DAPI, Brightfield, & SEM Imaging of TCP Fiber (ReBOSSIS)

Seeded with osteoprogenitor cells
separated from human bone marrow.

March 9, 2017

ORTHOReBIRTH USA

ReBOSSIS

ReBOSSIS is a cotton like material that, when hydrated, becomes a cohesive and moldable graft that easily conforms to fit and fill voids. It was cleared by the FDA in late 2014 and commercialized in 2015.

It is a product of composite materials, including β-tricalcium phosphate (β-TCP), siloxane-containing vaterite (SiV), and poly(L-lactide-co-glycolide) (PLGA).

The microfibers of ReBOSSIS are prepared by an electrospinning method.

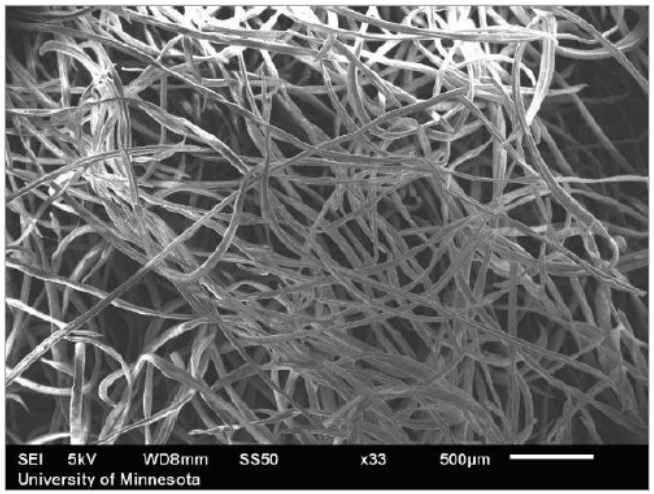
The diameter of these fibers is less than 0.25 mm and the content of silicon in ReBOSSIS is approximately 0.5-1.0 percentage by weight.

The entwined structure of its fine fibers makes ReBOSSIS an ideal scaffold for cell infiltration and colonization.

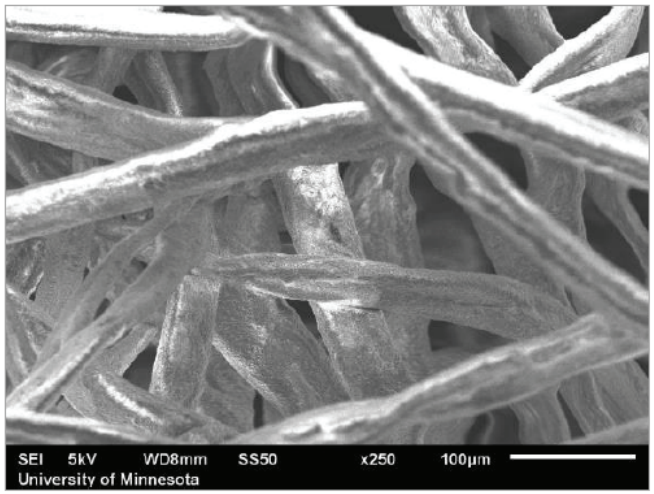
ReBOSSIS is manufactured and distributed by ORTHOReBIRTH.

