From Scalpel to Slide: The Effects of Air Exposure and Histologic Processing on Surgical Margin Assessment in a Murine Model

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Background: Accurate measurement of surgical margins is essential to confirm complete tumor excision and to guide appropriate postoperative oncologic treatment. However, post-resection tissue shrinkage, resulting from factors such as air exposure and histologic processing, can lead to underestimation of true margins. This distortion may contribute to overtreatment with unnecessary adjuvant therapies. This study aimed to evaluate the impact of various post-resection factors on the accuracy of margin measurement.

Methods: Seventeen euthanized mice had their arms, thighs, and legs dissected. Specimens were either processed immediately or exposed to ambient air for three or 24 hours. Micro-CT imaging was performed on arm specimens to measure cross-sectional muscle area and calculate radius at humeral mid-diaphysis. Lower limbs were processed using frozen sectioning, paraffin embedding after formalin fixation and EDTA demineralization, or MMA embedding with or without demineralization. Muscle fiber cross-sectional areas were measured from histology sections using ImageJ. Statistical analysis was performed using GraphPad PRISM version 10.5.0.

Results: Arm mass decreased by 15.7% at three hours ($p < 0.0001$) and 57.3% at 24 hours ($p < 0.0001$). Thigh and leg specimens showed similar time-dependent reductions in mass. Micro-CT analysis revealed a 25.5% decrease in muscle area at three hours ($p = 0.0199$) and 67.3% at 24 hours ($p < 0.0001$), corresponding to reductions in calculated radius of 14.2% and 43.2%, respectively. Differences were observed in muscle fiber area of paraffin embedded specimens only at 24 hours ($p=0.0178$). No differences were observed in muscle fiber area between frozen vs paraffin embedded thighs at zero, three, or 24 hours ($p=0.4848$, 0.3095, 0.4206) or between non-demineralized and demineralized legs (2261.4 vs 1940.4 um^2, $p= 0.0649$).

Conclusions: Air exposure causes significant tissue and margin shrinkage. Early changes likely result from interstitial fluid loss rather than muscle atrophy. Clinical processing and demineralization do not likely affect margin size. Adjustments for air exposure are therefore necessary to improve margin accuracy and provide appropriate post-surgical cancer patient care. Future research will confirm these effects in clinical specimens.