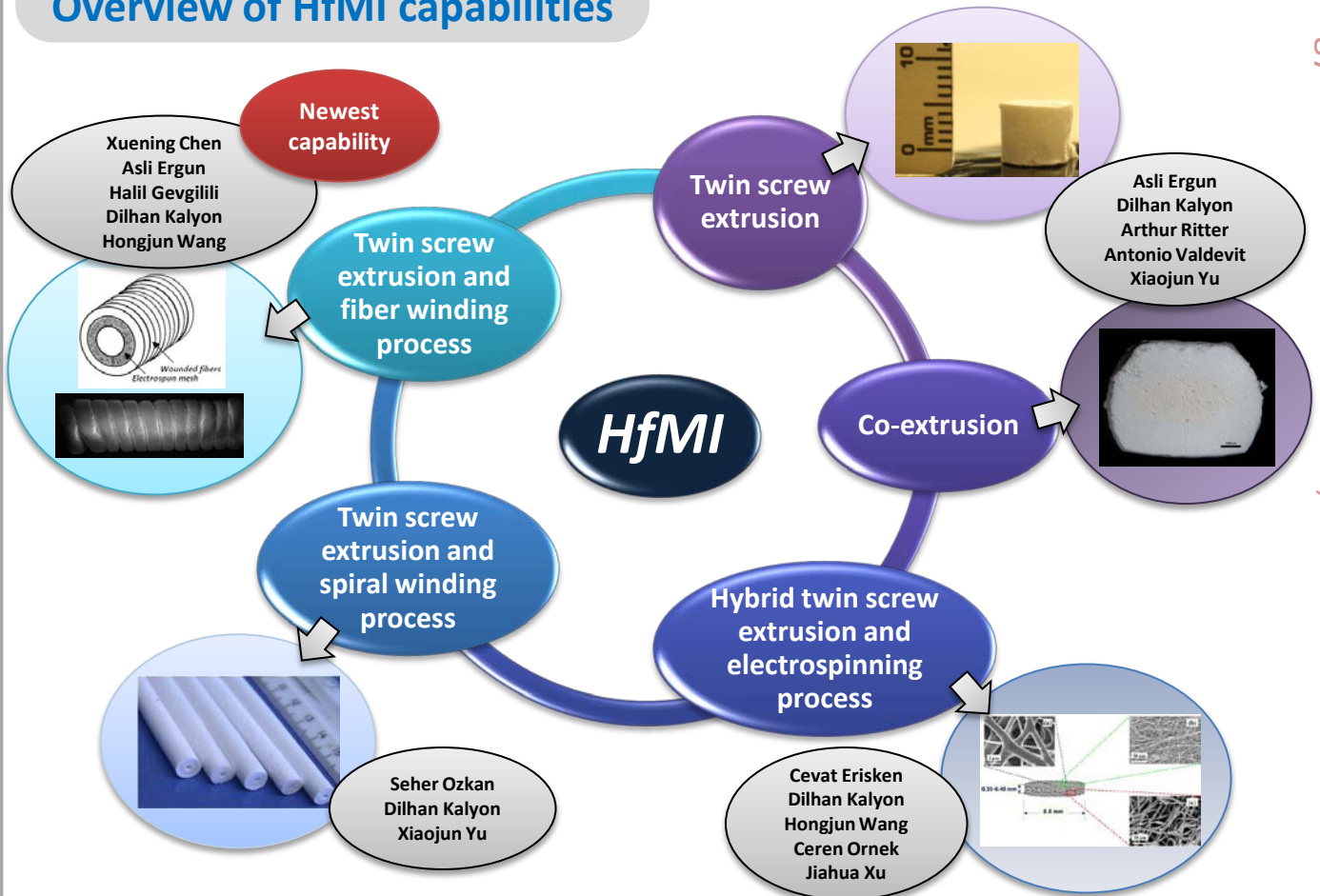
Tissue Engineering

Tissue engineering involves the in vitro culturing of human cells on biodegradable scaffolds, which are then implanted into the body, where cells continue to proliferate and differentiate while the underlying scaffold degrades.