SEM Native ReBOSSIS

Actual Size



33x



250x

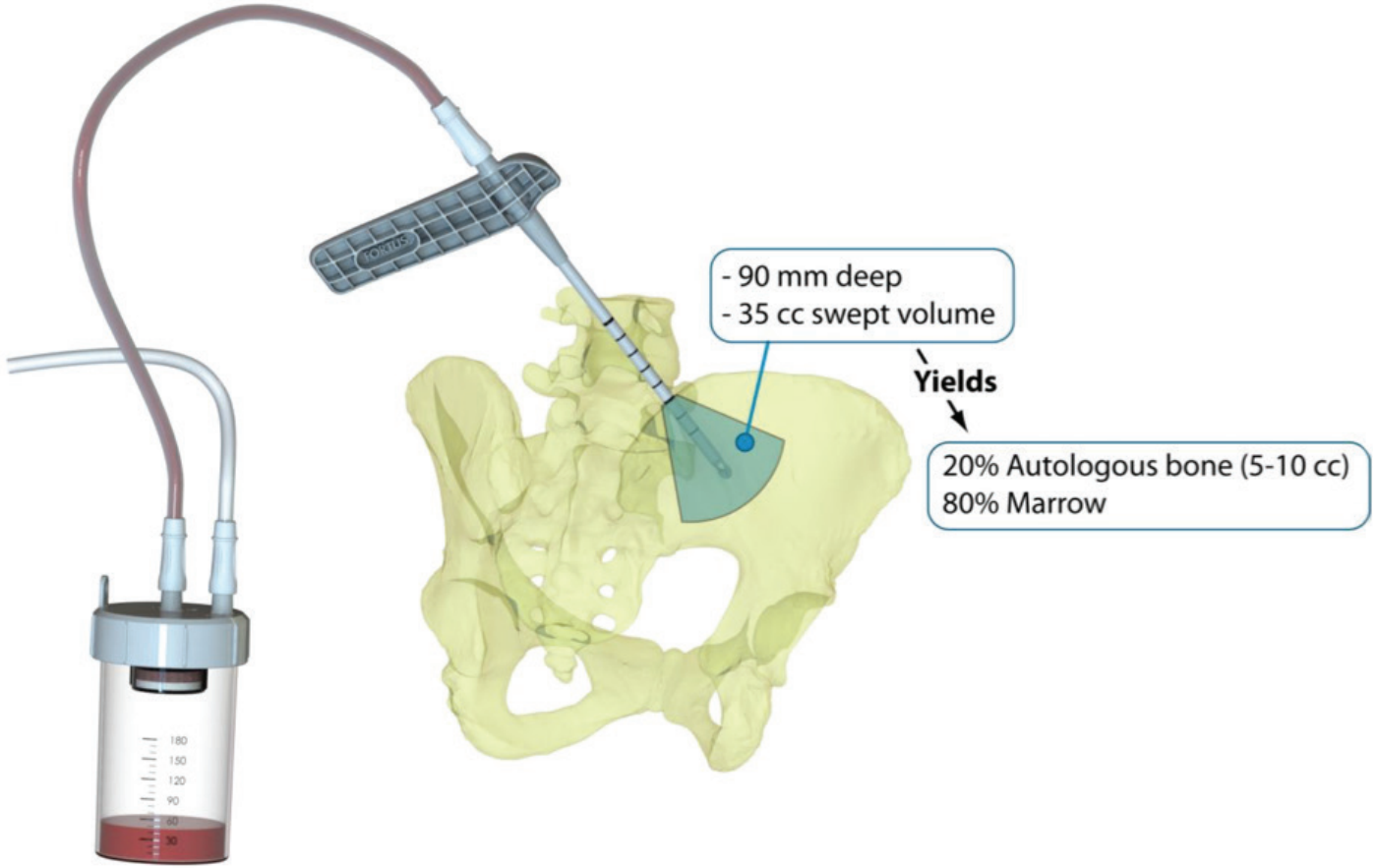
ReBOSSIS supplied and manufactured by ORTHOReBIRTH USA

Cells

- Cells were separated from human bone marrow.
- Testing was performed at Cleveland Clinic, in the laboratory of George Mushler, MD.
  (Note: Dr. Mushler has no affiliation with ORTHOReBIRTH)
- Fortus Medical supplied Red Blood Cell Separation and Processing System*

