Comparison of Preservation Solutions for Preventing Cell Death in Pig Vascularized Myofaciocutaneous Flaps During Prolonged Cold Storage

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BACKGROUND

- Vascularized composite allografts (VCAs) are transplants that are composed of a combination of different tissue types. This makes them particularly useful in the transplantation of structures like whole limbs.
- Maintenance of VCA long-term is complex because different tissues have different immunogenetic properties.
- The transplantation process itself can affect limb longevity due to subjecting the tissue to various periods of ischemia, which can contribute to cell death.
- Two commonly used preservation solutions include Belzer UW® Cold Storage Solution (UW) and Custodiol® HTK (Custodiol), which are usually used as stand-alone methods of preservation.
- Necrostatin-1 (Nec-1), an inhibitor of the receptor-interacting protein 1 kinase, is a known inhibitor of necroptosis, a type of programmed cell death that causes inflammation. Nec-1's potential to act as an adjunct to preservation not been explored.
- This study aims to understand which combinations of preservation solution (UW and Custodiol) and additive (Nec-1) best decrease cell death in pig VCA flaps.

METHODS

Abdominal flaps from six Yucatan pigs were preserved with either Custodiol, UW, Custodiol + Nec-1, or UW + Nec-1 at 4°C for 72 hours. Aperio ImageScope, a whole-slide digital scanner, was used to quantify the TUNEL-stained nuclei in different tissue types for each of the samples. Data was analyzed using Welch's unpaired t-test to determine significant differences between each tissue type. Statistical analyses were performed in GraphPad Prism. Statistical significance was set at $p < 0.05$.

Necrostatin-1 reduces apoptosis in VCA flaps, with a significant effect observed in UW-preserved epidermis.

