



# Flap Preconditioning with Adipose Derived Stem Cells and Hyperbaric Oxygen Treatment: A Guinea Pig Model

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## INTRODUCTION

Chronic wound care requires consistent management ranging from excision and debridement to timely wound dressing changes. In the case of non-healing chronic wounds, supplementary surgical management relies on skin grafting as well as tissue flap reconstruction. Ultimately, tissue flap success rates remain susceptible to potential postoperative risks, including hypoperfusion and tissue ischaemia. As a result, adjunctive therapies that may improve tissue flap survival must be explored. Thus, we propose a small animal model to explore the role of adipose-derived stem cells (ADSCs) and hyperbaric oxygen treatment (HBOT) in tissue flap reconstruction while quantifying their effects on flap survival. Additionally, research findings stand to identify the most favorable time for preoperative delivery of these adjunctive therapies.

Acting as a molecular chaperone, heat shock protein (HSP) 70 is considered a regulatory molecule providing cell protection. Wound healing studies incorporating HBOT report the up-regulation of cytoprotective genes and consequently an increase in cell proliferation and oxidative stress resistance.

Furthermore, recent research studies have indicated that hyperbaric preconditioning and stem cell transplantation prior to elevation of the flap enhances new vessel formation and stem cell mobilization.

A guinea pig animal model for stem cell harvesting and flap design was employed to elucidate the effects of HBO preconditioning treatment on the viability of ADSCs and tissue flap ischaemia.

## OBJECTIVES

1. Explore the role of HBO treatment and ADSCs in tissue flaps
2. Assess whether preconditioning of ADSCs increases the survival rate of tissue flaps
3. Determine the optimal time for ADSC injection during preoperative care
4. Improve and advance the current surgical protocol for tissue flaps
5. Optimize patient healthcare and health outcomes by improving tissue flap survival rates

## MATERIALS & METHODS

This prospective randomized control trial consisted of twelve guinea pigs that were assigned to one of two groups. Researchers administered ADSC injections subcutaneously to animals assigned to the experimental group, whereas those in the control group received saline injections. Subsequently, half of the animal subjects were exposed to four consecutive HBO treatments. Following preconditioning, a pedicled flap was elevated to assess the postoperative tissue survival.

