

Articular Cartilage Engineering Using Human Mesenchymal Stem Cells and Nanostructured Biomaterials

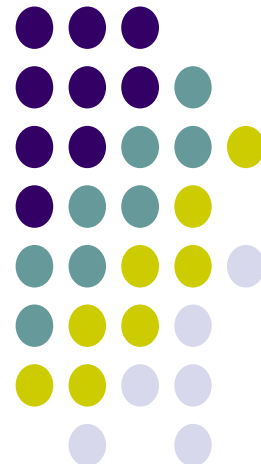
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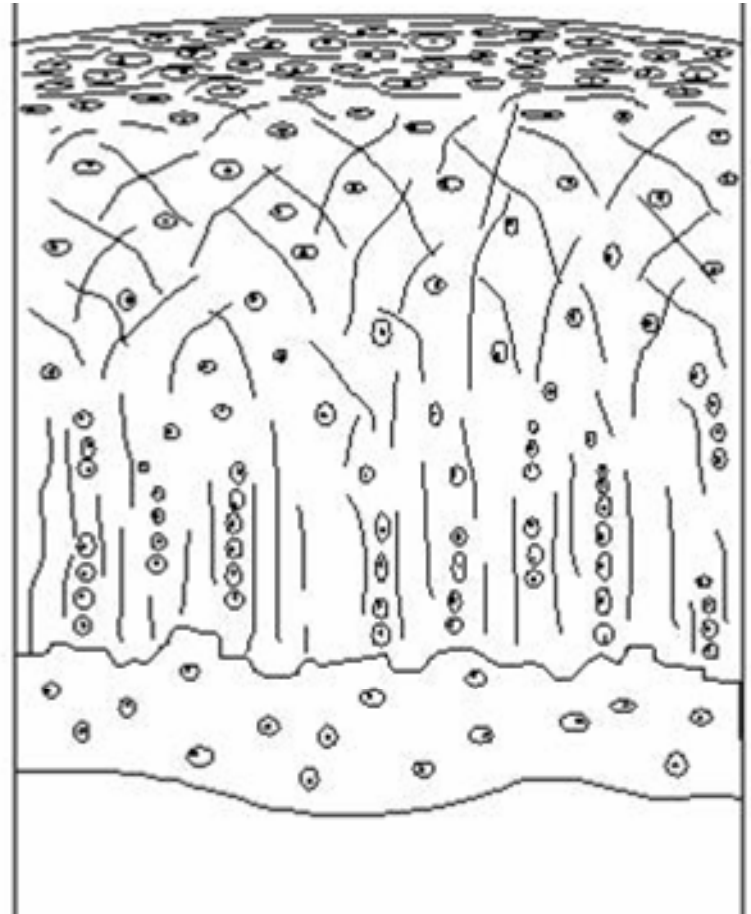
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Articular Cartilage Tissue

- Superficial (top) layer
 - Cells are flattened
 - Cells and ECM aligned and parallel to articular surface
 - High tensile strength
- Regulate orientation of cells and ECM, as in the superficial zone of articular cartilage, using polymer nanofiber scaffolds and hMSCs
- Engineered tissue could be used to regenerate cartilage tissue in patients with articular cartilage disease or damage