Schematics of tissue engineering



Overview of HfMI capabilities



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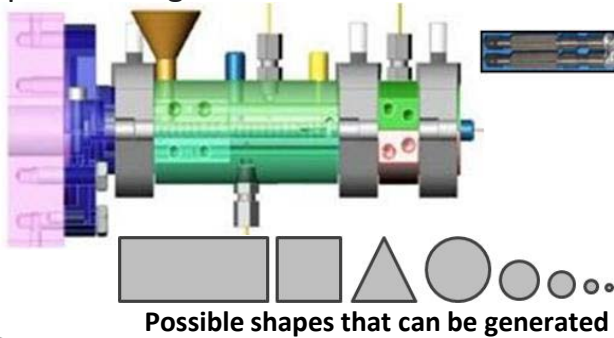
HfMI Technologies to Fabricate Tissue Engineering Scaffolds

TECHNOLOGY #1

Twin Screw Extrusion (TSE): 7.5 mm, L/D=15, fully-intermeshing co-rotating twin screw extruder with multiple feed ports for solid and liquid feeding.

Capability: allows multiple unit operations in a single equipment

- solids conveying
- melting
- distributive and dispersive mixing of particles
- deaeration
- shaping of the scaffolds



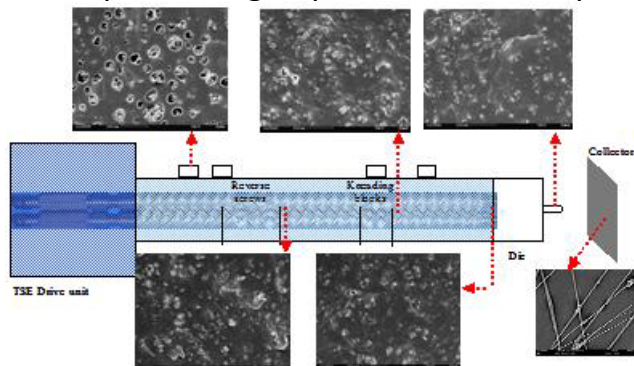
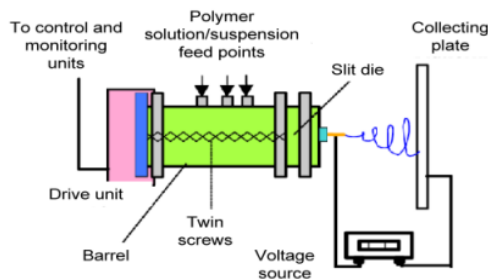
Twin screws

Typical Scaffolds



TECHNOLOGY #2

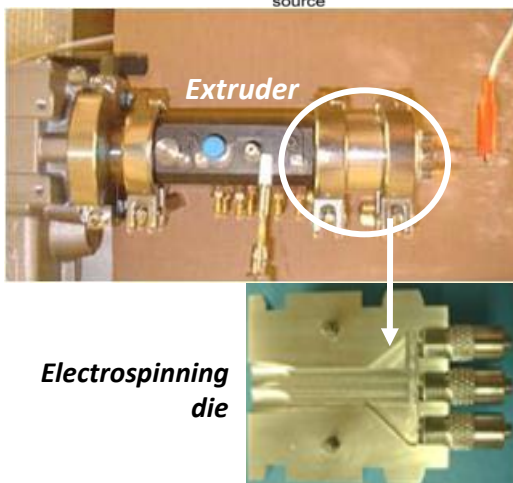
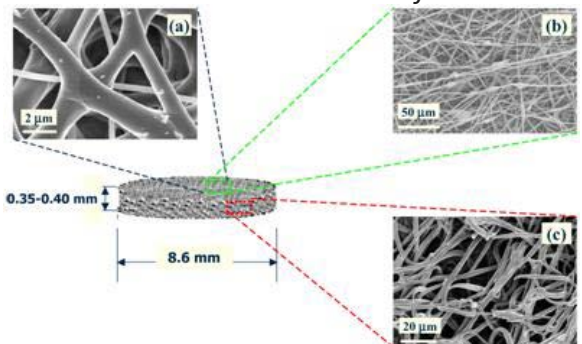
Hybrid Twin Screw Extrusion and Electrospinning (TSEE) Process: a new hybrid technology that fully integrates the processing capabilities of TSE process with electrospinning.



