Evaluation of Collagen Coated PCL Electrospun Scaffold for Bone Tissue Regeneration Using Human Adipose-Derived Adult Stem Cells

Carla Haslauer, Ajit Moghe, Bhupender S. Gupta, and Elizabeth G. Loboa

BME
BIOMEDICAL ENGINEERING
UNC NCSU

Background and Objective

- Nanofibrous structures have high surface area, high porosity, and a 3D interconnected pore network conducive to cell growth and proliferation

- **Objective:** to determine if the addition of a collagen coating enhanced cell viability and differentiation potential as opposed to uncoated PCL

Hypothesis

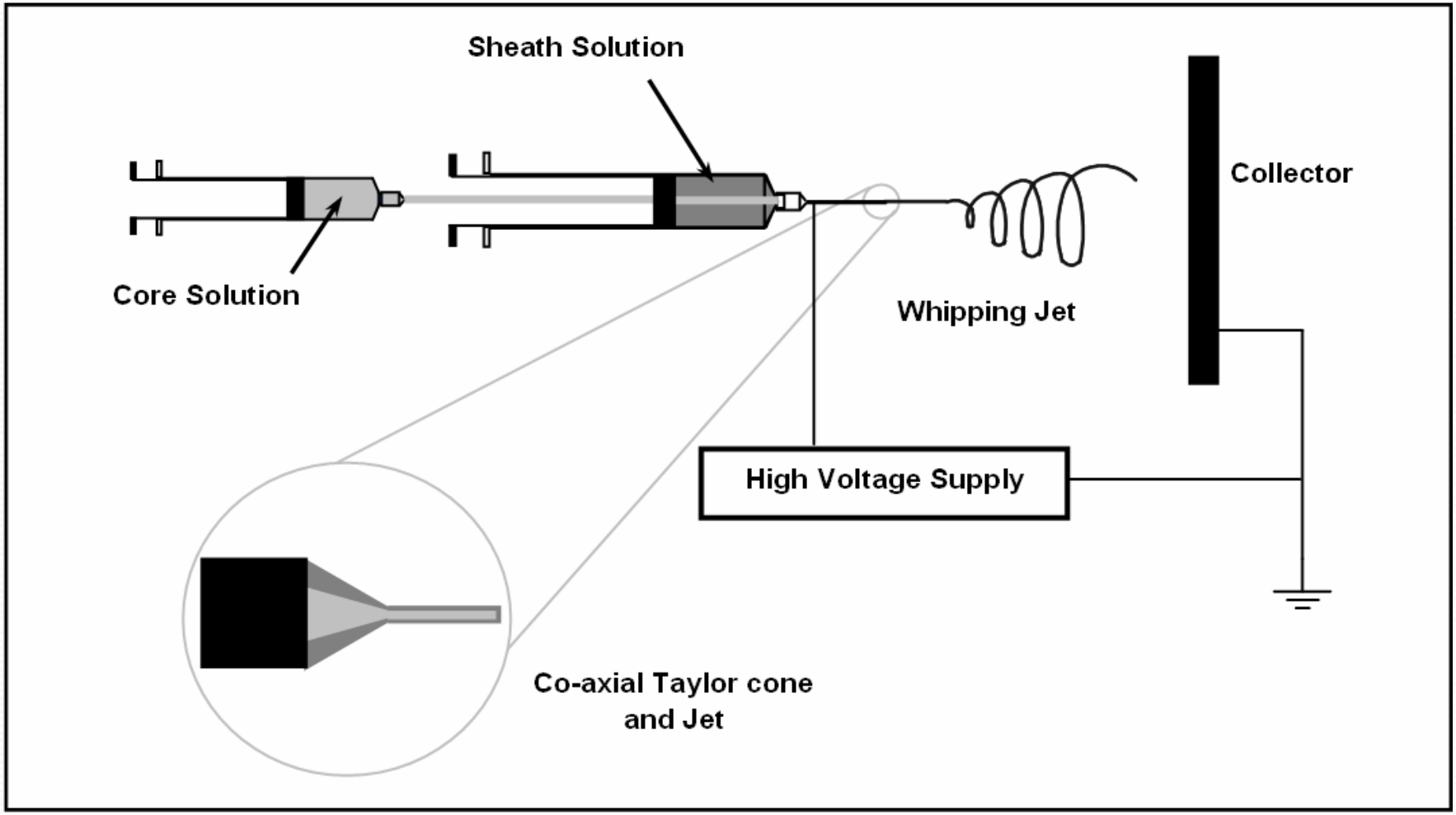
The collagen coating will enhance initial cell attachment and spreading, leading increased calcium deposition on the composite scaffold

