Abstract

Despite extensive research and the implementation of modern surgical methods, the incidence of anastomotic leaks remains high. The exact pathophysiological mechanisms responsible for these leaks are still unknown, making prevention a challenge. The use of animal models is extensive in the research of intestinal anastomotic leakage. The literature review of this thesis, therefore, presents a brief history of the usage of experimental animals as well as major differences in terms of anatomy and histology between humans and the experimental mammals utilized in intestinal healing studies. The thesis is based on four studies describing the possibilities of using quantitative histological methods in different applications, animal models, and experimental surgical techniques in the research of intestinal anastomotic healing. The conclusions of these four studies can be summarized as follows:

Conclusion 1: TeIGen software is readily accessible to scientists researching fibrous and porous materials using micro-CT analysis. This software aids in producing virtual image sets with defined morphometric attributes, and it can also help with fine-tuning of quantification tools required to examine micro-CT scans. The software also enables the identification of material morphological properties and image characteristics in micro-CT scans that may compromise the results of quantitative analysis. TeIGen software's capacity to generate virtual image sets significantly bolsters the reliability and conclusiveness of measurements, thereby elevating the research's value and reproducibility.

Conclusion 2: Histological research can benefit from the efficient use of scanning conventional slides in digital form combined with unbiased sampling of regions of interest. By adhering to unbiased random sampling principles, researchers follow the "do more less well" rule, leading to more valid, ethical, and efficient results. Digital microscopy has proven particularly useful for proper sampling at the slide level. To attain uniform distribution, one may opt to scan entire slides and carefully select fields of view according to a chosen pattern, or alternatively, use a motorized stage microscope. Such principles are not limited only to quantitative research, but are also crucial in qualitative studies, as they enhance both precision and replicability of the research. Our discoveries indicate that it is imperative for all motorized microscopes and digital histology slide scanners used for scientific purposes to incorporate support for unbiased random sampling.

Conclusion 3: Despite the promising potential of biodegradable nanomaterials, their use in the field of intestinal anastomoses remains largely uncharted. We have found out, that administering of nanomaterials in anastomoses in the small intestine of an animal model did

not impede normal healing. A standardized animal follow-up procedure post-surgery was established, completed with a new perianastomotic adhesion scoring system. Furthermore, a robust combination of histological and stereological methods was employed to assess healing. While nanomaterials appear safe, their efficacy in improving anastomosis healing requires further investigation.

Conclusion 4: Through a quantitative histological mapping of various factors, we have discovered the following about porcine small and large intestinal anastomoses: (i) the anastomosis had a higher proliferation index, microvessel density, and collagen volume fraction, but not neutrophil volume fraction; (ii) the healing of porcine small and large intestinal anastomoses was not interchangeable, with the presence of the experimental defect significantly affecting healing despite the healing process being complete at 21 days after the surgery; and (iii) proximity to the anastomosis had a more significant impact on healing in the small intestine than in the large intestine.

To summarize the conclusions of the literature review and presented studies, automated software used in image data analysis must undergo proper calibration. Stereology is a reliable calibration method that can help with validating such data. Our review paper on digital microscopy procedures and multilevel sampling strategies gives practical recommendations and considerations for optimal quantitative histological assessment. Clinical research projects involving surgery of experimental animals are essential to yield reliable results that can be translated into human medicine. The significance of such studies is highlighted in the experimental healing of intestinal anastomoses.