Using laboratory µCT for assessing peripheral nerve regeneration

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INTRODUCTION: Peripheral nerve injuries are increasing in number, with approximately 300,000 cases reported annually in Europe. With the existing therapeutic interventions, axonal regeneration frequently remains challenging and the functional outcome is unsatisfactory.[1] Research on biodegradable nerve conduits (NC) gained increasing importance. Thus, different materials are used with or without growth promoting agents. To assess various approaches, the nerve regrowth must be visualized inside the NC at different time points[2] This task is usually performed after standard histological preparation and immunohistochemistry, requiring dedicated equipment and considerable expertise. Resulting data are two-dimensional, consequently volume measurements are associated with sampling error and often require time-consuming serial sectioning. We thus tested a micro computed tomography (µCT) laboratory system as a complementary approach for fast and reliable imaging and quantification of nerve regeneration through the NC. Additionally, µCT is a powerful tool to select the histology cutting plane for validation purposes.

METHODS: The µCT laboratory system used was the phoenix nanotom®m (phoenix|x-ray, GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany), operating at the Biomaterials Science Center at the University of Basel. It is equipped with a 180 kV source and a 3072×2400 pixels detector. The test specimen was prepared from a collagen NC implanted in a rat sciatic nerve gap model. Following formalin fixation and paraffin embedding, the NC-nerve complex was scanned. A voltage of 60 kV and a current of 310 mA were selected for the X-ray beam. In a range of 360º, 839 projections were acquired. Scanning time was less than 15 minutes. The reconstructed dataset had an effective pixel size of 27.6 µm. It was filtered with a median filter and a histogram-thresholding segmentation was then performed, using the VGL Studio Software (Volume Graphics GmbH, Germany).

RESULTS: A time-efficient scan reveals all structures of interest. Before filtering, NC and nerve dimensions can be measured manually. After filtering, the collagen tube, the nerve inside it and the surrounding paraffin have sufficient contrast for fully automatic intensity-based segmentation and volume measurements, as indicated in Figure 1. Scanning parameters will be optimized so that the entire nerve can be correctly segmented.

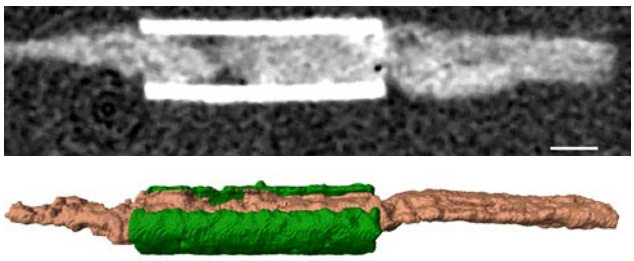

Fig. 1: The µCT scan results indicate the possibility of quantifying the growing nerve inside the collagen tube. A virtual slice of the initial, unfiltered dataset (top). The scalebar is 1 mm in length. A three-dimensional rendering of the same dataset after filtering (bottom). Region-growing segmentation allows for the three-dimensional visualization of the rat sciatic nerve (brown) inside the collagen tube (green). The surrounding paraffin has been made transparent.

DISCUSSION & CONCLUSIONS: The µCT laboratory system nanotom®m is a potent tool for the quick, non-destructive imaging of specimens and retains absolute compatibility with the established protocols. Parameters need to be adjusted for each application, after which, high automation is possible. Currently, the critical step for the full integration of such laboratory µCT systems is the increased collaboration of imaging specialists with groups working on the field of biomedical engineering and regenerative medicine.

REFERENCES: [1] EO. Johnson et al (2005) Injury **36(4):** 24-9 [2] S. Madduri et al (2010) Biomaterials **31**:8402-8409.