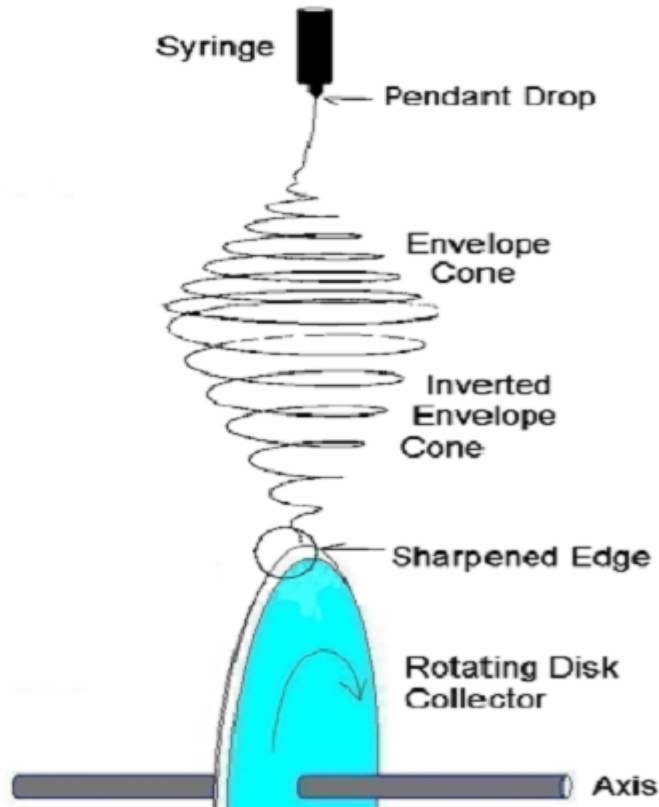


Summer Research



- Quantitative study of stem cell orientation and viability onto three different types of biocompatible polymer nanofibrous scaffolds during long-term culture
- Observe cell-nanofiber adhesions and cytoskeletal reorganization
- Comparison of results to stem cells differentiated into cartilage cells (chondrocytes)

Aligned polymer nanofiber scaffolds by electrospinning

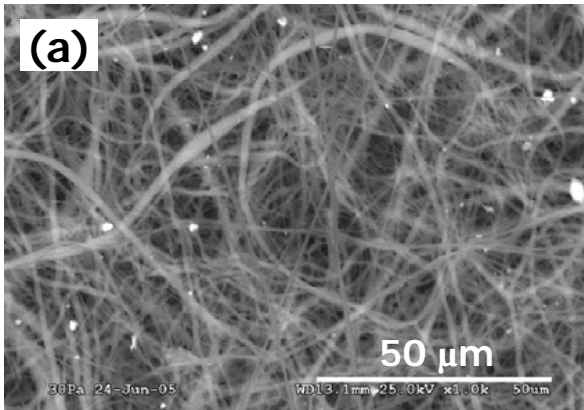


- Rotating disk collector can make aligned nanofiber scaffolds
- Can make fibers with diameters as small as several nanometers
- Experimental Scaffolds:
 - Diameter: several hundred nm
 - Poly(ϵ -caprolactone) fibers are biocompatible

