# Tissue Engineered Vascularized Bone Grafts from Human Stem Cells for Craniofacial Reconstruction

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## ABSTRACT

Repair of large voluminous bony defects in craniofacial reconstruction which relies on the ability to induce vascular networks remains a major challenge. This study tested biological and tissue engineering approaches to induce neovascualrization. Thrombin induced VEGF gene expression *in vitro*. A combination of human umbilical cord vascular derived endothelial cells, hematopoietic stem cells and mesenchymal stem cells were loaded in thrombin matrices. A biodegradable and biocompatible scaffold of TCP/PCL was developed and fabricated. Cell-thrombin composites were evenly seeded in scaffolds and implanted in mice for eight weeks. RT-PCR revealed up-regulated expression of thrombin treated HUVECs. Harvested implants demonstrated the formation of substantial amounts of microvascular beds in the MSC-HUVEC constructs as illustrated by micro-CT and vascular volumetric plotting. These findings demonstrate that through the use of a thrombin based MSC-HUVEC biomatrix, microvasculature can be created.

## BACKGROUND

### Large Bony Defects in Orthodontics

- Cleft palate repair
- Craniosynostosis repair
- Moving teeth into edentulous area
- Current gold standard: autologous grafts





**Drawbacks**

- Limited donor tissue availability
- Additional surgical risks and donor site morbidity

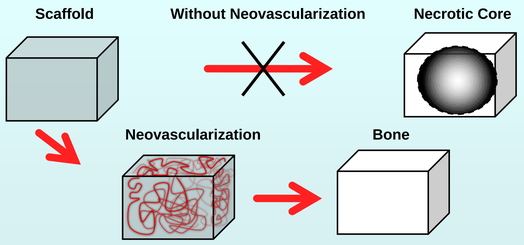
### Metallic Implants

- Readily available
- Immediate support at defect site
- Unable to grow and remodel, may serve as nidus for infection

### Ceramic Implants

- Osteoconductive
- Biocompatible
- Brittle; poor tensile strength limits locations of potential use

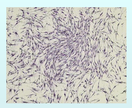
### Bone Growth Requires Microvasculature



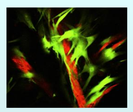

### Thrombin: Dual Roles

- Biomatrix for cell seeding: thrombin cleaves fibrinogen into fibrin
- Growth factor: thrombin promotes angiogenesis via G protein-coupled receptors

### Cell Types- Osteogenic : Mesenchymal Stem Cells



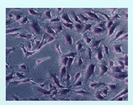
- Differentiate into osteoblasts
- Release vascular endothelial growth factor (VEGF) to promote angiogenesis



- Stabilize vascular network formation

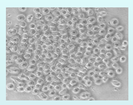
### Cell Types- Angiogenic :

**Human Umbilical Vein Endothelial Cells**



- Directly participate in vascular structure formation

**Hematopoietic Stem Cells**



- Promote endothelial cell migration for angiogenesis

## OBJECTIVE

To present study was designed to create a vascularized craniofacial implant from human stem cells.

## CLINICAL SIGNIFICANCE

Biosurgery, in the form of a scaffold-based tissue engineering approach, offers a viable approach to reconstruct large bony defects in orthodontic and craniofacial patients suffering from cleft palate, craniosynostosis, and edentulous sites.

## MATERIALS & METHODS

**Freeform fabrication of 3-D PCL-beta-TCP scaffold.** The porous scaffolds were fabricated from a mix of 30% PCL and 70% beta-TCP at Therics to a dimension of 6mm$^3$ with 70% porosity. The average pore size was 250 microns. All scaffolds were sterilized using ethylene oxide gas for 20 hours at 37C.




***Figure 1***: *PCL-TCP scaffold following fabrication* ***(A)*** *Micro-CT rendering of the same scaffold* ***(B)***

**Cell Phenotypes and Culture Conditions.** MSCs and HSCs were extracted from bone marrow of healthy donors. After isolation, MSCs were culture expanded using 89% DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic. HSCs were culture expanded in Iscove's Modified Dulbecco's Medium, 10% FBS, 100ng/mL of granulocyte colony stimulating factor, and 100ng/mL of stem cell factor, and 100ng/mL of thrombopoeitin. HUVECs were purchased and cultured in HUVEC supplemental medium from AllCells. Passage 5 cells were used and received media change two times per week.

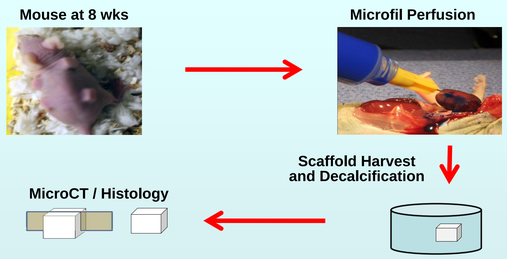
***In vitro* examination of angiogenic traits of thrombin.** MSCs and HUVECs in monolayer were exposed to 0, 0.5, 1.0, 1.5 u.ml of thrombin for 7 days prior to RT-PCR for VEGF expression.