Materials

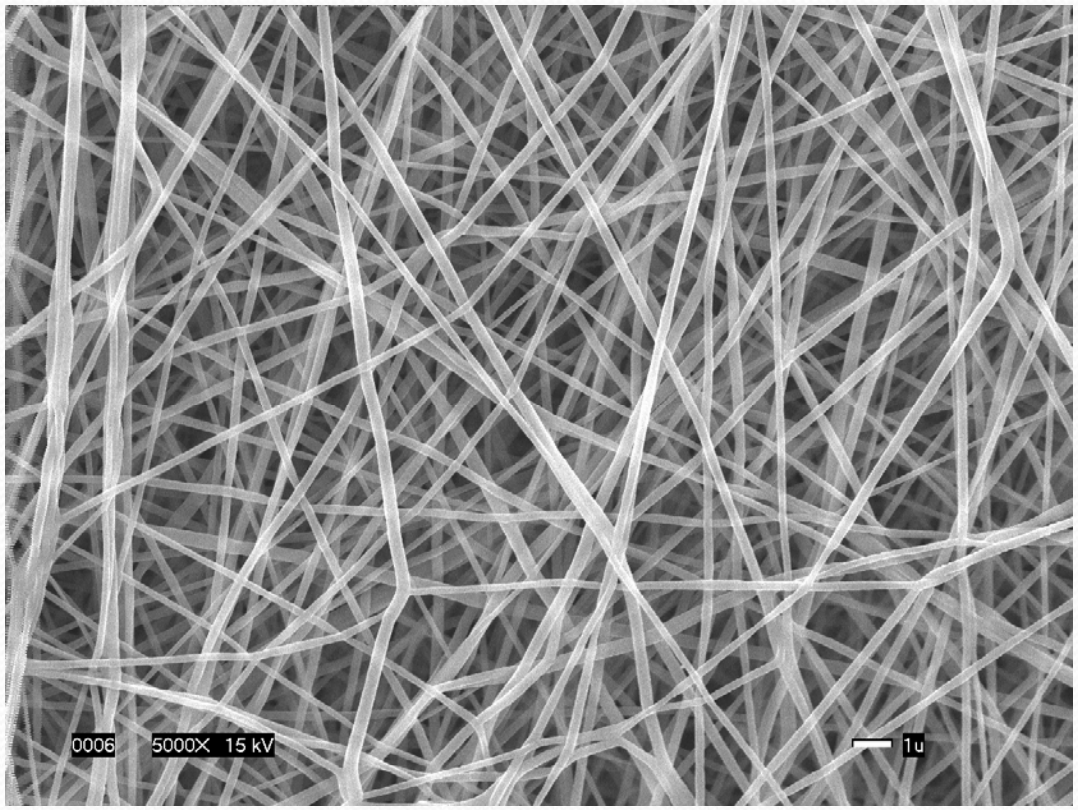
- Polycaprolactone (PCL)
- Collagen (Type I)

- Scaffolds were created using electrospinning, with glacial acetic acid and a small amount of pyridine as solvents