*Fortus system is not cleared by the FDA for use in the United States

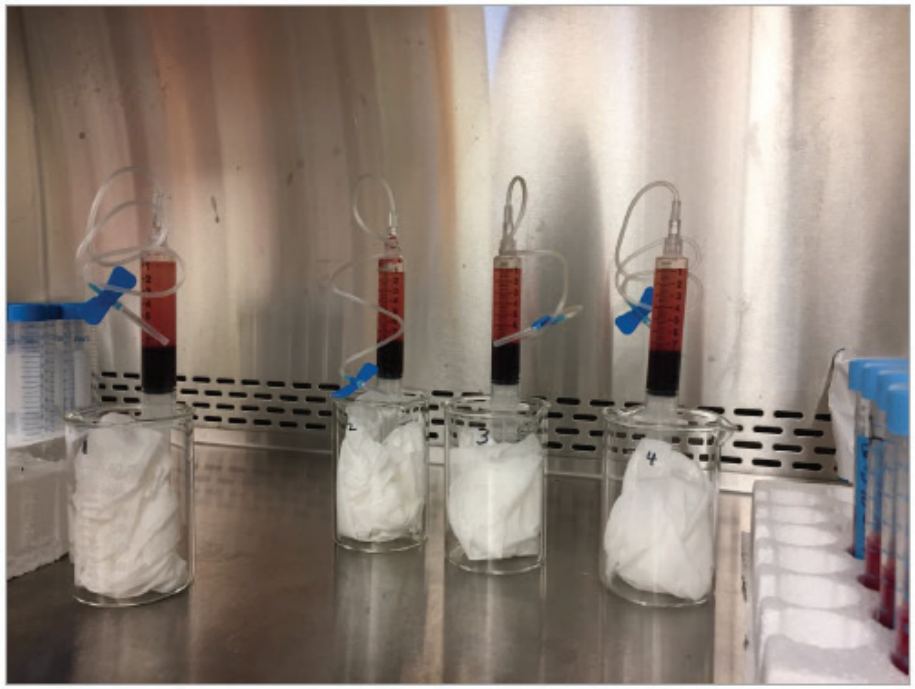
Autologous Bone & Marrow Harvesting System Including Red Blood Cell Separation & Processing by Fortus Medical



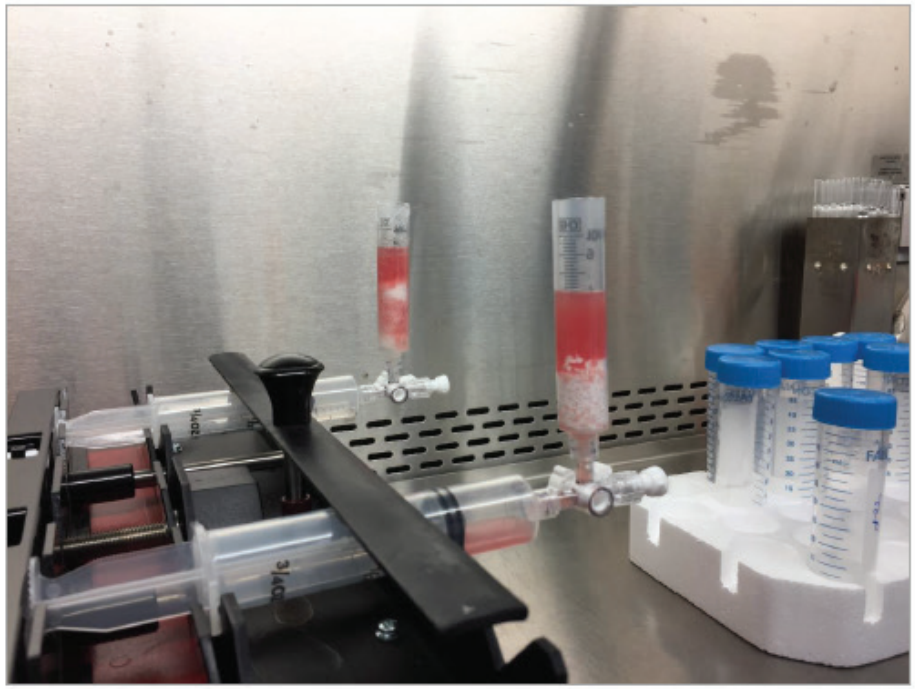
Methods

- Red blood cells were removed from bone marrow using the Fortus separation media
- Resulting progenitor-rich supernatant was divided in half
- Supernatant was drawn through TCP fiber (ReBOSSIS) and 1000-2000 mm granules at a controlled rate
- The cell-seeded graft (ReBOSSIS) was cultured for 14 days
- Viable cells were imaged by DAPI staining and Brightfield imaging
- SEM images were also acquired