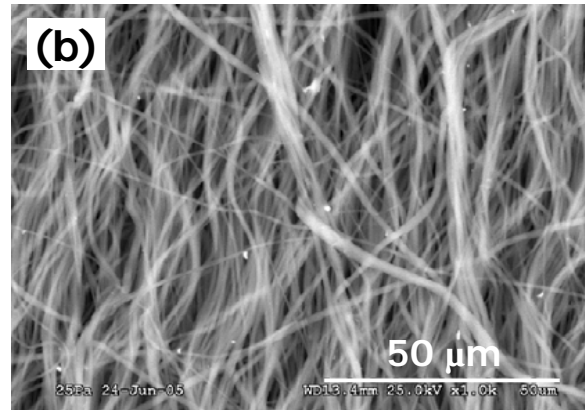


SEM images of scaffolds

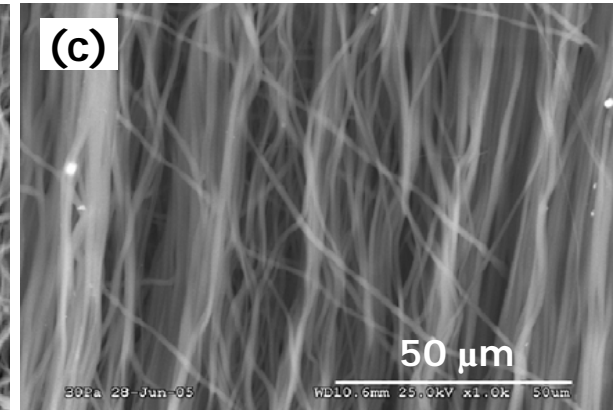
random



aligned ribbon



aligned rope



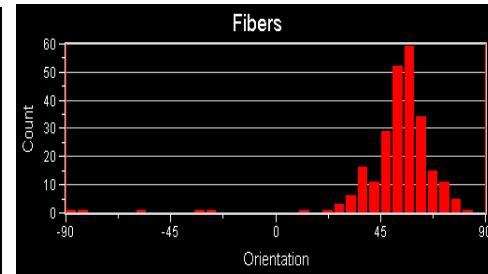
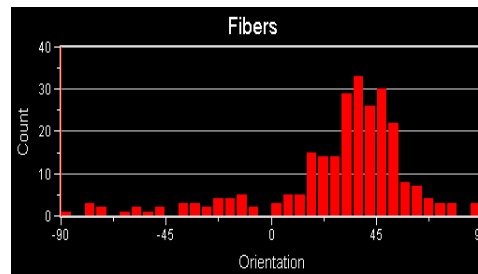
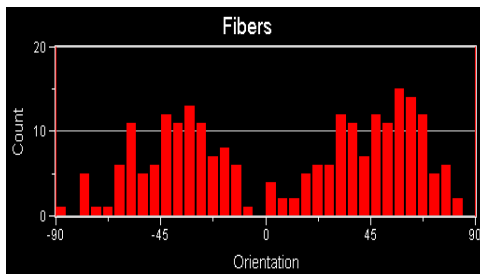
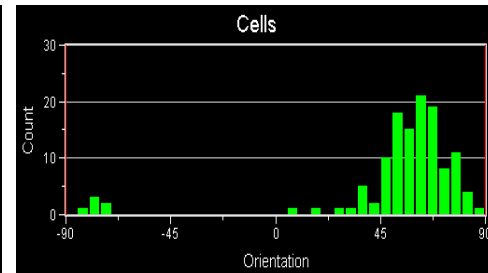
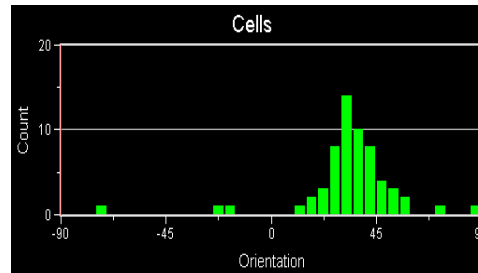
