Quantifying Tissue Shrinkage Throughout Histological Processing for Cutaneous, Parenchymal and Luminal Tissues

Shrinkage of tissues following excision and histological processing is a well-known phenomenon. However, in veterinary medicine, it is poorly quantified, particularly for internal organs.

In many circumstances, surgery is the primary treatment modality for cancer in veterinary patients. The success of surgical oncology is often based on the presence of complete or incomplete margins assessed on histopathology. However, if tissue shrinkage is significant during histological processing, then the clear margins reported by the pathologist will be less than in-situ. In addition, studies based on retrospective histopathological findings may recommend surgical margins that are too conservative. For these reasons, the aim of this study was to quantify tissue shrinkage more accurately during histological processing in cutaneous tissue, and the major thoracic and abdominal organs.

Four clinically normal ewe cadavers were extensively sampled at post-mortem examination. Samples were obtained from nine cutaneous bilateral locations to assess the effect of sample location on shrinkage. Half of the cutaneous samples were left freely floating in formalin, while the other half were stuck to cardboard during fixation. Five parenchymatous organs (the lungs, heart, liver, spleen, and kidneys) and ten luminal organs (entire gastrointestinal tract, uterus, and bladder) were also sampled. Samples were taken at different lengths to assess whether this correlated with overall shrinkage.

All samples were fixed in formalin, dehydrated, embedded into paraffin wax, and then mounted on to slides and stained with Haematoxylin and Eosin. Measurements were taken at five different time points: in situ (T0), post excision (T1), after 24-48 hours fixation (T2), post paraffin wax embedding (T3), and on slide, post H&E staining (T4). A total of 432 skin, 200 parenchymatous, and 200 luminal organ samples were collected and measured.

Shrinkage for skin was calculated from T0-T4, and T1-T4 for parenchymatous and luminal organs. Skin sample length was found to shrink by 20.75% in freely floating samples and 11.32% in samples fixed on cardboard. Parenchymatous organs on average shrank by 16.92%, and luminal organs by 14.35%. Interestingly, almost every organ showed a pattern of minor shrinkage after formalinfixation, most shrinkage then occurring after dehydration and paraffin wax embedding, and sample length increase after H&E staining. This trend is shown in *figure 1* and *figure 2*. Overall, each sample group showed significant shrinkage from T0/T1-T4.

This study demonstrates the importance of quantifying tissue shrinkage for surgical oncology. A 20% error in the margins used during surgery could be the difference between complete and incomplete margins. In order to create more evidence-based guidelines for excisional tumour margins, a good starting place is to fully quantify tissue shrinkage in domestic species.

Figure 1. Box plot of percentage Length change of parenchymatous organs compared to postexcisional length at time points T2-T4



Figure 2. Box plot of percentage length change of luminal organs compared to post-excisional length at time points T2-T4



Explanation for Names Listed

Due to the extensive nature of the methodology in this project, it was carried out by two veterinary students, myself (Murray Burns) and Mina Lee. We are very hopefully you will select our project to present, and if possible, we would like to present the research as a pair. However, if the presentation format only accommodates one student, then we would be happy to present individually.