Methods

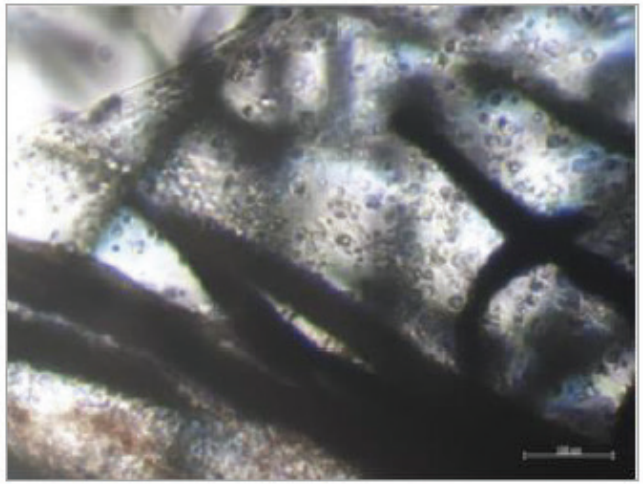
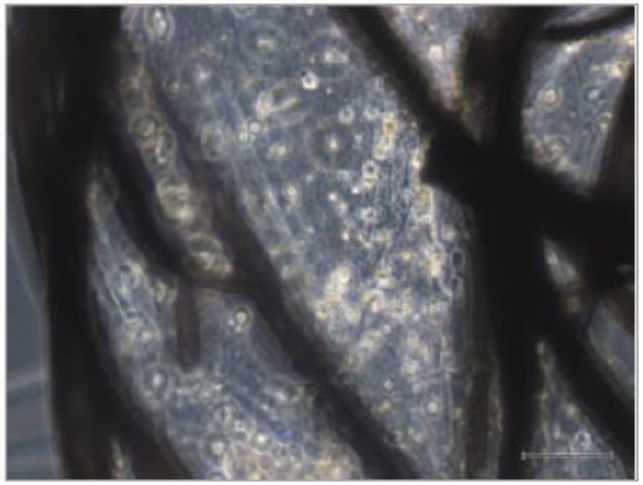
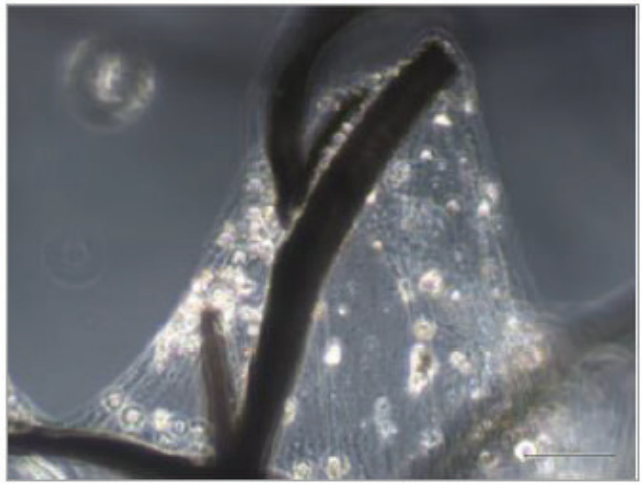