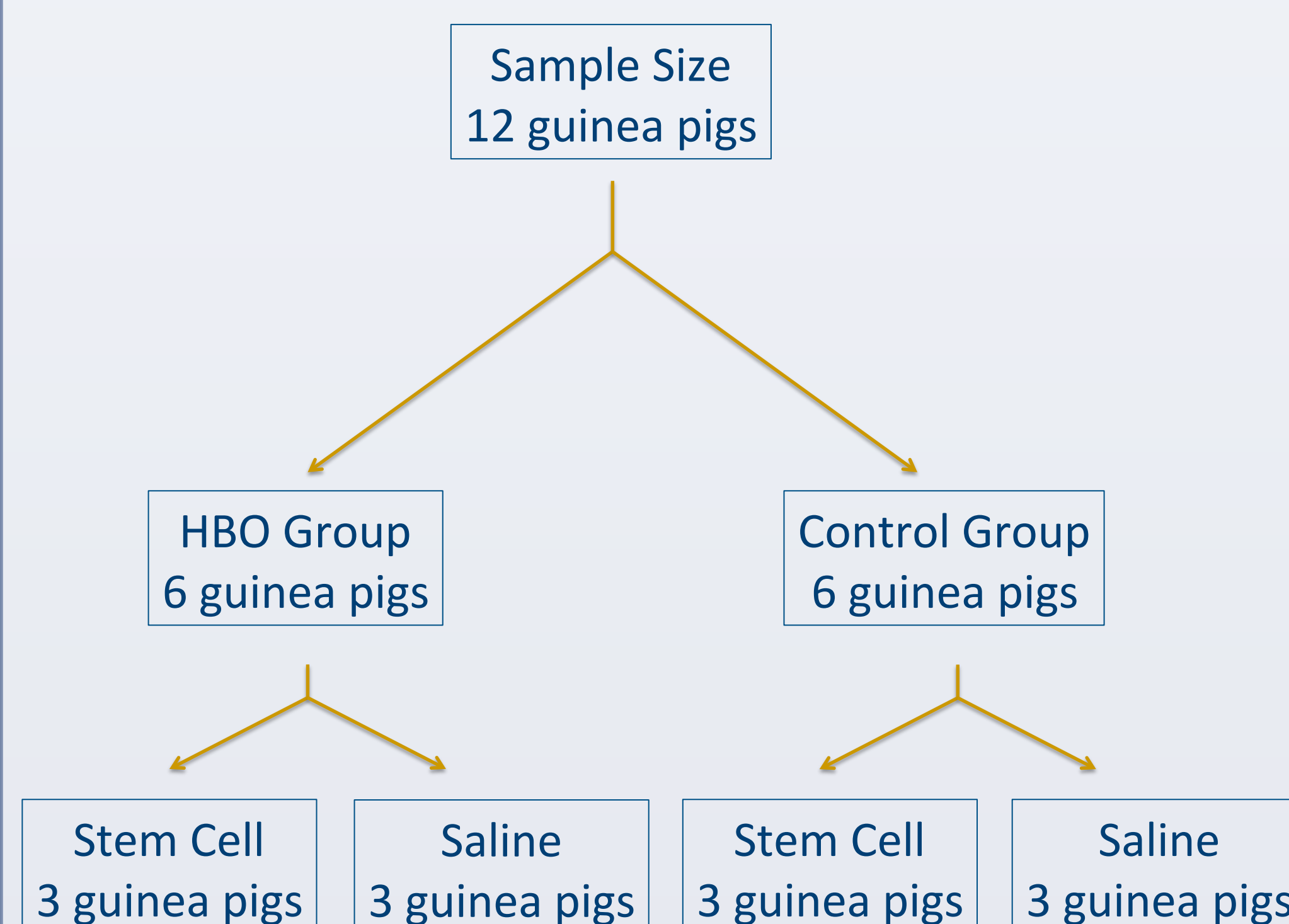
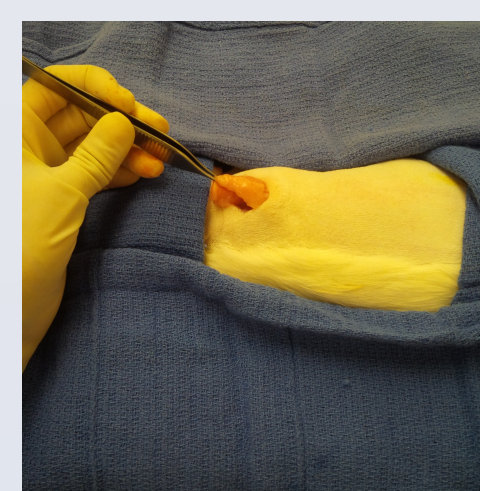


Figure 1: Schematic Representation of Study Groups

### ADSC Harvesting

A transverse incision was made directly over the fat pad and the pad was teased out. Once extracted, the stem cells were injected into the distal third of the flap.



### HBO Chamber Protocol

2.0 atmospheres absolute (ATA) for 90 minutes of 100% oxygen



### Flap Creation

In a prone position, a random vascular pattern 2 x 8 cm skin flap was elevated on the dorsum of the guinea pig, with the base at the level of the axilla. The skin near the dorsal midline receives a number of small perforators from the dorsal intercostal blood vessels.

## RESULTS

### Endpoint 1: Clinical Tissue Flap Assessment

The clinical appearance of the skin flap was assessed visually to detect signs of venous or arterial compromise. Serial examinations of the flap monitored the consistency and color of the flap and surrounding skin, tissue vascularity (e.g., capillary refill) as well as temperature of the flap.

