vascularity.\textsuperscript{1} Adjunctive measures have included free microvascular transfer, supercharging, caspase inhibitors and free oxygen radical scavengers. In this study, we explored the potential complementary effects of HBOT and stem cell delivery on cutaneous flap survival.

**MATERIALS AND METHODS:** The potential healing benefits of HBOT preconditioning and stromal vascular fraction (SVF) delivery on flap survival were examined in a guinea pig model.\textsuperscript{2} Animal subjects were randomly assigned to one of four study groups: SVF/saline injections followed by HBOT, SVF/saline injections only, HBOT only, or neither HBOT nor injections. In order to enhance clinical relevance, an additional group of animals underwent HBOT prior to SVF/saline injections. Thereafter, an unfavorably designed cutaneous flap was elevated and clinically assessed via study-blinded observer, as well as by quantification of TUNEL-positive cells.

**RESULTS:** Distal necrosis of the tissue flap was most often observed in the no intervention group (72.8% of the flap, \( p < 0.001 \)), similar to tissue flaps treated with HBO only (62.9%, \( p = 0.036 \)) and SVF injections (46.7%, \( p = 0.013 \)). The most significant difference occurred in the combination HBO and SVF delivery group, where distal necrosis was only visible in 24.6% of the flap (\( p < 0.05 \)). Most notably, SVF delivery immediately prior to flap elevation further minimized distal necrosis of the flap to 18 percent. These findings were mirrored by the TUNEL assay, indicating the highest percentage of cell death in the no intervention and HBO only groups (\( p < 0.05 \)).

**CONCLUSION:** Findings not only indicate that combining HBO treatment and SVF improves flap viability, they also suggest that it may be more appropriate to deliver SVF at the time of tissue elevation, providing a more clinically relevant way to treat these patients.

**DISCLOSURE/FINANCIAL SUPPORT:** Supported by an unrestricted gift provided by Oxyheal Health Group Inc. None of the authors has any financial interest in the products or devices mentioned in this manuscript.

**REFERENCES:**

1. Morris, S.F., Taylor, G.I. (1992). Predicting the Survival of Experimental Skin Flaps with a Knowledge of Vascular Architecture. Plast Reconstr Surg, 92(7): 1352–61.
2. Frolich, K., Scherzed, A., Mlynski, R., et al. (2011). Multipotent Stromal Cells for Autologous Cell Therapy Approaches in the Guinea Pig Model. Otorhinolaryngol Relat Spec, 73(1): 9–16.

---

**Fabrication of a Tissue-Engineered Pre-Vascularized Perfusable Skin Flap**

**Ross H. Weinreb, MS; Kerry A. Morrison, BA; Julia Jin, BS; Xue Dong, BA; Yoshiko Toyoda BA; Adam Jacoby, MD; Sushmita Mukherjee, PhD; Jason A. Spector, MD**

**INTRODUCTION:** Currently, there remains no clinically translatable tissue engineered skin flap with the ability to provide whole tissue perfusion. Herein, we fabricate a pre-vascularized full-thickness cellularized skin equivalent containing a three-dimensional vascularized network of interconnected macro and microchannels lined with vascular cells, within a collagen neodermis containing encapsulated fibroblasts, and an epidermis comprised of human keratinocytes capable of providing whole tissue perfusion.

**METHODS:** Pluronic F127 was used for network preparation: 1.5 mm diameter “U” shaped macrofibers and 100–500 \( \mu \)m-interwoven microfibers were heat extruded and then embedded within Type I collagen into which CFP-tagged human placental pericytes (HPLP-CFP) and human foreskin fibroblasts (HFF1) at a density of \( 1 \times 10^6 \) cells/mL, respectively had been encapsulated. After pluronic sacrifice, channels were intraluminally seeded with \( 5 \times 10^5 \) RFP-tagged human aortic smooth muscle cells (HASMC-RFP) and \( 5 \times 10^5 \) GFP-tagged human umbilical vein endothelial cells (HUVEC-GFP). The construct was then topically seeded with \( 1 \times 10^6 \) human epidermal keratinocytes (HEK). Constructs were incubated for 7, 14 and 28 days and subsequently live flaps were analyzed using multiphoton microscopy (MPM) or fixed and processed for histology. Flaps were microsurgically anastomosed to rat femoral artery and vein and perfused, \textit{in vivo}.

**RESULTS:** MPM imaging demonstrated a hierarchical vascular network containing macro and microvessels lined by endothelial and smooth muscle cells, supported by perivascular pericytes, all in appropriate microanatomic arrangement. Neodermal HFF1 proliferated throughout the observation period and the HEK neoepidermis developed into a stratified epithelium along the superficial aspect of the construct. MPM images indicated angiogenic sprouting from the nascent vascular network into neovessel like structures. Skin flaps were successfully anastomosed and perfused while withstanding the physiological pressures and maintaining their inherent vascular network architecture.