Co-axial Electrospinning System

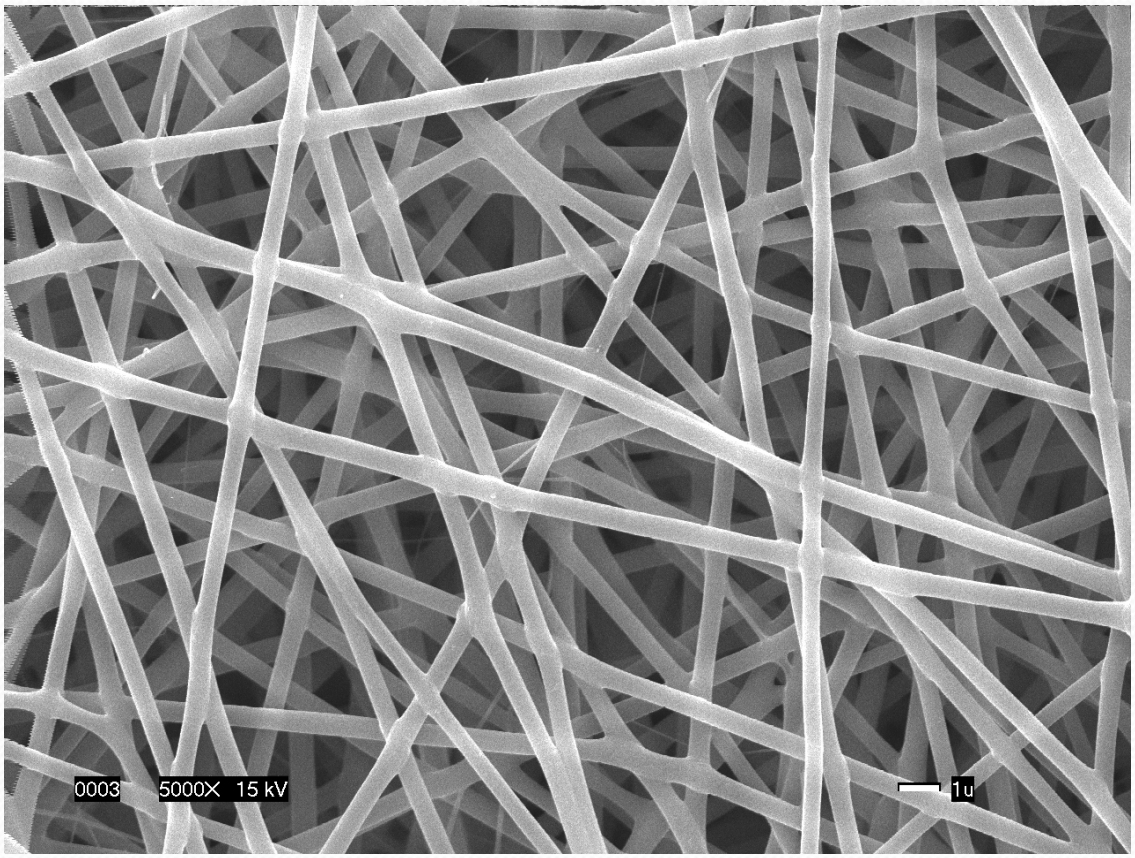


SEM Image of PCL Scaffolds



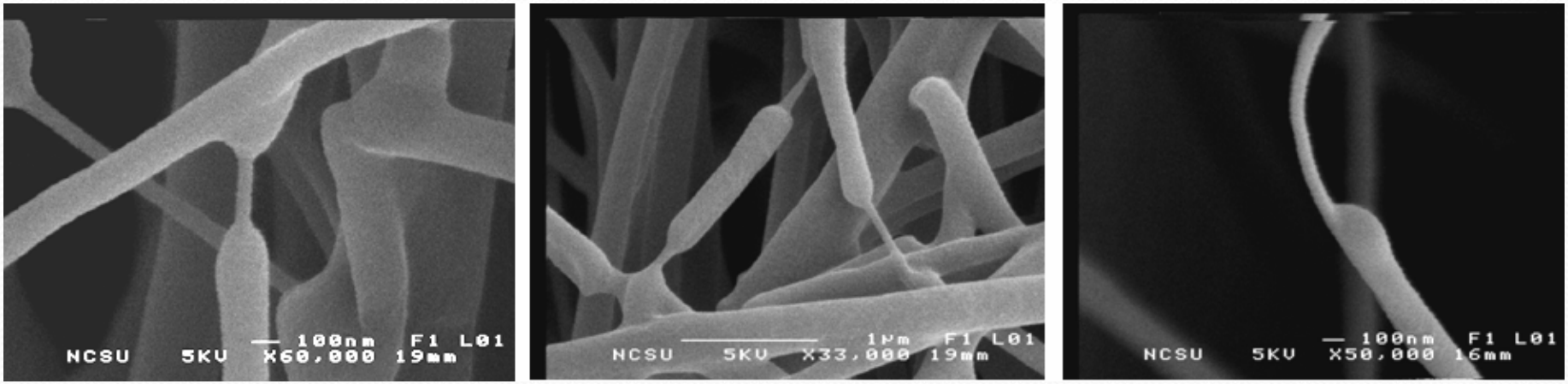
Avg fiber diameter 250 nm

SEM Image of Collagen Coated PCL Scaffolds



Average fiber diameter: 442± 45nm

SEM Image of Sheath/ Core Fibers



Freeze-fractured nanofibers (Sheath-Collagen; Core- PCL)

Methods

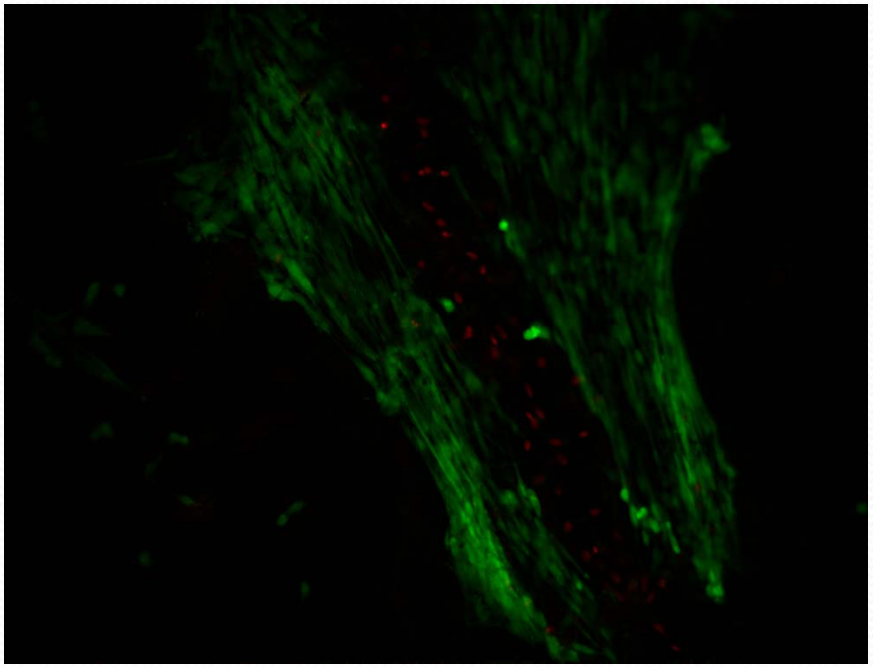
