Verslag Buitenlandse Stage Boston – Alix Matton

During my project at Dr. Korkut Uygun's research laboratory at the Center for Engineering in Medicine, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, USA, I learned a lot. The initial project that I went there for did not succeed unfortunately, though I did initiate and complete another study successfully instead.

The first months were spent obtaining extrahepatic bile duct segments from human donor livers that were rejected for transplantation and attempting to isolate cholangiocytes from them. Based on the rat cholangiocyte isolation protocol that was already being used at the institute, I tried to isolate human cholangiocytes. Many cultures did not have high enough yields, as was expected as more than 90% of donor livers that are transplanted have severely injured or absent biliary epithelium, and many have injured peribiliary gland cells. Logically, discarded livers were of even lower quality and result in even lower yields. After contact with Eliane Wauthier, a renowned researcher in this field from North Carolina, several protocol modifications were made. Eventually, six different batches of cells were grown and cultured, some for up to several months. In the meantime, I designed the Amnis Imaging Flow Cytometry panels to test the isolated and cultured cells for the following antibodies: LGR5, EpCAM, SOX2 (stem cell), CD62p (endothelial cell), ASGR1 (hepatocyte), anti-fibroblast, secretin receptor (cholangiocyte), aSMA (smooth muscle cell), MAP2 (neuron), Ki-67 (proliferation), SOX17 (cholangiocyte precursor) and AFP (hepatocyte progenitor). All antibodies had to be tested first, which was done using cell lines (cells have to be in suspension to be run in the Amnis Flow Cytometer). Many cell lines, however, had to be ordered and cultured for several weeks as they were not readily available through the research institute. Not all cell lines worked and antibodies therefore had to be changed again, some taking up to 6 weeks to arrive. Next to that, many problems were encountered with the antibodies themselves when run in the Amnis. The fact that many markers were intracellular meant that many first required permeabilization, which resulted in strange unusable staining patterns in the Amnis. In the end, it was impossible to create the appropriate panels for the Amnis and attempts were done to run the antibodies one by one instead of in panels, but there were insufficient cells for this many runs. Running the Amnis one by one instead of in panels also took much longer, for which reason I returned in July to try and finish the running experiments. In the end, unfortunately this project was left unfinished and will hopefully be picked up again by another colleague, advisably after first having gained experience at a research center with more expertise in this particular field.

As I had encountered so many problems with the initial project, I initiated another project (aside from helping many researchers with their own machine perfusion projects). Worldwide, no consensus has been reached regarding the optimal flushing and preservation solution for protecting against biliary injury during preservation. Again using livers that were discarded for transplantation, sixteen extrahepatic bile ducts were dissected and cut into six equal segments, flushed and preserved in five different ice cold preservation solutions for a median of 8 hours. Study groups were University of Wisconsin (UW) solution, UW + PEG15-20 solution, histidine-tryptophan-ketoglutarate (HTK) solution, HTK + PEG15-20 solution, and HTK + PEG35 solution. Histological bile duct injury was assessed using a clinically relevant scoring system. Results suggest that HTK solution was worse than UW solution in protecting the biliary tree. The addition of PEG15-20 and PEG35 to HTK resulted in slightly less, but non-significant, histological injury. The addition of PEGs to preservation solutions may be a readily implementable and affordable method to protect the biliary tree and warrants further investigation. The manuscript is currently being prepared for submission.