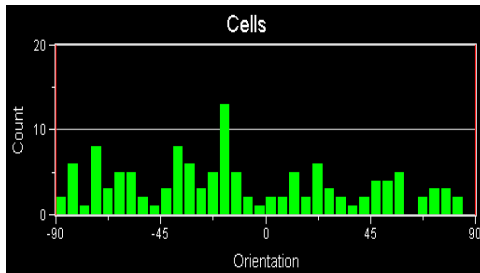
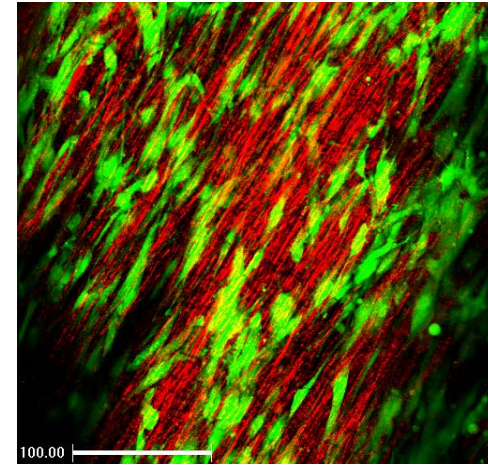
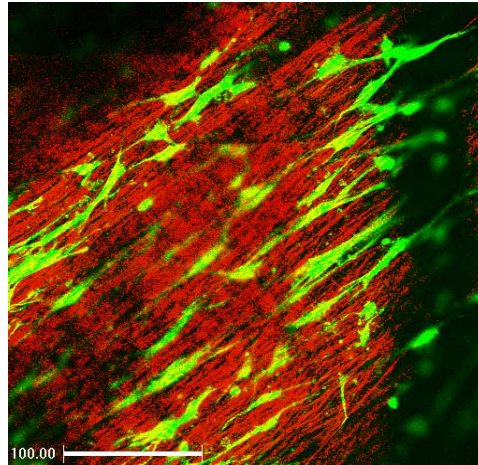
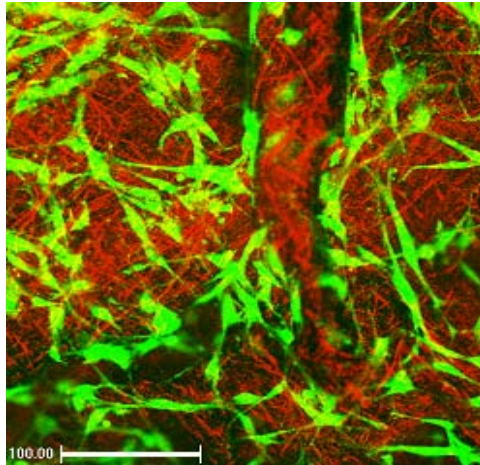
- Random – polymer spun onto flat aluminum surface
- Aligned Ribbon – polymer collected onto yarn on rotating disk
- Aligned Rope – polymer collected on edge of rotating disk