**CONCLUSION:** We have successfully fabricated and microsurgically anastomosed the first ever tissue-engineered
pre-vascularized full thickness skin flap, which recapitu-
lates the inherent hierarchical vasculature found within the
human skin and is suitable to provide whole tissue perfu-
sion. We provide the platform for an on-demand, geo-
metrically tunable tissue engineered skin equivalent with
an anastomosable vascular network which will transform
reconstructive surgical practice by eliminating the conse-
quences of donor site morbidity and enabling rationally
designed, patient specific flaps for each unique wound envi-
ronment and anatomic location.

**DISCLOSURE/FINANCIAL SUPPORT:** None

Vacuum Assisted Flap Delay, a Novel Strategy to Increase the Flap Survival: An Experimental Study in Rabbits

Osman E. Aydin, MD; Said Algan, MD; Onder Tan, MD; Elif Demirci, MD; Osman N. Keles, MD; Abdulmecit Kantarci, MD

**INTRODUCTION:** Flaps are the work horse in daily plas-
tic surgery practice. To overcome the major complication of
flap necrosis in risky groups, delay procedures have been
defined. The golden standard method of delay is surgical
delay. On the other hand, it has a major drawback; the two
sessions of operations. Efforts have been made to omit one
session and increase the patient safety and decrease the cost
of the treatment. Topical negative pressure has been used
for assisting the wound closure. However, the mechanisms
of action of the negative pressure are not still clearly elu-
cidated. The topical negative pressure has been shown to
induce neovascularization and increase vascular density. With
this respect, writers aimed to use topical negative
pressure to prospective flap area prior to flap elevation and
compare the survival and perfusion results, with the golden
standard technique “Surgical Delay”.

**MATERIALS AND METHOD:** Thirty rabbits were
equally divided in 3 groups; control group, surgical delay
group (SD) and vacuum assisted flap delay group (VAFD).
In a cranially based, 25x5 cm random flap model on the lat-
eral thoracic region the study was conducted. In the VAFD
group, a topical negative pressure system with an 80 mmHg
pressure was applied prior to flap elevation, where the flap
was planned to be elevated, for seven days. Surgical delay was
conducted in the same flap model seven days before eventual
flap elevation in the SD group. The flap area, necrosis area,
necrosis ratio, histomorphometric vascular density, immu-
nohistochemical evaluation of neovascularization (CD31/
CD34), Laser Doppler images and computerized tomogra-
phy contrast uptake were used to compare the groups.

**RESULTS:** In all the parameters, the VAFD group was
equivalent to the SD group. Both were superior to the con-
trol group.

**CONCLUSION:** The main disadvantage of surgical delay
is that it is a surgical procedure. Morykwas and Argenta
showed that, TNP successfully increased the flap survival
rates even after the elevation. However, their results did
not imply the use of the TNP systems as a delay procedure.
Results of this study suggest that topically applied negative
pressure induces vacuum assisted flap delay. This VAFD
phenomenon is superior to surgical delay as it is cheap,
practical and less morbid. Further studies are needed to elu-
cidate its clinical significance.

**DISCLOSURE/FINANCIAL SUPPORT:** Supported by
Ataturk University Scientific Research Projects, with the
project number 2012/040 (to Dr. Osman E. Aydin and Dr.
Said Algan). None of the authors has a financial interest
in any of the products, devices, or drugs mentioned in this
manuscript.

**REFERENCES:**
1. Ghali S, Butler PE, Tepper OM, Gurtner GC. Vas-
cular delay revisited. *Plast Reconstr Surg* 2007;
119(6):1735–1744.
2. Argenta LC, Morykwas MJ. Vacuum-assisted closure:
a new method for wound control and treatment: clinical
experience. *Ann Plast Surg.* 1997;38(6):563.
3. Morykwas MJ, Argenta LC, Shelton-Brown EJ, McGuirt
W. Vacuum-assisted closure: a new method for wound
control and treatment: animal studies and basic founda-
tion. *Ann Plast Surg.* 1997;38(6):553–562.

Burn Scar Regeneration with the “Sufa”
(Subcision and Fat Grafting) Technique. A Prospective Clinical Study

Francesco Gargano, MD, PhD; Scott T. Schmidt, MD; Richard J. Zienowicz, MD; Silvio Podda, MD; Paul Liu, MD

**INTRODUCTION:** Treatment of burn scars with tradi-
tional surgical techniques is challenging due to recurrent