From Scalpel to Slide: The Impact of Air Exposure and Histologic Processing on Surgical Margins

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Significance & Hypothesis/Techniques: Accurate measurement of surgical margins is essential to confirm complete tumor excision and guide postoperative oncologic treatment. However, post-resection tissue shrinkage from air exposure and histologic processing may systematically underestimate true margins. This distortion may contribute to overtreatment with unnecessary adjuvant therapies. We hypothesized that air exposure results in rapid, clinically meaningful tissue contraction that alters margin assessment. This study evaluated the impact of post-resection handling and processing on margin accuracy.

Innovation: This study demonstrates that surgical margin measurements may be substantially underestimated due to rapid tissue shrinkage following air exposure. Quantifying this effect introduces a previously underrecognized, modifiable source of error in margin assessment with direct implications for surgical decision-making and adjuvant therapy selection.

Approach: Seventeen euthanized mice had their arms, thighs, and legs dissected with skin removed. Specimens were either processed immediately or exposed to ambient air for three or 24 hours. Micro-CT imaging of arm specimens was used to measure cross-sectional muscle area and calculate radius at the humeral mid-diaphysis. Lower limbs underwent frozen sectioning, standard-of-care paraffin embedding following formalin fixation and EDTA demineralization, or MMA embedding with or without demineralization. Muscle fiber cross-sectional area was quantified using ImageJ. To enhance clinical relevance, human soft tissue sarcoma resections were weighed immediately and after three hours of air exposure. Statistical analysis was performed using GraphPad PRISM.

Results: Arm mass decreased by 15.7% at three hours ($p < 0.0001$) and 57.3% at 24 hours ($p < 0.0001$), with similar time-dependent reductions observed in thigh and leg specimens. Micro-CT demonstrated 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 in muscle fiber area were only observed at 24 hours in paraffin-embedded specimens ($p = 0.0178$). No differences were identified between frozen versus paraffin-embedded thighs at any time point ($p = 0.4848$, 0.3095, 0.4206) or between demineralized and non-demineralized legs (2261.4 vs 1940.4 μm^2, $p = 0.0649$). In human specimens ($n = 4$), median mass decreased by 4.23% at three hours and trended toward significance ($p = 0.12$).

Discussion & Impact: Air exposure results in rapid, measurable tissue contraction that can lead to clinically meaningful underestimation of surgical margins. Early changes are likely driven by interstitial fluid loss rather than true structural shrinkage, while histologic processing and demineralization appear to have minimal additional impact. These findings identify time-to-fixation as a modifiable variable in margin assessment. In practice, delays on the back table or during grossing may artifactually convert negative or adequate margins into “close” margins, potentially altering adjuvant treatment decisions. Standardizing rapid specimen handling and fixation may improve margin accuracy and reduce unnecessary escalation of care. Ongoing studies are focused on expanding the human specimen cohort to improve statistical power and validate these findings in clinically relevant samples.

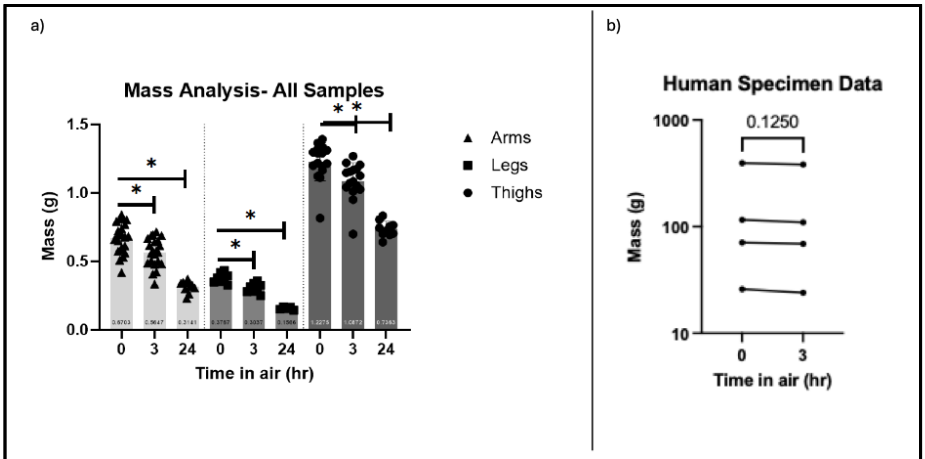

Figure 1. Changes in mass over time seen in mouse samples (a) and human tumor samples (b). * indicates statistical significance.