Ability to disperse nanoparticles

Typical Scaffolds

Fabrication of nanoparticle incorporated nanofiber meshes

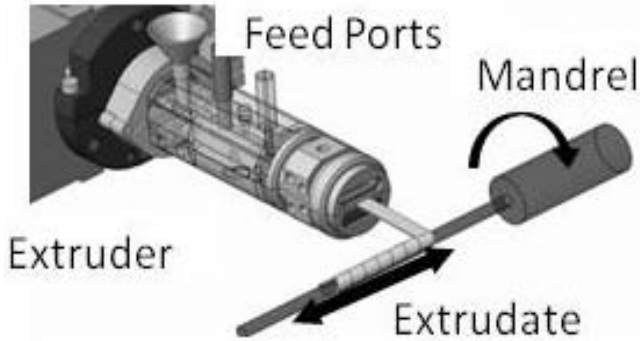


Electrospinning die

HfMI Technologies to Fabricate Tissue Engineering Scaffolds

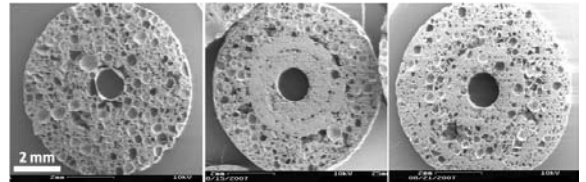
TECHNOLOGY #3

Twin screw extrusion spiral winding process (TSESW): A novel process, suitable for generating relatively large scaffolds with gradations in porosity, pore size, chemical composition and mechanical properties.



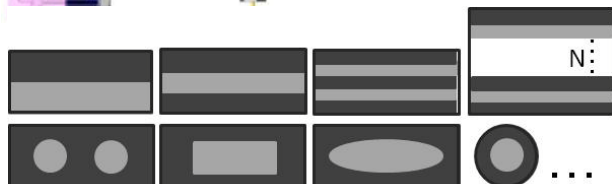
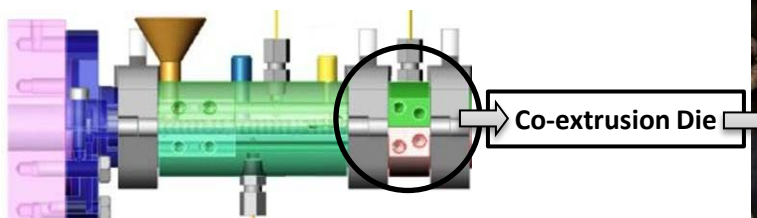
Ozkan, Kalyon, Yu, 2010 J Biomed Mater Res A. 2010 Mar 1;92(3):1007-18

Typical Scaffolds



TECHNOLOGY #4

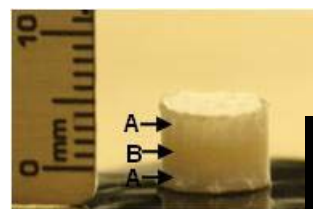
Co-extrusion: A process using twin screw extrusion in conjunction with a co-extrusion die designed to provide multi-layered extrudates to generate myriad shapes and sizes from any of the currently FDA approved biodegradable polymers.



Possible shapes that can be generated

Ergun, Yu, Valdevit, Ritter, Kalyon, 2010 submitted to J Biomed Mater Res A.

