Introduction: Biomaterials that recapitulate the intrinsic architecture of *in vivo* tissue are vital for study diseases, as well as to facilitate the regeneration of lost and malformed soft tissue. We have developed a novel biofabrication technique that combines state of the art imaging, 3D printing, and selective enzymatic activity to create the new generation of biomaterials for research and clinical application. The purpose of this work is to create a new *in vitro* model that mimics the true three dimensional architecture which are created with flow channels or tissue voids that represent the *in vivo* niches. This model is composed of collagen, which is a natural and abundant extracellular component, to mimic the native architecture. To demonstrate our new technology, we have developed a 3D branched vessel using type I collagen. This scaffold can be cultured with vascular endothelial cells and smooth muscle cells. Using human renal CT angiograms, we obtain the true architectural features of the renal artery which has been implemented in our biofabrication technique. The proposed research will yield a novel fabrication technique and fill a notable technological void. These new biomaterials will enable the modulation of cell potential, and thus, accelerate discovery in the field of regenerative medicine.

Materials and Methods: We are able to recreate the 3D architecture of the tissue of interest by creating a model in Amira®. We used Mastercam Art ® and other techniques to bring the images to Mastercam®. Using the Microlution 363-S micro milling machine, we use the G-code to machine the specific features of the model into a stainless steel mold. We also used Makerbot Replicator2 3D Printer to create polylactic acid (PLA) molds. The BSA rubber is reaction injection molded into the geometries created in the mold. Our prototype is composed of collagen type I at a concentration of 14 mg/mL casted around this BSA mold which was selectively enzyme digested away leaving the inner flow paths within the scaffold.

Results and Discussion: We have successfully determined the adequate BSA and glutaraldehyde ratio that will uphold the intrinsic geometry. We created a branched architecture and we machined a stainless steel mold as seen in figure 1. We reaction injected 30% BSA and 3% Glutaraldehyde in a 4:1 ratio to the stainless steel mold. After 24 hr, we release the BSA rubber and casted 14 mg/mL collagen concentration. Using trypsin, this rubber was digested and left specific channels. We also fabricated molds that mimic the human renal artery.



Figure 1: On the left, is the solid created in Mastercam with an inflow tract diameter of 4 mm and outflow of 3 mm each. On the right, is a stainless steel mold created using the Microlution.

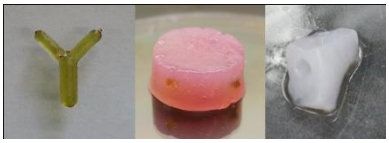


Figure 2: On the left is the BSA rubber made using the mold in figure 1. The center shows the rubber embedded in the collagen hydrogel. On the right, we can see the channels left within the collagen scaffold after the rubber was enzyme digested.

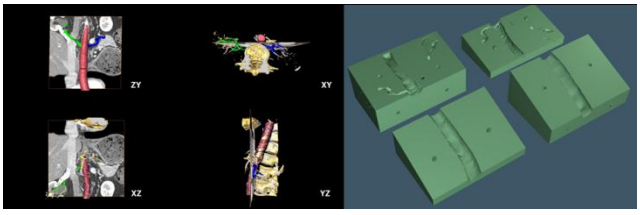


Figure 3: Renal Artery. We used de-identify Renal CT Angiography images from healthy human donors. The left image shows the ortho slices of the Renal CT Angiograms with the Amira 3D model reconstruction. Using the Makerbot, we created a mold based on the model as seen on the right.

Conclusion: Previous studies and our data using natural ECM components suggest that the mechanical environment plays a significant role in the cell behavior. Using this biofabrication technique, we can create3D collagen hydrogels that closely mimic the native tissue. We can create a specific architecture as well as obtain the specific dimensions of the native architecture using 3D models such as the renal artery model. By recapitulating the *in vivo* tissue niche and adopting the precise architecture, stiffness, and cell population, we can obtain an ideal *in vitro* model to further study of malformation and diseases.