

Advances in Pre-treatments of the Ex-vivo Vascularized Composite Allograft: A Systematic Review

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INTRODUCTION

- Vascularized composite allografts (VCAs) are comprised of multiple tissue types each with a unique physiological profile.
- Management of tissue viability throughout the transplantation process is necessary for safe and successful patient outcomes.
- A key opportunity for intervention is graft pre-treatment (PT), in the window between harvest and transplant.
- The current gold standard PT is a graft flush followed by static cold storage with the University of Wisconsin solution (UW). Graft viability is limited to 4-6 hours.¹
- In this short time, donor-recipient matching, graft allocation, and tolerance induction are limited or impossible.
- This purpose of this systematic review of the literature is to present novel PT strategies and their results, along with practical considerations.**

METHODS

Following PRISMA guidelines, a literature search was conducted with the following parameters

- Databases:** PubMed, Cochrane and Embase
- Key Terms:** 33, related to VCA grafts and transplantation
- Exclusion Criteria:** abstracts, commentaries, editorials, non-English articles, review papers, and PT interventions targeting immunogenicity rather than cell physiology
- Based on the findings from data extraction, articles were organized into three categories: **solution-based alterations to the gold standard, ex-vivo perfusion, or other novel techniques**

RESULTS

- Our search identified 3,125 unique articles and resulted in 13 studies for data extraction with 12 distinct methods of PT, as shown in Table 1.

Table 1. Study design and protocol of each experimental pre-treatments within their categorical distinctions

Category	Study Identifier	Animal Model	Transplant Type	Novel PT Protocol
Solution-based Alterations to the Gold Standard (N = 2)	Fries et. al, 2019 ²	Swine	Heterotopic, allogeneic	Flush w/ H2S + SCS w/ H2S venous effluent and UW (3 hrs)
	Rostami et. al., 2021 ³	Rat	Heterotopic, allogeneic	Flush w/ heparinized saline, UW, HTK, or Perfadex + SCS (6 hrs)
Perfusion (N = 7)	Constantinescu et. al., 2011 ⁴	Swine	None	Flush + warm ischemia (1 hr) + perfusion with oxygenated autologous blood (32 deg C, 12 hrs)
	Ozer et. al, 2015 ⁵	Swine	Orthotopic, allogeneic	Flush w/ saline and heparin + perfusion with oxygenated autologous RBCs and plsma (27-32 deg C, 6 hrs)
	Ozer et. al, 2016 ⁶	Swine	Orthotopic, allogeneic	Flush w/ saline and heparin + perfusion with oxygenated autologous RBCs and plasma (27-32 deg C, 6 hrs)
	Gok et. al., 2019 ⁷	Rat	None	Flush w/ heparinized saline + perfusion with oxygenated swine RBCs in STEEN solution (30-35 deg C, 6 hrs)
	Kruit et. al., 2019 ⁸	Swine	Orthotopic, replant	Flush w/ heparine-saline + perfusion w/ oxygenated UW or HTK (8-10 deg C)
	Haug et. al., 2020 ⁹	Human	None	SCS w/ UW (mean: 67 min) + perfusion with acellular STEEN solution (10 deg, 24 hrs)
	Burlage et. al, 2022 ¹⁰	Rat	Heterotopic, syngeneic	Perfusion w/ skeletal muscle media alone, with PEG, or with HBOC-201, PEG, and prostaglandin (21 deg C, 6 hrs)
Other Novel Techniques (N = 4)	Rinker et. al., 2008 ¹¹	Rat	Orthotopic, syngeneic	Flush w/ RL and UW + SCS w/ RL and UW (4 deg C) + cryopreservation w/ 2.0 DMSO (-140 deg C, 2 weeks)
	Liu et. al., 2010 ¹²	Canine	Orthotopic, replant	Gold Standard (24, 36, or 48 hrs) + flush w/ RL
	Amin et. al. 2018 ¹³	Swine	None	Flush w/ UW and heparin + SCS (2 or 6 hrs) + flush w/ RL
	Gok et. al., 2020 ¹⁴	Rat	Orthotopic, syngeneic	Group 1: Static warm storage (6 hrs) + transplant Group 2: Flush w/ HTK + SCS (6 hrs) + transplant

Notes: Gold standard is flush w/ UW + SCS w/ UW. H2S = hydrogen sulfide solution; SCS = static cold storage; UW = University of Wisconsin solution; HTK = histidine-tryptophan-ketoglutarate solution; RBCs = red blood cells; HBOC-201 = hemoglobin substitute; PEG = polyethylene glycol; RL = Ringer's lactate solution; DMSO = dimethyl sulfoxide. Details regarding time, temperature, amount, or solution not listed were not reported by the study.

Solution-based Alterations to the Gold Standard

- Solutions analyzed were hydrogen sulfide (H2S), Perfadex, and histidine-tryptophan-ketoglutarate (HTK), in addition to UW and saline.^{2,3}
- H2S-treated grafts demonstrated a 6-day delay in rejection and consistently lower grades of clinical rejection to follow.²
- HTK- and saline-treated grafts showed more apoptosis and inflammatory markers than UW and Perfadex. UW or Perfadex was recommended.³
- Skin, blood vessels, nerves, and muscle showed damage most quickly. Bone was fairly resilient.³

Ex-vivo Perfusion

- All flaps showed weight gain, up to 50%.⁸
- Neuromuscular stimulation and force generation were continuously demonstrated throughout perfusion (up to 24 hours) and following transplantation. Muscle function declined or was lost in SCS-stored flaps.^{2,3,4,5,6,7,8,9,10,11,12,13,14}
- Muscle still showed more injury, immunoglobulins, and complement than SCS.^{4,7,9,10}
- The addition of HBOC-201, PEG, and prostaglandin to perfusate showed lower mortality rates by graft failure than immediate transplant.¹⁰

Other Novel Techniques

- Cryopreserved grafts had a percent viability of 91.9% after two weeks, but 90% of grafts failed after transplantation.¹¹
- The preservation limit for the gold standard was recommended to be 24 hours before statistically significant graft damage occurs.¹²
- Muscle was highly vulnerable to ischemia and showed diminished function.¹⁴ Muscle and nerve viability decreased most quickly, and skin least.¹²
- A post-PT flush to decrease graft damage due to antigenicity should be explored further.¹³

DISCUSSION

- The easiest intervention to integrate into current practice, clinical studies should explore H2S and Perfadex in PT.
- Individual tissue analyses should be more widely used in VCA research to assess PT success in this heterogeneous setting.
- Ex-vivo perfusion most closely mimics the corporeal environment. Perfusion yielded exceptional results for muscle function over PT up to 24 hours. Weight gain is a drawback.
- Viability pre- versus post-transplant and histological versus clinical outcomes do not necessarily correlate, and additional studies must assess the significance of such discrepancies.

CONCLUSIONS

- The 13 PT strategies reviewed demonstrate significant innovation from previous methodologies with improved VCA function and prolonged viability.
- Incorporating these ideas into clinical studies, and eventually clinical practice, will expand the donor pool and increase time for graft allocation and recipient tolerance induction.
- Given its success in preserving muscle, one of the most vulnerable tissues, ex-vivo perfusion is particularly promising.

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