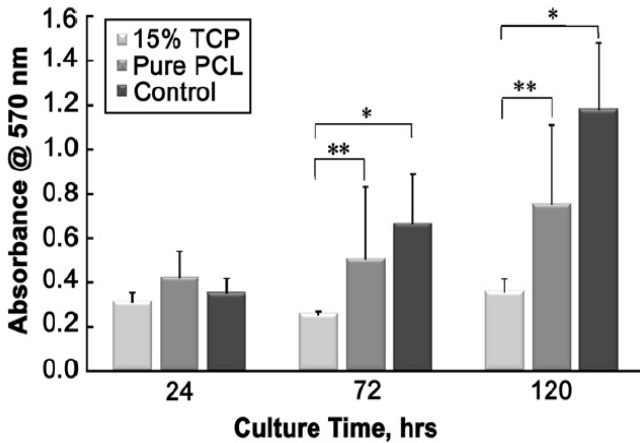
Typical Scaffolds



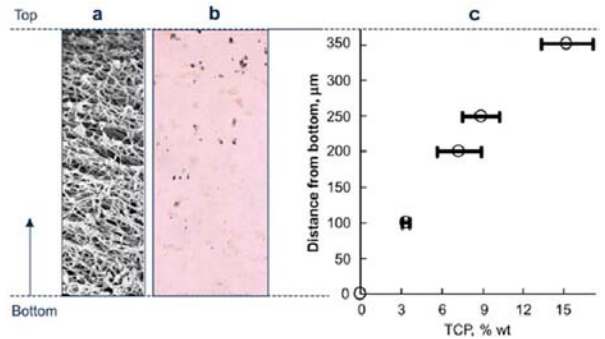
Recent Projects & Applications in bone and cartilage tissue engineering

Functionally-graded electrospun polycaprolactone (PCL) and β -tricalcium phosphate (β -TCP) meshes to serve as scaffolds for bone tissue engineering were fabricated using **hybrid twin screw extrusion and electrospinning** process.

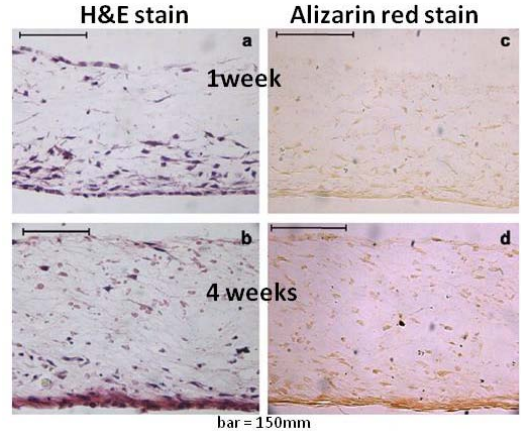
The process allowed the generation of a linear concentration gradient of the β -TCP nanoparticles between the two surfaces of the scaffolds.



in vitro evaluation of bone regeneration on functionally-graded β -TCP/PCL scaffolds (MC3T3-E1 cells)



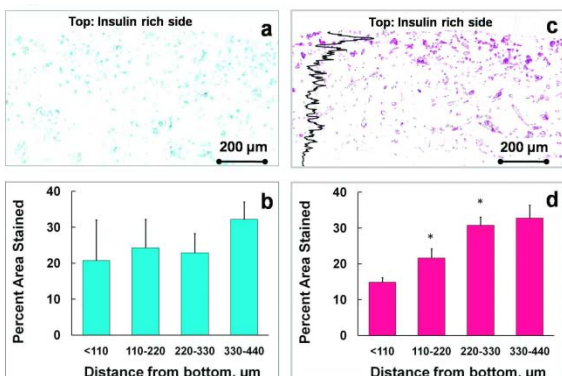
(a) SEM micrograph of cross-section of the functionally graded non-woven mesh, (b) von Kossa staining for distribution of β -TCP nanoparticles along distance, (c) and the quantification of β -TCP concentration.



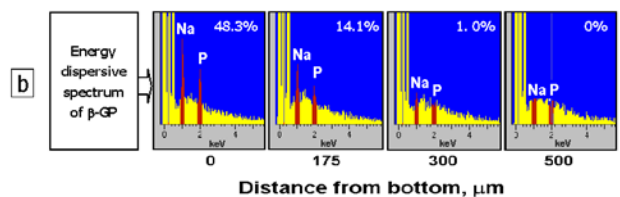
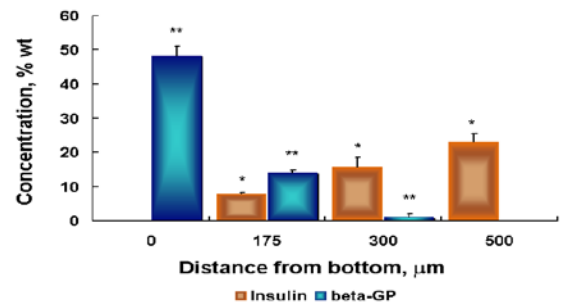
Histological examination of functionally-graded tissue samples

Erisken, Kalyon, Wang, 2008 Biomaterials, 29: 4065-4073

Same methodology was also used to fabricate functionally-graded electrospun meshes with graded **insulin and β -glycerol phosphate (β -GP) concentrations** for osteochondral tissue formation through adipose-derived stem cell differentiation on scaffolds.