Days 4, 5, 6, 8, 11



Group	Flap Assessment Criteria	Statistical Analysis
HBO: Stem cells vs. Saline	All criteria	p > 0.05
No HBO: Stem cells vs. Saline	All criteria	p > 0.05
HBO vs. No HBO: All	Tissue Flap color Congestion of the flap was significantly greater in the No HBO group (26.7%) as compared to the HBO group (0%).	p = 0.004*
HBO vs. No HBO: Stem cells	Necrotic Tissue Type Thick, black, leathery tissue was more often noted in the No HBO group (46.7%) as compared to the HBO group (6.7%).	p = 0.013*
HBO vs. No HBO: Saline	Tissue Flap Color The tissue flap was more often congested (blue) in the No HBO group (33.3%) as compared to the HBO group (0%).	p = 0.03*

Table 1: Clinical Assessment Findings

### Endpoint 2: Heat Shock Protein 70

Preconditioning with HBO treatment has been shown to provide cytoprotective effects, through the up-regulation of HSP 70, a regulatory molecule that protects cells from environmental insults.

Days: 1, 4, 11

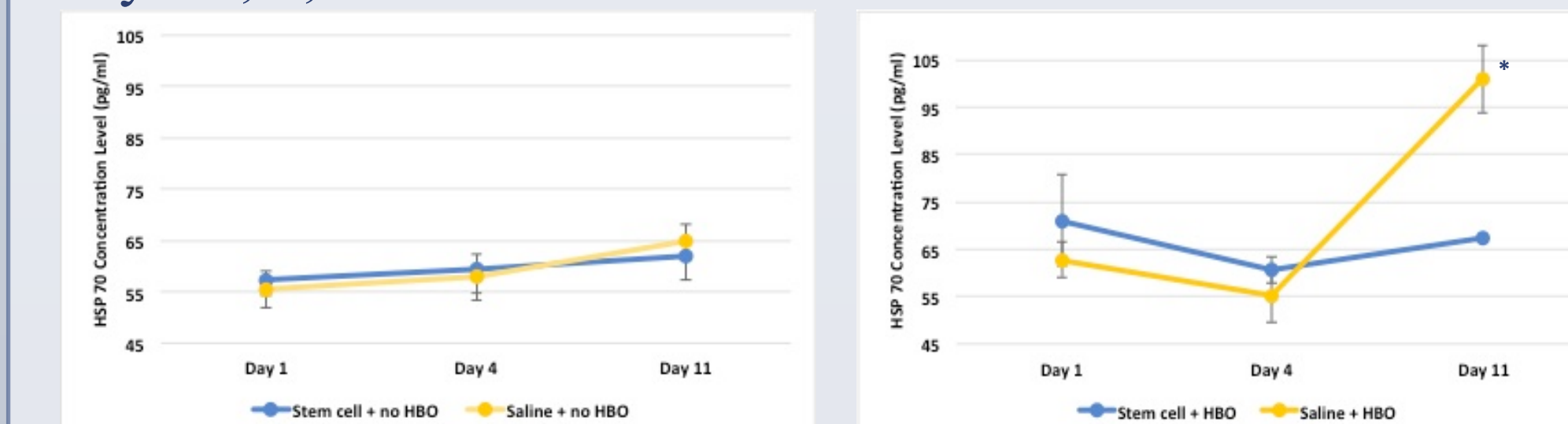


Figure 2: HSP 70 levels, (a) No HBO and (b) HBO groups

Consistent with research and clinical findings, HSP 70 levels did not rise in the No HBO group.

An Independent Samples T-test revealed a significant difference in the HSP 70 levels in the HBO saline group at Day 11 ( $p = 0.042$ ).

### Endpoint 3: Tissue Apoptosis

TUNEL Apoptosis assays are one of the most widely used methods for detecting DNA damage in situ.