Separation of red blood cells from bone marrow



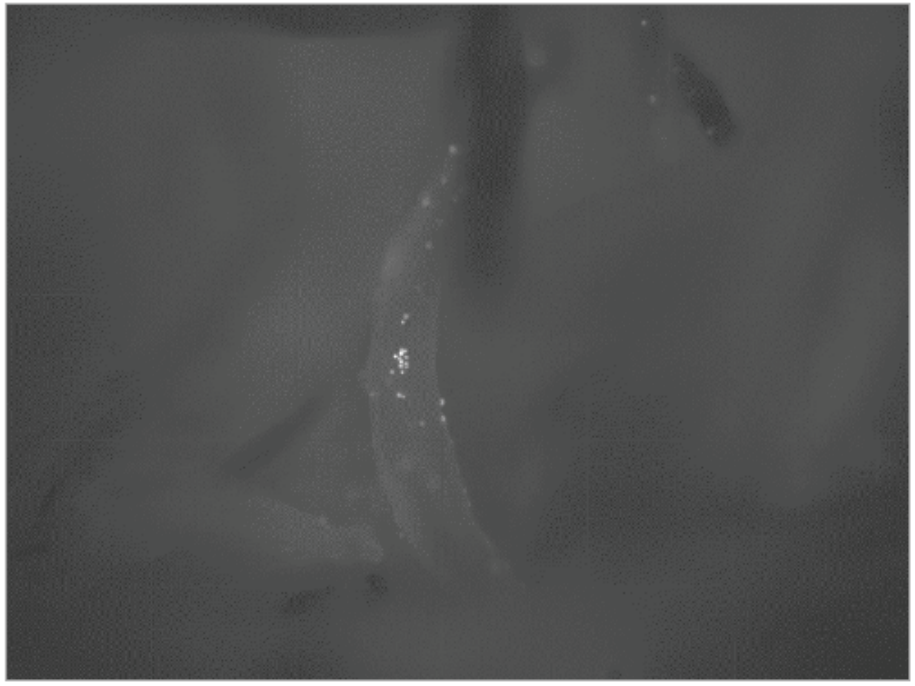
Syringe pump to draw supernatant through ReBOSSIS (rear)

ReBOSSIS Fibers: Brightfield Images – 3 days

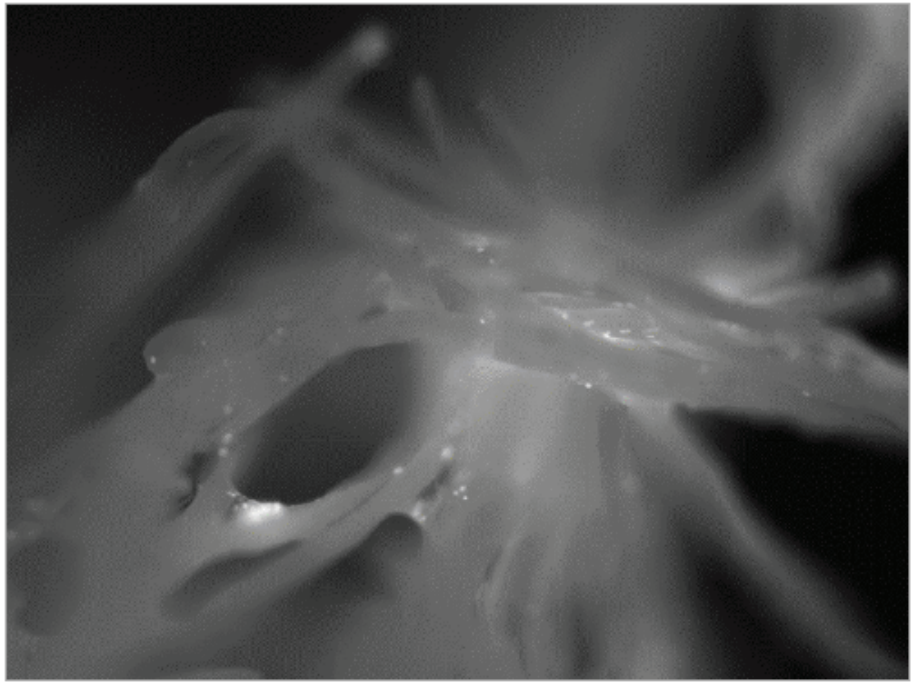




- ReBOSSIS fiber Brightfield images after 3 days in culture
- Robust cell growth
- The cells are known to be biologically active. This is evident based on the growing cell colonies. The formation of an ECM membrane and the subsequent migration of cells onto this membrane indicates the cells are on the pathway to bone formation