Data Analysis



- Prove fibers affect orientation of cells
 - Compare standard deviations of cells on different scaffolds
- Prove ribbon and rope scaffolds are aligning stem cells better than the random scaffolds
 - Compare difference between individual cells and average fiber orientation

Day 1 of stem cells on nanofibers

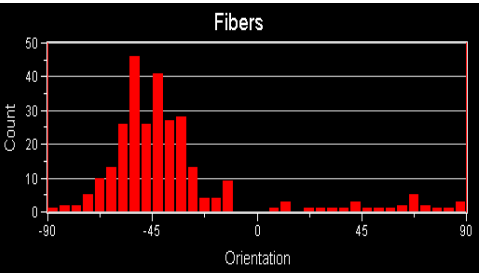
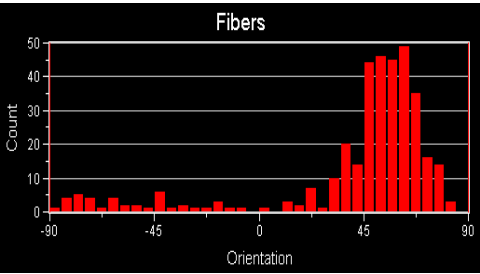
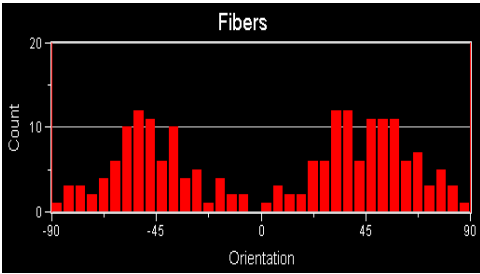
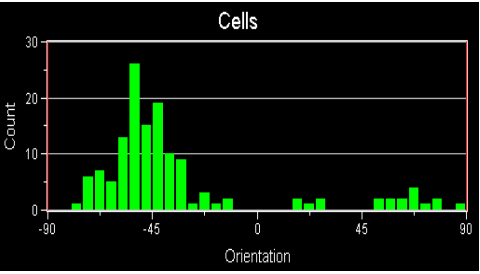
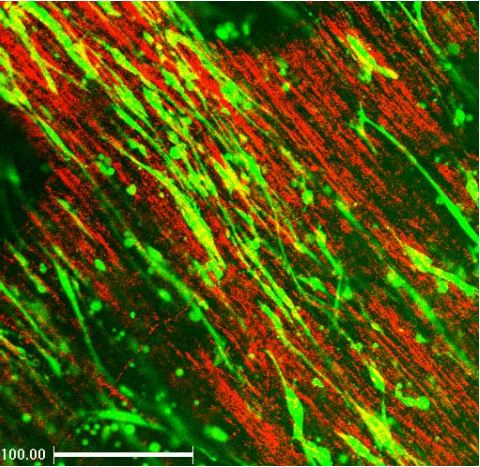
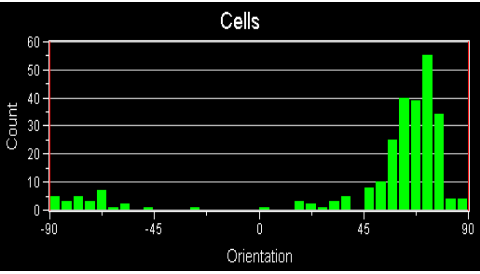
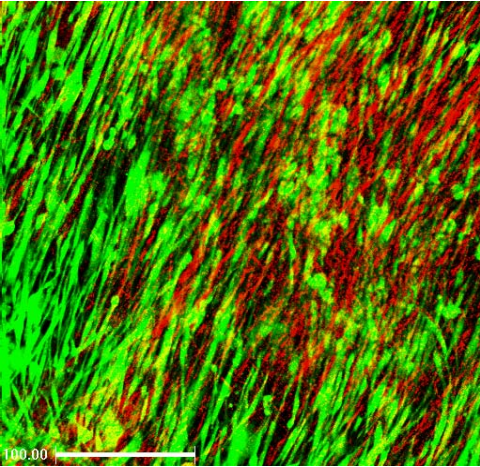
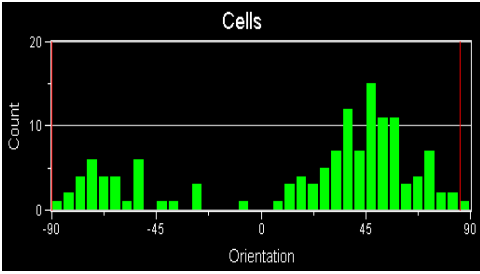
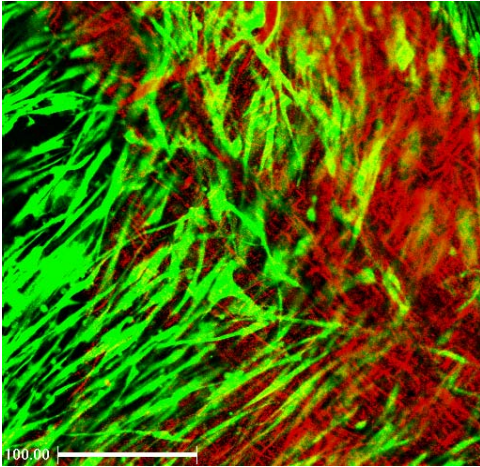
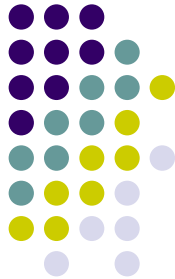