**In vitro examination of osteogenesis.** MSC seeded scaffolds were treated with osteogenic media for 7 and 14 days and stained using alkaline phosphatase and alizarin red.

**Implantation.** The Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia approved all animal procedures. A liquid biomatrix of 4U/mL of thrombin was used to encapsulate 3 million cells/mL in the construct and then sealed in 20mg/mL of fibrinogen. 4 dorsal subcutaneous pockets were made in 6 week old athymic nude mice and the constructs were implanted for 8 week

**In Vivo Microfil Perfusion and Micro-CT.** Microfil is a silicone-based vascular contrast agent for micro-CT studies. To examine vasculogenesis within the implanted constructs, microfil was systemically injected via the left ventricle at week 8. The samples were subsequently fixed and decalcified, and imaged using micro-CT.

**Histology.** Following 8 weeks in vivo, implants were harvested, fixed in formalin, and processed for hematoxylin and eosin histology. Additionally, microfil perfused implants were formalin fixed, processed, and sectioned in half for microscopic photographic analysis of engineered blood vessels.




## RESULTS

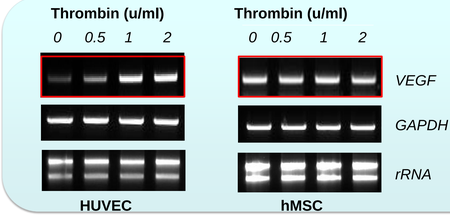



***Figure 2:***. *RT-PCR demonstrated that thrombin triggered an up regulation of VEGF in a dose-dependent manner, from the HUVEC cells, but not from the MSCs. Levels of GAPDH and ribosomal RNA were consistent among each sample group, demonstrating that overall RNA production remained unchanged*

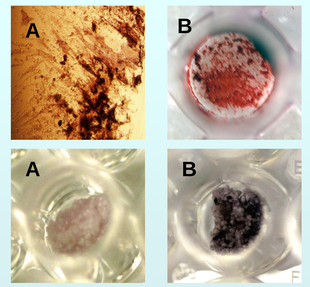



***Figure 3:*** *Positive alkaline phosphatase stained mesenchymal stem cells at 7 days* ***(A)*** *and positive alizarin red stained cells at 14 days illustrating production of osseous markers following exposure to osteogenic culture medium* ***(B)***.

***Figure 4:*** *Sectioned scaffolds loaded with stem cell-thrombin biomatrix. Culture medium treated control* ***(A)*** *stained negative while the scaffold treated with osteogenic culture medium stained positive for alkaline phosphatase at 7 days* ***(B)***.

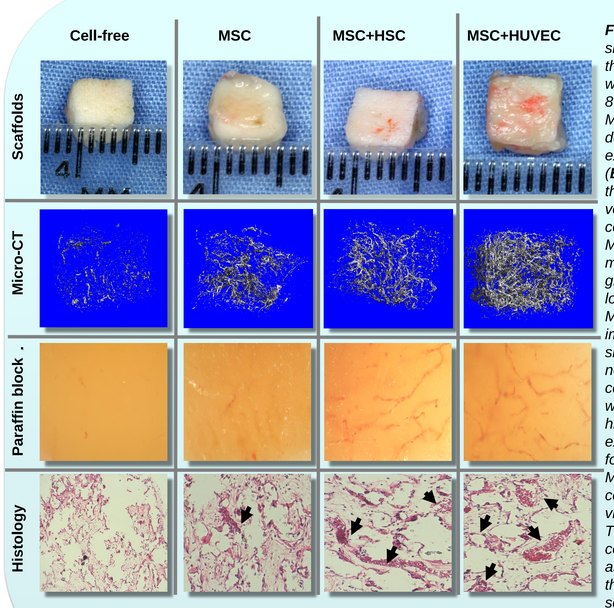



***Figure 5:*** ***(A)****. The general shape was well preserved but the dimension of the construct was decreased by 16% after 8 weeks in vivo. The extent of Microfil perfusion was readily detectable by gross examination of the samples.* ***(B)****. Quantitative analysis of the samples for blood vessel volume showed that the constructs loaded with MSC+HUVEC cells showed most extensive blood vessel growth followed by those loaded with MSC+HSC and MSC alone. The scaffold implanted without cells showed minimal neovascularization within the constructs. The constructs were further analyzed by histology which conformed extensive blood vessel formation in the MSC+HUVEC cell loaded constructs, confirming the visual and micro-CT data (****C****). The paraffin blocks of the constructs were prepared to assess the vascular formation through the length of the scaffold center (****D****). Note prominent neovasculature in both HSC+MSC and HUVEC+MSC.*

## CONCLUSIONS

- Through the use of a biocompatible scaffold with interconnected pores containing a thrombin based MSC-HUVEC biomatrix, a viable craniofacial implant can be created.
- The development of neovascularization from noninvasive sources constitutes an important step in the development of clinical strategies for tissue vascularization and restoration of large craniofacial defects in orthodontics.

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