(a) Alcian Blue stain and (c) picosirius red stain after 4 weeks revealed GAG (b) and increasing collagen (d) deposition as a function of increasing insulin concentration from bottom to top.

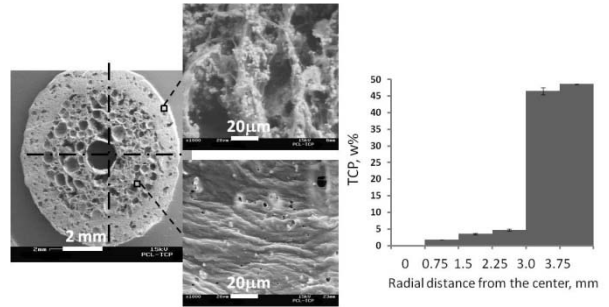


(a) Quantification of biofactors at various scaffold thickness, (b) EDS analysis to indicate Na and P peaks of β -GP

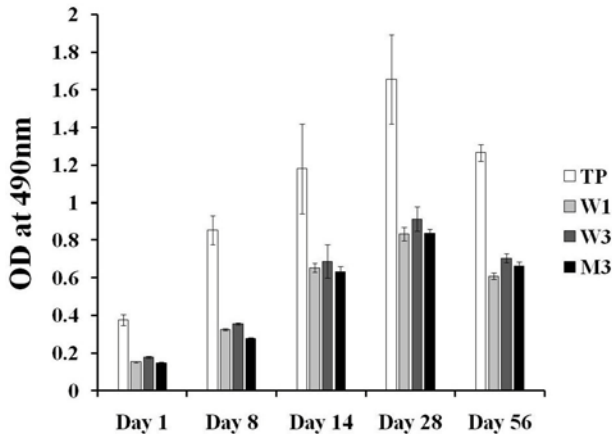
Recent Projects & Applications in bone and cartilage tissue engineering

Functionally-graded relatively large β -TCP/PCL nanocomposite scaffolds were fabricated using **twin screw extrusion and spiral winding** process for bone tissue engineering applications.

The process allowed the generation of scaffolds with **radially-graded** pore size, porosity and β -TCP concentration.

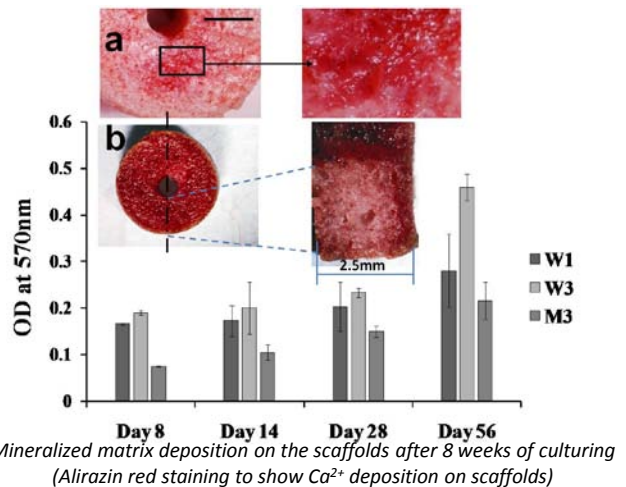


SEM micrograph of the radially graded β -TCP/PCL scaffolds and the quantification of β -TCP nanoparticle concentration along radial direction



in vitro evaluation of bone regeneration on functionally-graded β -TCP/PCL scaffolds with human fetal osteoblast cells

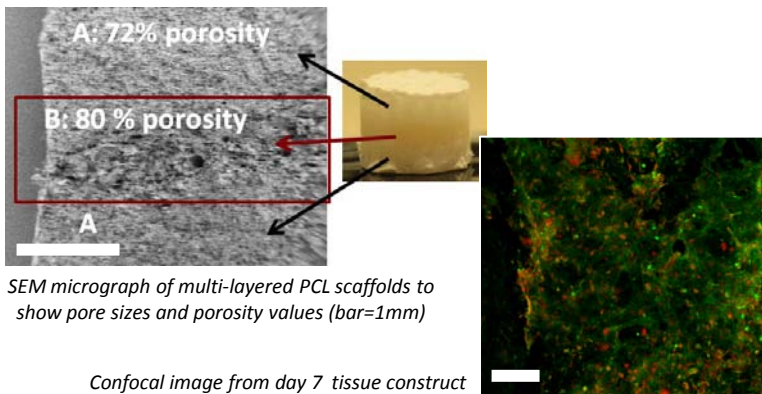
Ozkan, Kalyon, Yu, 2010 *J Biomed Mater Res A*, 2010 Mar 1;92(3):1007-18 & Ozkan, Kalyon, Yu, McKelvey, Lowinger, *Biomaterials*, 30(26):4336-47