ReBOSSIS Fibers: DAPI staining – 3 days



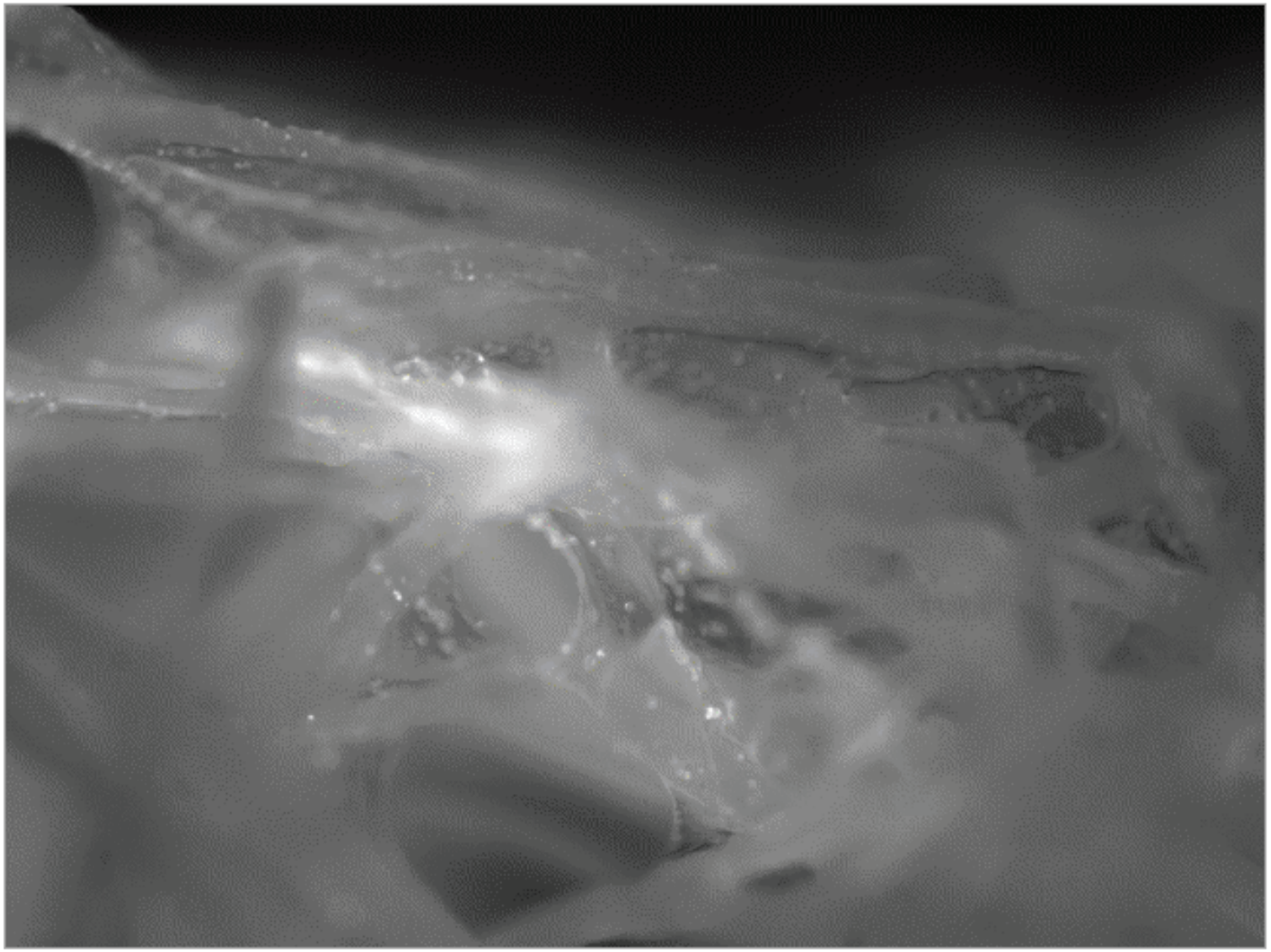
Top of graft column – first exposure area to cells