random

aligned ribbon

aligned rope

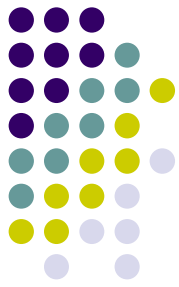
Day 18 of stem cells on nanofibers



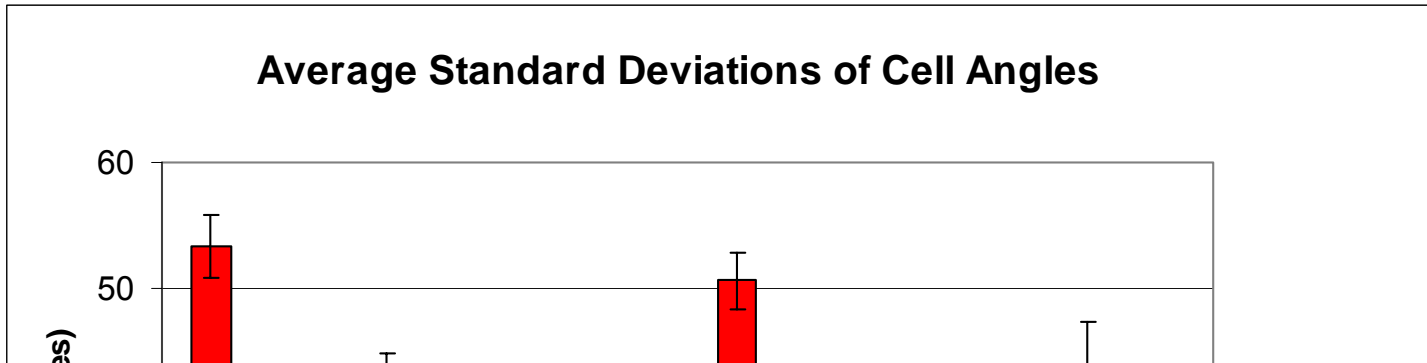
random

aligned ribbon

aligned rope

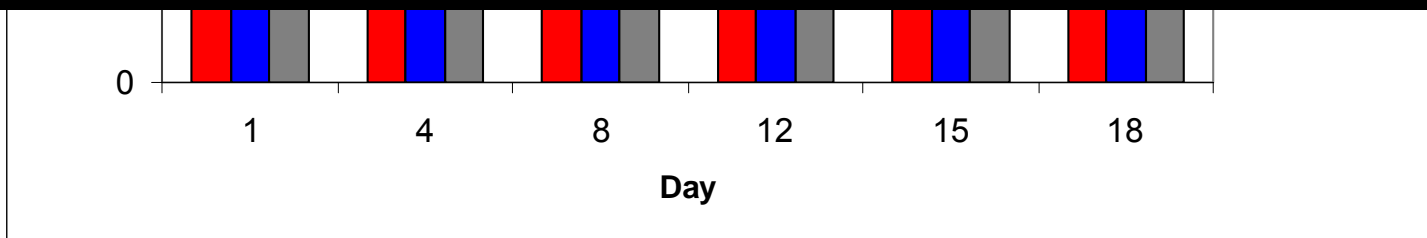


Data Analysis: Standard Deviation



- T-test statistics for unpaired two-tailed test (for $p < .05$)
 - Random vs. Ribbon: p-value = .00449
 - Random vs. Rope: p-value = .000997
 - Ribbon vs. Rope: p-value = .312

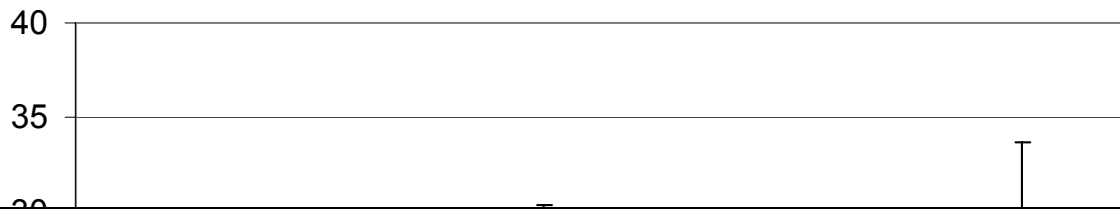
... over 95% confidence that either aligned scaffold will produce cells with smaller standard deviation



Data Analysis: Deviation of Cells from Average Fiber Angle

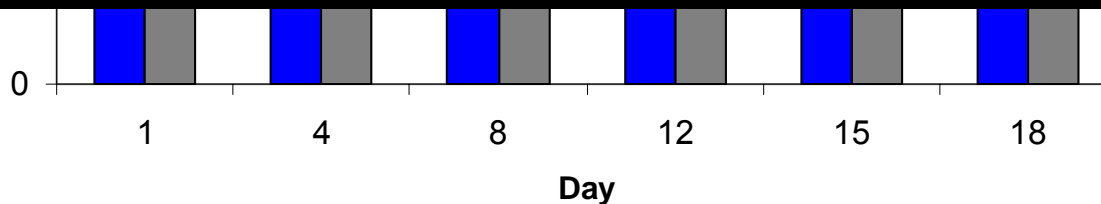


Average Angular Deviation of Cells from Fibers



- Rope cells average deviation from fibers = 18.3°
- Ribbon cells average deviation from fibers = 22.1°
- Random not included (no average random fiber angle)

... observation that rope seems to align cells better than ribbon can be explained by previous SEM images, which show the rope scaffold is made of straighter, more aligned bundles of fibers





Cell viability on Days 4 and 18

Day	Sample	% Live	Total # Cells Viewed
4	random	75.79%	318
	ribbon	89.19%	319
	rope	79.49%	525
18	random	72.54%	1487
	ribbon	73.99%	1461
	rope	76.45%	913

- % Live lower than expected- attributed to the age of the stem cells (passage 6) and the high initial seeding density (7.5×10^4 cells/cm²)
- Steady % Live and increase in total number of cells viewed confirms that a PCL scaffold is reasonable for short or long-term cell culture experimentation



Conclusions / Future Research

- Nanofiber organization has an effect on cell orientation
- Aligned scaffolds orient cells better than random scaffolds
- In the near future, conduct the same experiment with stem cell-derived cartilage cells
 - Does collagen-type II produced by chondrocytes also align?
 - Do chondrocytes maintain alignment over time as stem cells did?
 - Do the fibers degrade over time?



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