Mineralized matrix deposition on the scaffolds after 8 weeks of culturing (Alirazin red staining to show Ca^{2+} deposition on scaffolds)

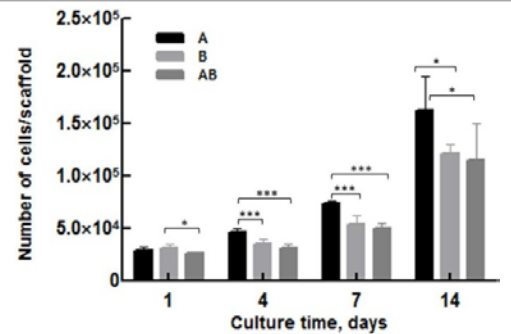
The **co-extrusion** process was used to form of **multi-layered** PCL scaffolds with distinct and tailorable porosities and pore sizes.

Human fetal pre-osteoblastic cells, hFOB, were *in vitro* cultured on the co-extruded scaffolds to evaluate the suitability of their use as bone graft substitutes especially for repair of critical-sized defects or non-union fractures.

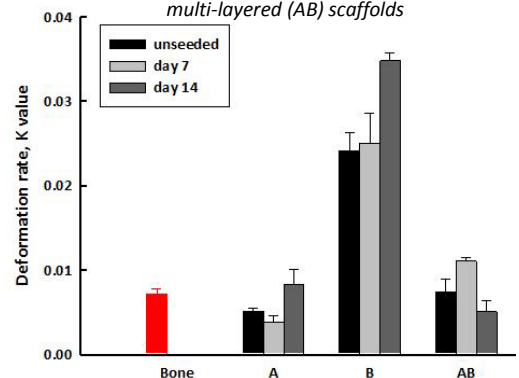


SEM micrograph of multi-layered PCL scaffolds to show pore sizes and porosity values (bar=1mm)

Confocal image from day 7 tissue construct (green: cytoskeleton, red: nuclei - bar=100 μ m)



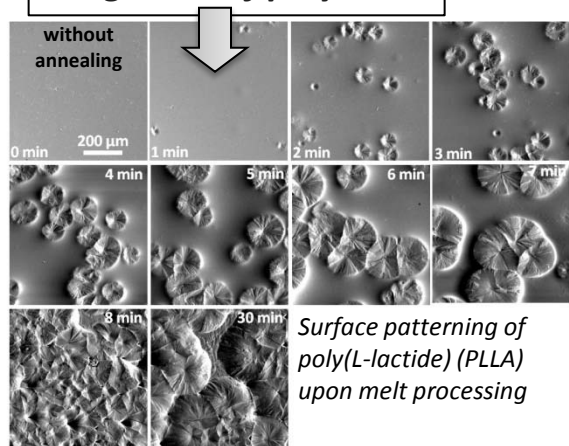
hFOB proliferation on single-layered (A&B) and multi-layered (AB) scaffolds



Deformation rate of scaffolds compared to human trabecular vertebral bone (calculated from cyclic fatigue compression)