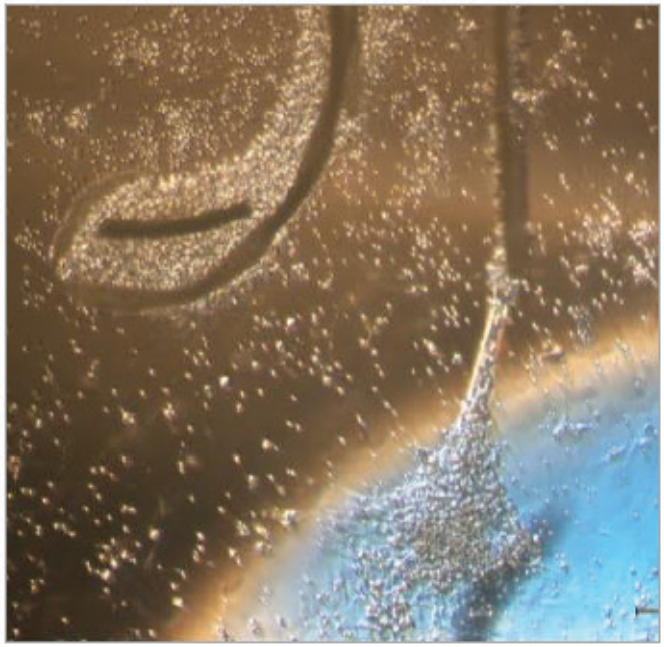
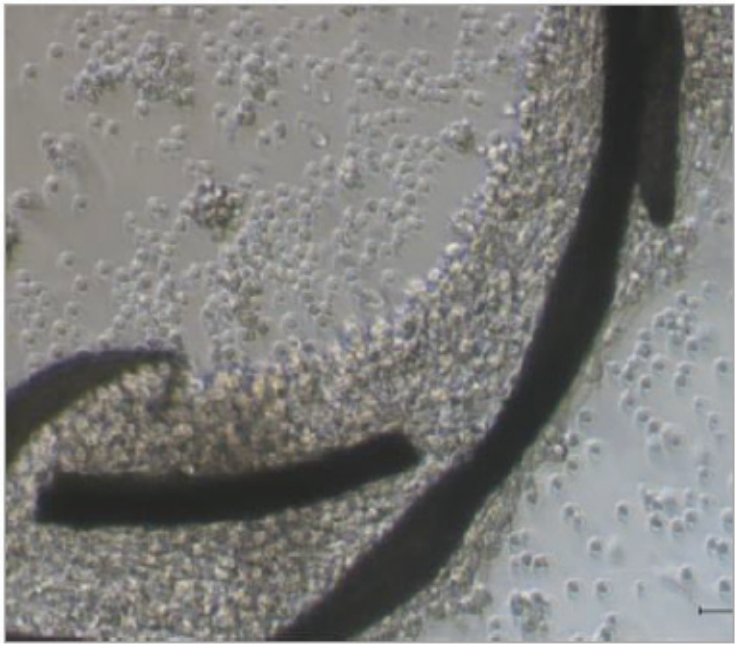
Top of graft column – first exposure area to cells