- Human Adipose Stem Cells (hASCs) from three donors were isolated and seeded at a density of 20,000 cells / cm^2
- Cells were kept in complete growth medium consisting of α-MEM, 10% fetal bovine serum, Penicillin/ Streptomycin, and L-glutamine
- To examine cell differentiation, the cells were grown in growth medium for one week, then the medium was supplemented with dexamethasone, ascorbic acid, and β-glycerolphosphate

Methods Continued

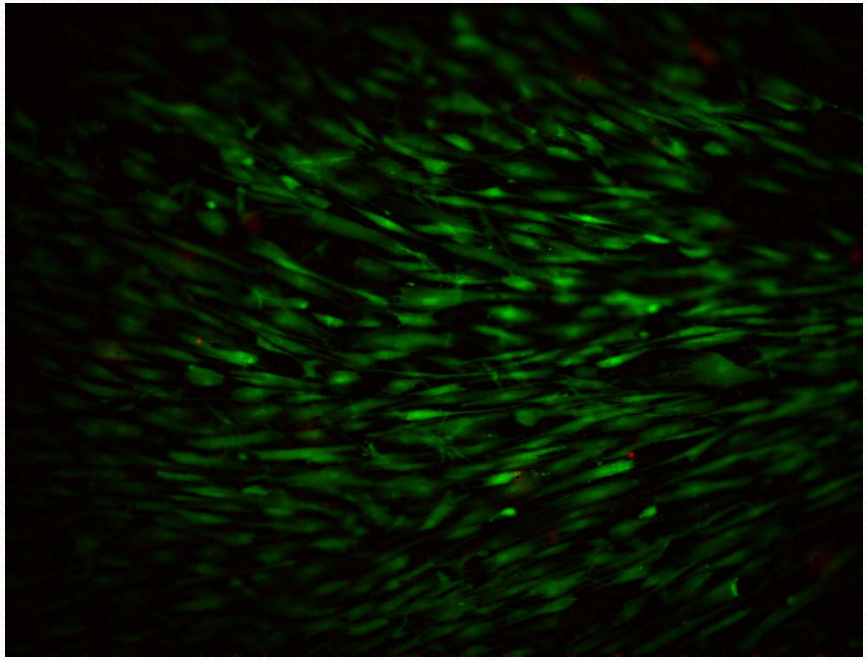
Examined:

- Cell viability (Live/Dead Stain)
- Cell proliferation (AlamarBlue)
- Differentiation (Calcium LiquiColor)

Live/ Dead Images – 72 hours

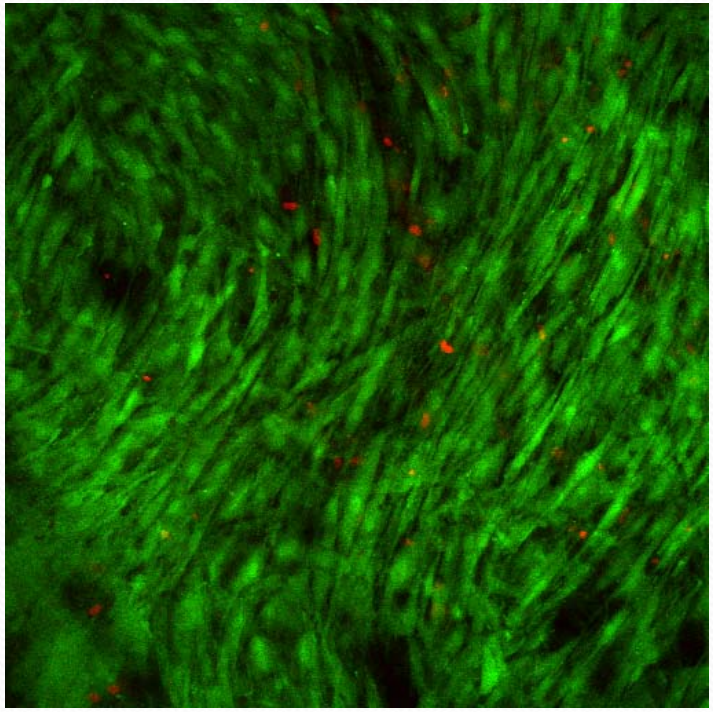


Uncoated PCL

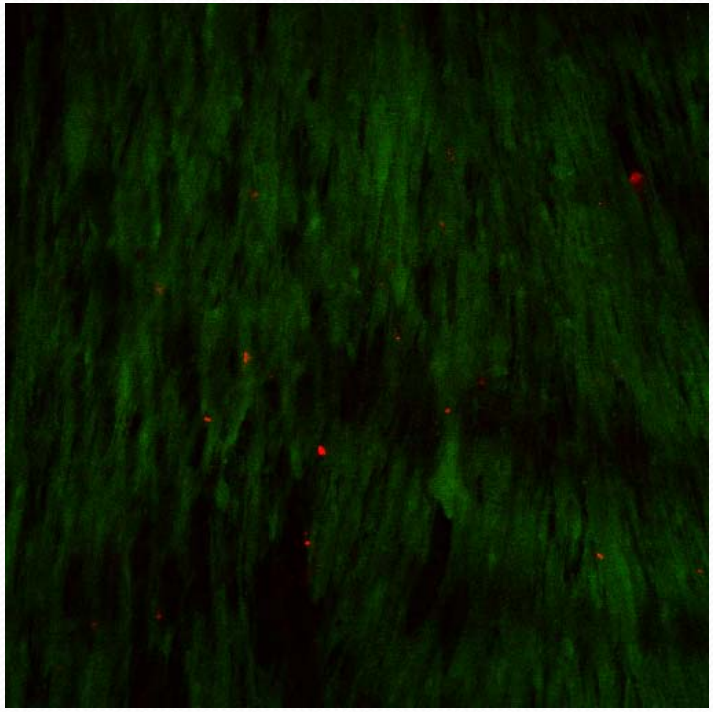


Collagen Coated PCL

Live/ Dead Images- 1 week

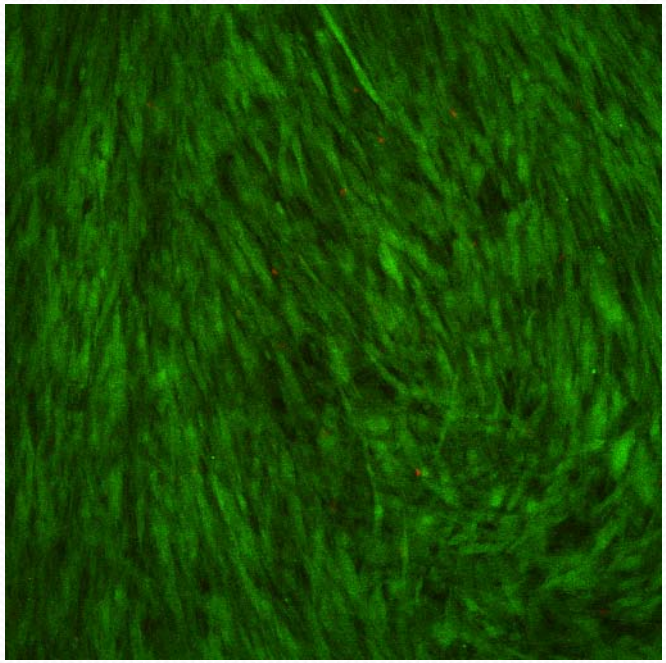


Uncoated PCL

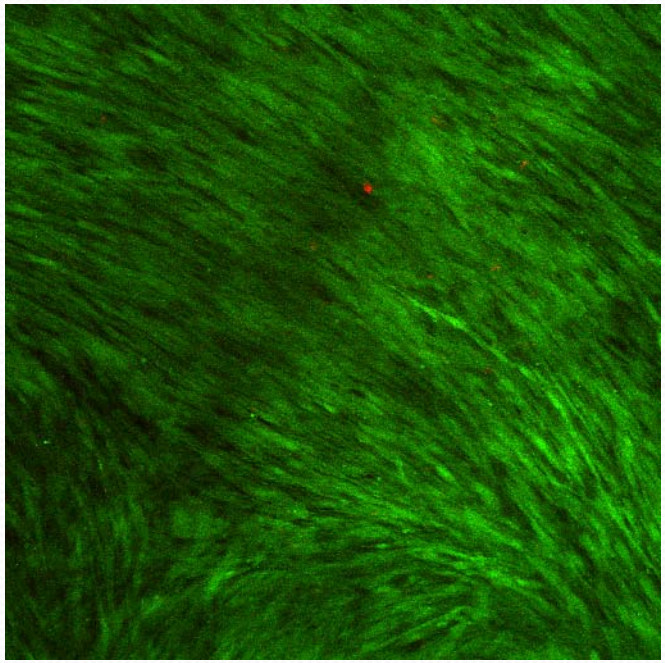


Collagen Coated PCL

Live/ Dead Images- 2wks



Uncoated PCL