Days: 4, 14

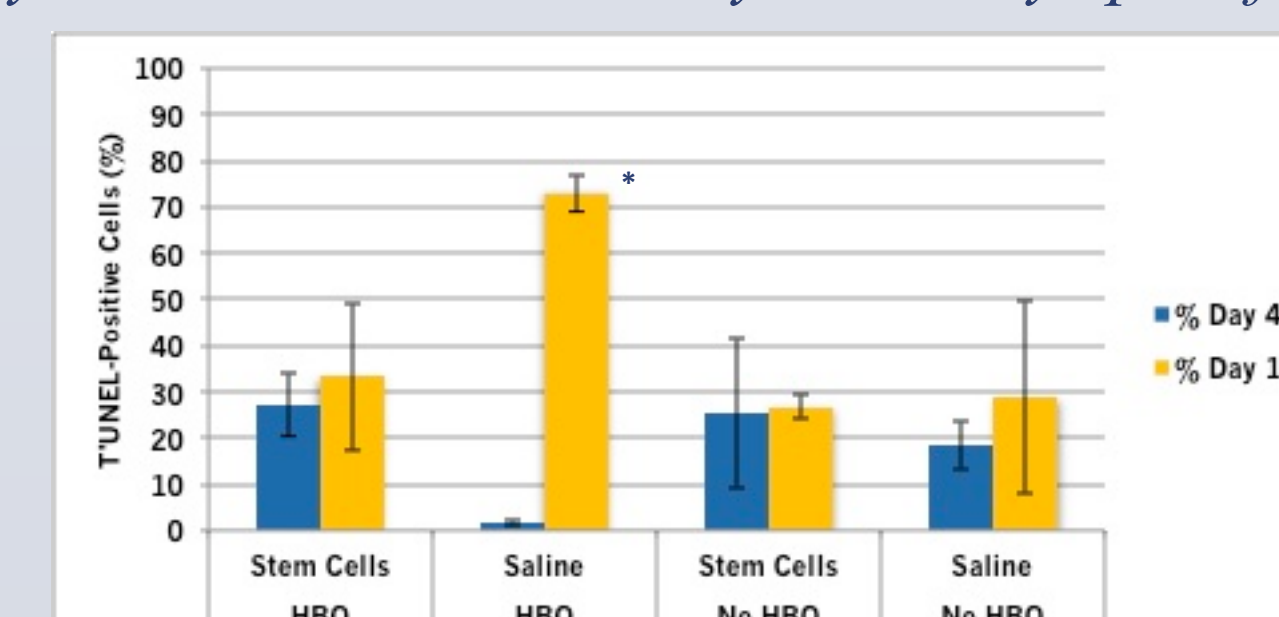


Figure 3: Percentage of TUNEL-Positive (apoptotic) cells (\*  $p = 0.003$ )

## CONCLUSIONS

Preliminary study findings support that HBO preconditioning of tissue flaps improves survival rates. In the presence of HBOT and ADSCs, necrosis of the distal portion of the flap was markedly reduced. Yet, the postulated cytoprotective effects of HSP 70 following HBOT did not support this finding. Although a statistically significant rise in HSP 70 levels was detected in the HBOT and saline group at Day 11, this rise was not mirrored in the HBOT and ADSC group. Tissue flap clinical appearance assessments were consistent with the TUNEL assay results, both demonstrating that tissue survival in the HBOT and saline group was markedly reduced. While preliminary results show that both HBOT and ADSCs play a role in tissue viability, further investigation with a larger sample size is necessary to elucidate the true effects of HBOT and ADSC combination on tissue flap survival.

## FUTURE DIRECTIONS

1. Address study limitations (e.g., sample size, surgical procedures, animal environment) in future groups.
2. Complete remaining study groups to determine the optimal time for preoperative ADSC injection
3. Complete HBOT only control group (no injections)
4. Quantify CD34 marker in the peripheral circulation to assess ADSC proliferation.
5. Enhance research design to include HBO treatment following tissue flap elevation in addition to tissue preconditioning with HBOT and ADSCs.

## ACKNOWLEDGEMENTS

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