- ReBOSSIS fibers after 3 days in culture stained with DAPI

ReBOSSIS Fibers: DAPI staining – 3 days

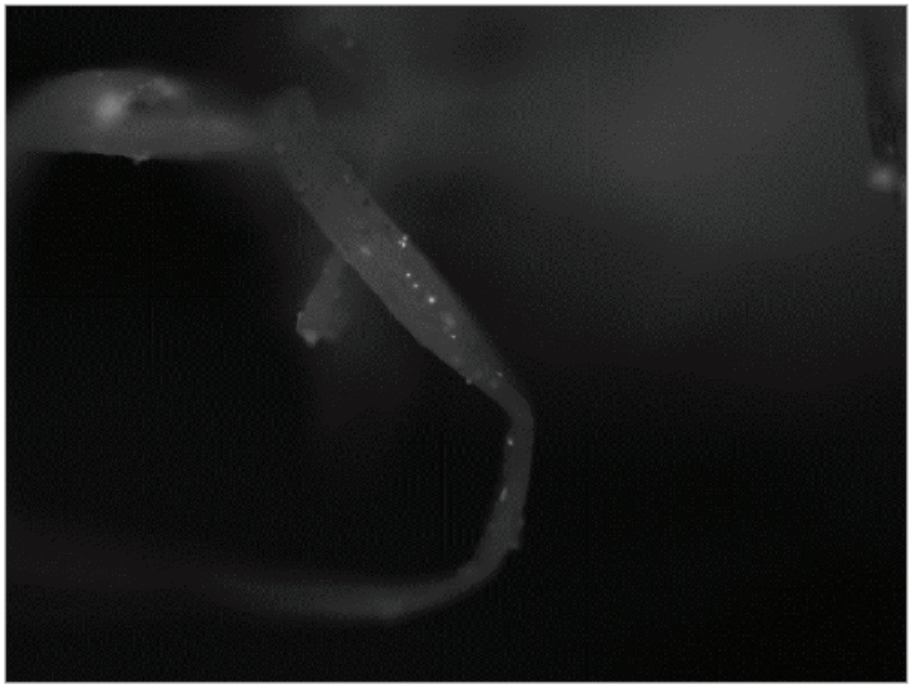


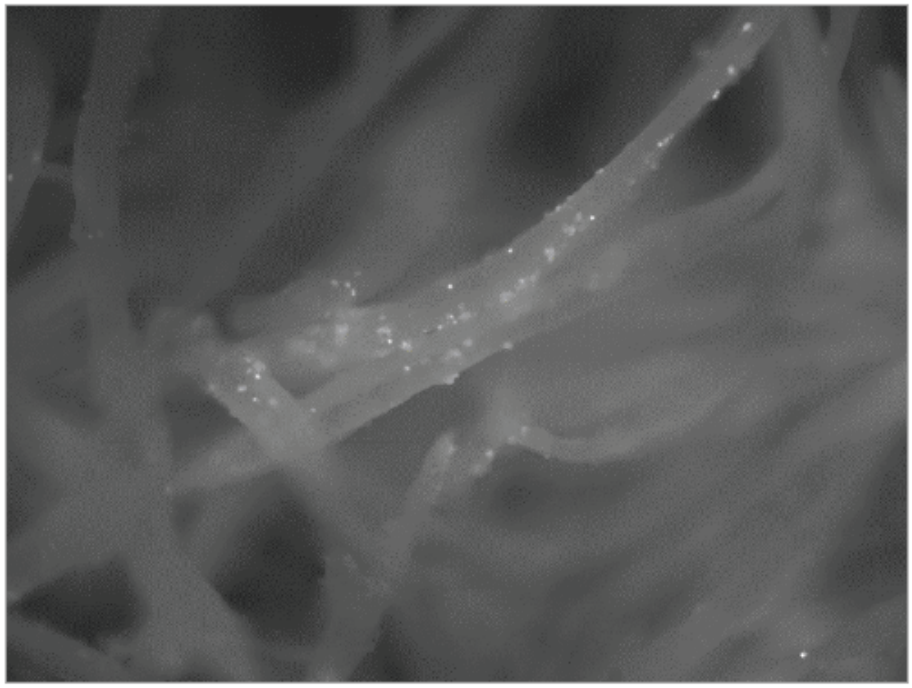
ReBOSSIS Fibers: Brightfield Images – 11 days


- ReBOSSIS fiber Brightfield images after 11 days in culture
- Robust cell growth
- A very dense concentration of cells indicates that cells are alive, growing and expanding

ReBOSSIS Fibers: DAPI staining – 14 days





- ReBOSSIS fibers after 14 days in culture stained with DAPI
- The cells are adhered, as the daily media exchange washes away any cells that are not adhered

Conclusion

SEM images and Brightfield images of ReBOSSIS at 3, 11, and 14 days indicate that ReBOSSIS supports cell vitality (ECM formation at 3 days) and enhances cell proliferation as well as cell adherence to the scaffold.