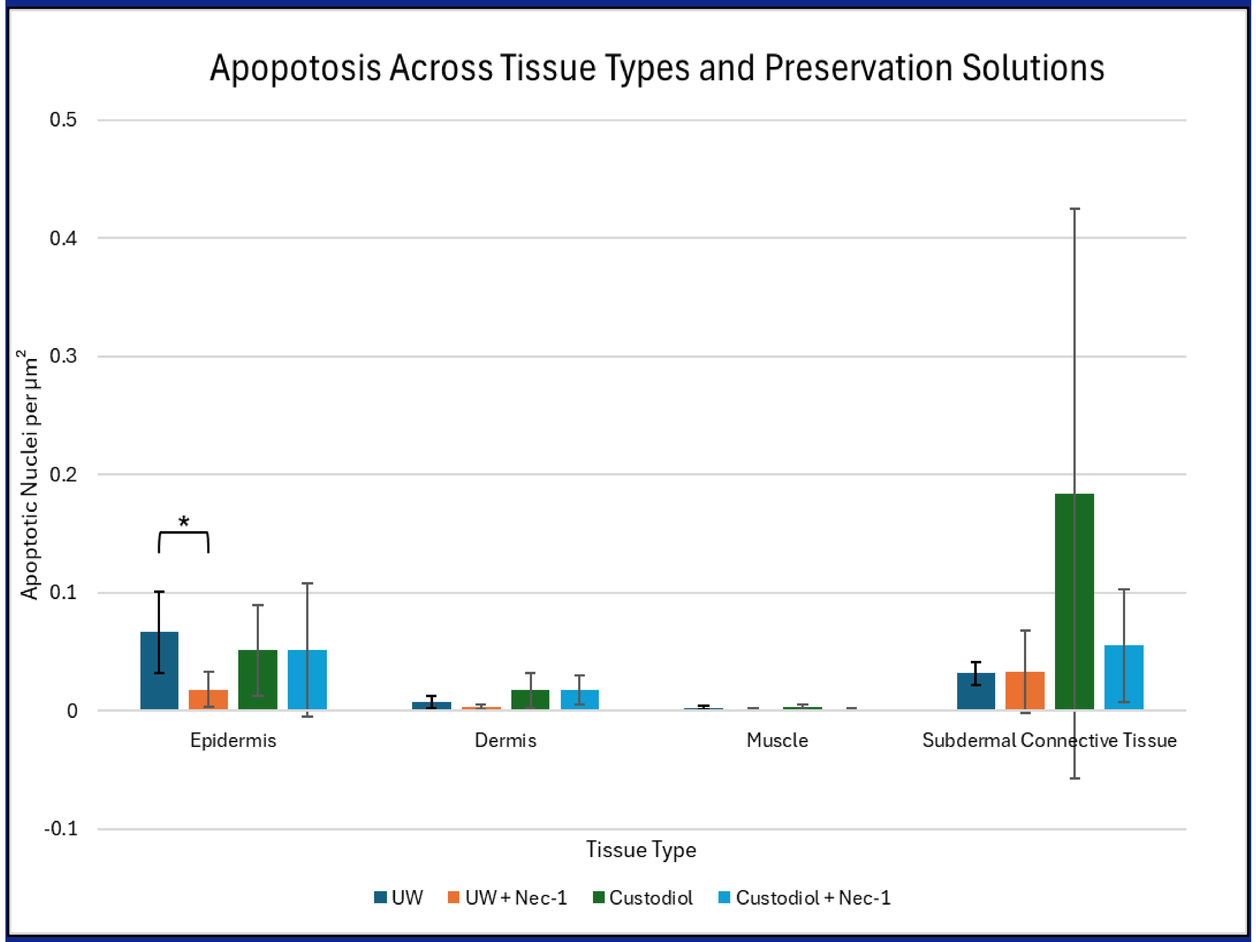

Figure 1. Mean apoptotic nuclei per µm² across tissue types following preservation in UW or Custodiol solutions with or without Necrostatin-1 (Nec-1). Data are presented as mean ± SD (n = 3–7 per group). Nec-1 treatment was associated with reduced apoptosis across multiple tissues, reaching statistical significance only in UW-preserved epidermis (*p = 0.0151).

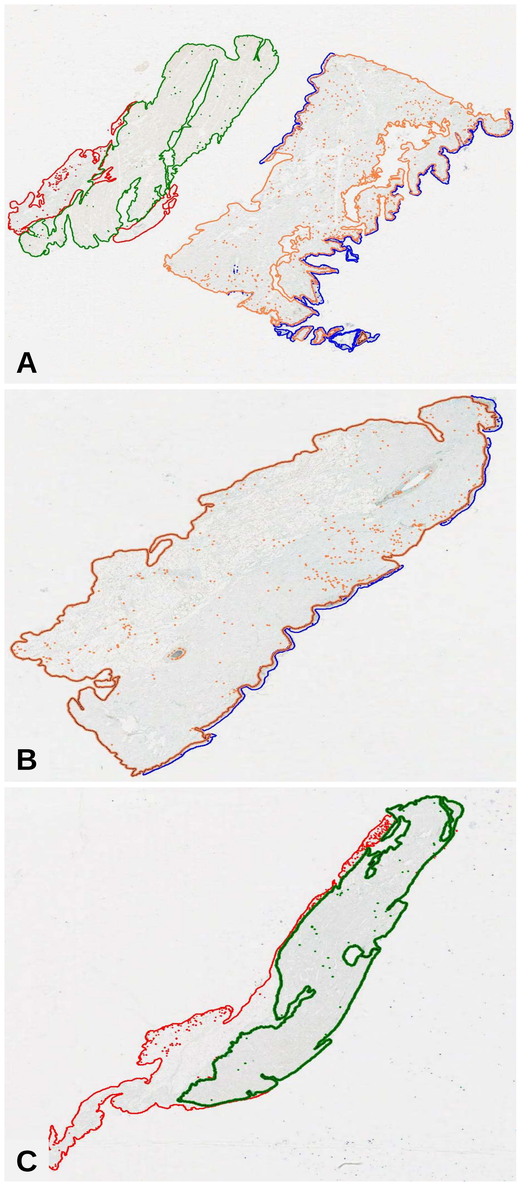

Figure 2. TUNEL-stained slides from Pigs 35506 (2A) and 35507 (2B, 2C) are annotated to show apoptotic nuclei and areas for skin slides: epidermis (blue), dermis (orange) and muscle slides: connective tissue (red), muscle (green).

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