Examples of complementary capabilities

Ability to alter surface roughness of polymers

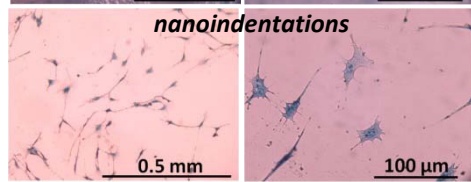
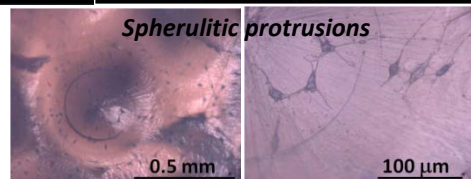
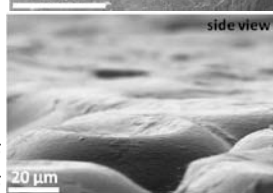
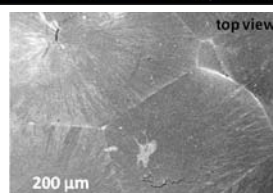
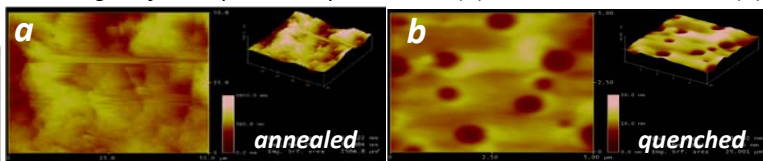


Surface patterning of poly(L-lactide) (PLLA) upon melt processing

Degirmenbasi, Ozkan, Kalyon, Yu, 2009. *J Biomed Mater Res A*, 88(1):94-104

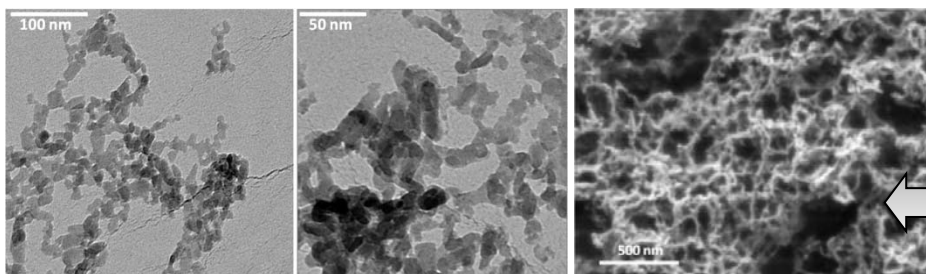
SEM micrographs of the spherulites

AFM images of the spherulitic protrusions (a) and nanoindentations (b)



MC3T3-E1 cell proliferation

SEM micrograph of the biocomposite



TEM micrographs of the biocomposite

Degirmenbasi, Kalyon, Birinci, 2006. *Colloids and Surfaces B: Biointerfaces*, 48(1):42-49

Ability to synthesize mediators in situ

Recent Publications

- Kalyon DM, Erisken C, Wang H. Viscoelastic and biomechanical properties of osteochondral tissue constructs generated from polycaprolactone and beta-tricalcium phosphate composites. *J Biomech Eng* 2010; doi:10.1115/1.4001884.
- Degirmenbasi N, Ozkan S, Kalyon D, Yu X. Surface patterning of poly (L-lactide) upon melt processing: in vitro culturing of fibroblasts and osteoblasts on surfaces ranging from highly crystalline with spherulitic protrusions to amorphous with nanoscale indentations. *J Biomed Mater Res A* 2009, 88A (1):94-104.
- Ozkan S, Kalyon DM, Yu X, McKelvey C, Lowinger M. Multifunctional polycaprolactone (PCL) scaffolds integrated for controlled release and tissue engineering: In vitro evaluation of released protein structure stability, release profile and biocompatibility. *Biomaterials* 2009, 30:4336-4347.
- Ozkan S, Kalyon D, Yu X. Functionally graded β -TCP/PCL nanocomposite scaffolds for bone tissue engineering: *In vitro* evaluation with human fetal osteoblast cells. *J Biomed Mater Res A* 2009;92A(3):1007-1018.
- Erisken C, Kalyon D, Wang H. A hybrid twin screw extrusion/electrospinning method to process nanoparticle-incorporated electrospun nanofibers. *Nanotechnology* 2008;19:165302.
- Erisken C, Kalyon DM, Wang H. Functionally and continuously graded electrospun polycaprolactone and β -tricalcium phosphate nanocomposites for interface tissue engineering applications. *Biomaterials* 2008;29:4065-4073.
- Degirmenbasi N, Kalyon DM, Birinci E. Biocomposites of Nanohydroxyapatite with Collagen and Poly(vinyl alcohol). *Colloids and Surfaces B Biointerfaces* 2006, 48:42-49.

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