Collagen Coated PCL

Live/Dead Images

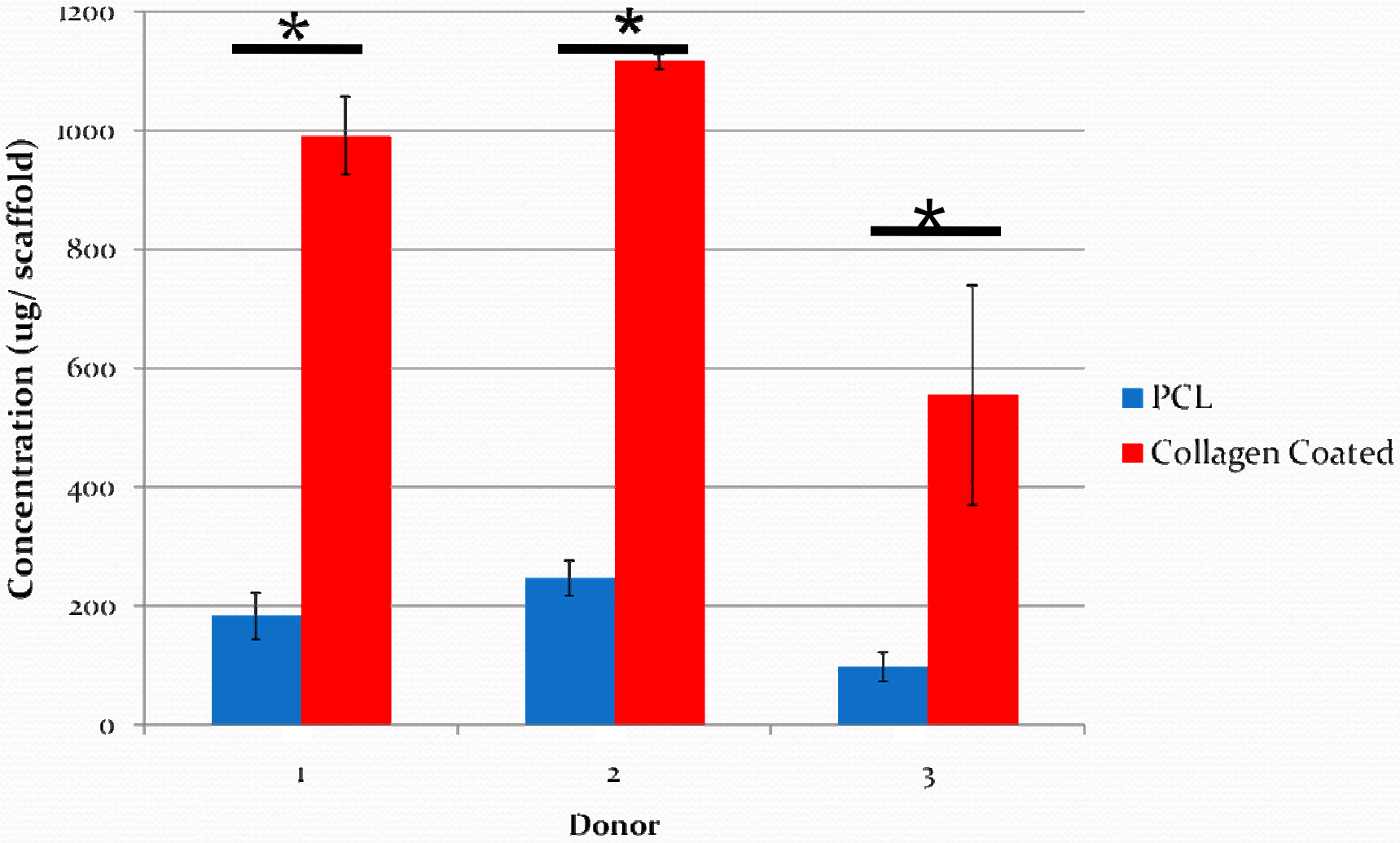
- Confocal images revealed cells that were well-spread on the scaffold surface
- Some dead cells were present on each type of scaffold
- Confirmed viable cells present after two weeks of culture in complete growth medium

AlamarBlue

Time	Average (%)
24 hrs	102.9±5.6
48hrs	104.7±5.1
72hrs	105.5±13.8
1 wk	106.1±11.5
2 wks	101.5±11.7

Comparison of Collagen Coated PCL Scaffolds to Uncoated PCL Scaffolds

Calcium Production - Day 14



Conclusions

- Viable cells on both scaffolds after two weeks in culture
- Viability and proliferation was the same on the PCL and the collagen coated scaffolds
- Increased cell spreading on collagen coated scaffolds
- Increased calcium production suggests increased osteogenic differentiation on collagen coated scaffolds

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