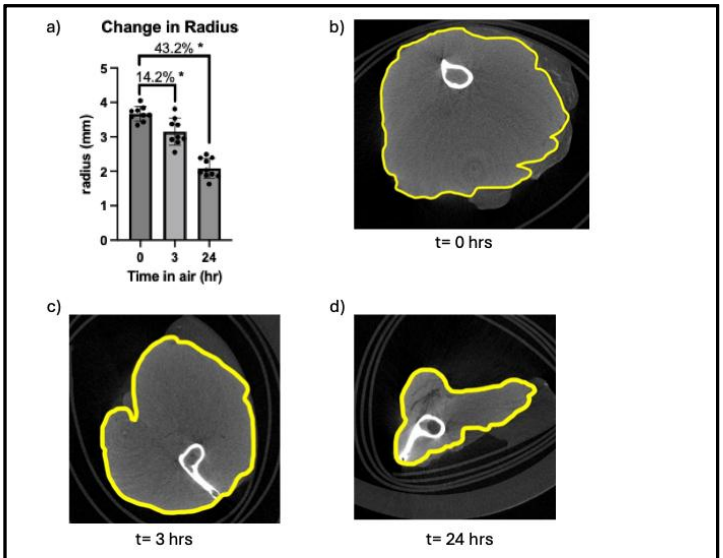

Figure 2. Changes in muscular radius over time (a) with representative uCT images (b-d). * indicates statistical significance.

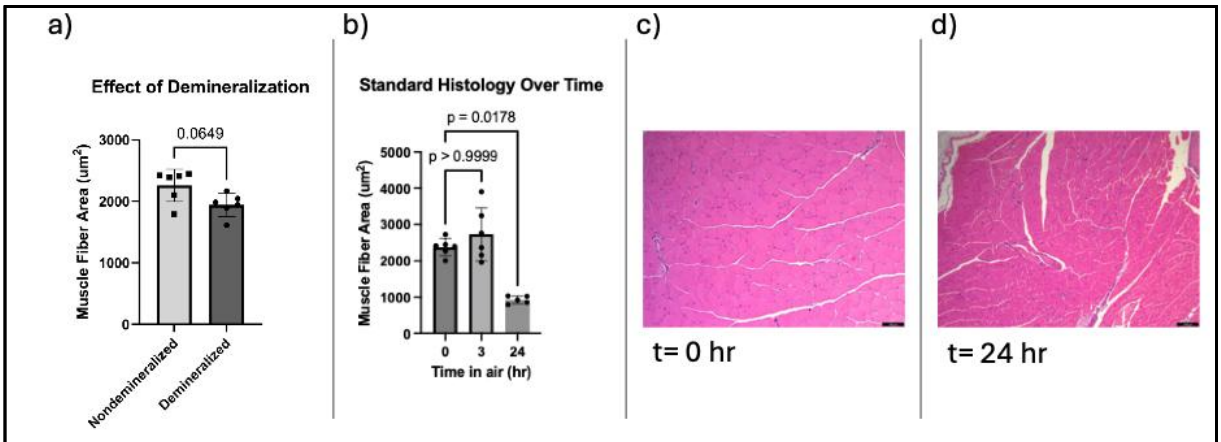

Figure 3. Results of demineralization (a) and standard-of-care (formalin fixed paraffin embedded and demineralized (EDTA)) histology over time (b) with representative standard-of-care histology images (c and d).