A Human Placenta-Derived Decellularized Connective Tissue Matrix (CTM) Supports Cellular Functions Involved in Wound Healing Processes

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STATEMENT OF PURPOSE

The purpose is to analyze the role of human placenta-derived decellularized connective tissue matrix (CTM) in wound healing and its potential benefits in foot and ankle surgery. Our hypothesis was that CTM may act as a potential scaffold to accelerate wound repair and closure.

INTRODUCTION

Wound healing involves complex cellular and molecular responses and interaction of cells, including their proliferation and interaction with an extracellular matrix (ECM). In slow-or non-healing wounds, both cellular activity and the ECM are compromised. Placental Connective Tissue Matrix (CTM) is a human placenta-derived ECM which is decellularized, and is available in a particulate form; it can also be re-constituted with normal saline into an injectable form. This in vitro study was conducted to better understand the mechanism of action of CTM and its potential role in the replacement of abnormal ECM in slow- or non-healing or slow healing wounds. The biochemical composition of CTM was characterized, and its interaction with three major cell types involved in wound healing including fibroblasts, keratinocytes and endothelial cells was investigated.

❑ Wound healing is a complicated process involving a variety of cell types that work together to repair damaged skin and grow new skin.

❑ New biomaterials for wound healing should have positive effects on the different cell types involved in wound healing processes, establishing healthy inflammatory and proliferation phases to promote a re-epithelialization, angiogenesis.

❑ Uncontrolled pro-inflammatory (M1) macrophage activation is present during chronic inflammation and has recently been shown to lead to impaired wound healing[3].

❑ Likely, a vicious cycle exists between abnormal ECM and uncontrolled M1 macrophages in chronic wounds. Application of natural, healthy ECM products may allow for re-establishing normal functions of ECM thereby helping to resolve chronic inflammation.

RESULTS

CTM promotes adhesion and viability of cell types involved in wound healing

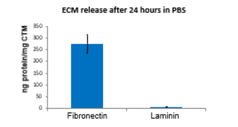


Fig 1. CTM releases ECM adhesion molecules fibronectin and laminin. The release of fibronectin and laminin was measured by ELISA.

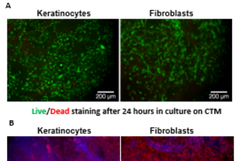


Fig 2. Adhesion and viability of Keratinocytes and Fibroblasts on CTM. Human Keratinocytes and Fibroblasts were seeded on CTM. (A) The viability of the cells was detected using Live/Dead staining. Live cells are green fluorescent. (B) The cells on CTM were immunostained with DAPI (blue) and actin (red).

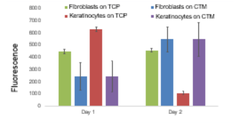


Fig 3. Proliferation of Keratinocytes and Fibroblasts on CTM. The viabilities of Keratinocytes and Fibroblasts on tissue culture surface (TCP) (green and blue) and on CTM (red and purple) were measured by alamarBlue assay at Day 1 and Day 2.

CTM promotes endothelial cell attachment and viability

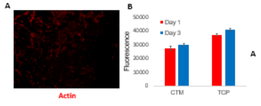


Fig 4. Adhesion and proliferation of Endothelial Cell on CTM. Human adult microvascular endothelial cells (HMVEC) were seeded on CTM. Cells were stained with rhodamin-phalloicin (A). The viabilities of cells on CTM or TCP were measured by alamarBlue assay at Day 1 and Day 3 (B).

CTM supported the migration of endothelial cells in a scratch wound assay

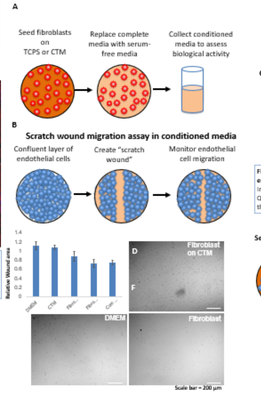
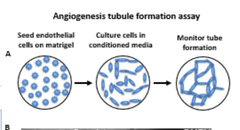


Fig 5. Conditioned media from fibroblasts cultured on CTM promoted the migration of endothelial cells in a scratch wound assay. The conditioned media from fibroblasts cultured on TCP or CTM were collected (A). The "wound closure" of endothelial cells in the scratch assay was measured (B). The wound areas in conditioned media from Fibroblast on CTM (D), DMEM control (E) and Fibroblast on TCP (F) were quantified at 3 h and 10 h (C). The conditioned media from fibroblast on CTM or TCP showed similar activities to the complete medium, which promoted more closure than DMEM control did.

CTM promotes tube formation of endothelial cells in matrigel



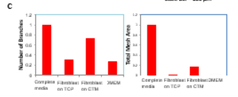


Fig 6. Conditioned media from fibroblast cultured on CTM promoted endothelial cells tube formation in matrigel. (A) Tube formation assay. (B) Images of tube formation at Day 1 in different conditioned media. (C) Quantification of branches and total mesh areas of the tube formation using the Angiogensis Analyzer plugin for ImageJ.

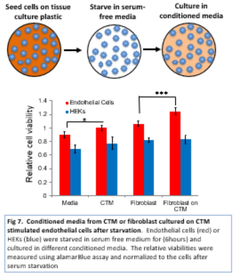


Fig 7. Conditioned media from CTM or fibroblast cultured on CTM stimulated endothelial cells after starvation. Endothelial cells (red) or HEKs (blue) were starved in serum free medium for (6hours) and cultured in different conditioned media. The relative viabilities were measured using alamarBlue assay and normalized to the cells after serum starvation

Human monocytes cultured on CTM show good viability and are pushed towards M1 macrophages

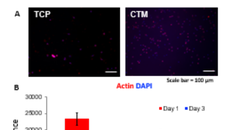


Fig 8. Monocyte viability on CTM and TCP. (A) More monocytes adhered to CTM than to TCP at 24 h. (B) The viability of monocytes on CTM or TCP was measured using alamarBlue assay at Day 1 and Day 3. There were significant more monocytes on CTM than on TCP at both time points .

Polarization of macrophages on CTM

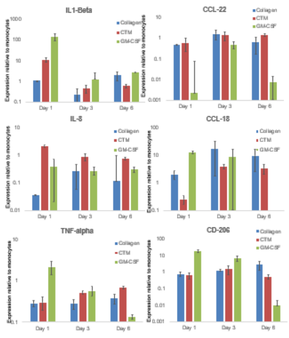


Fig 9. Gene expression profiles of M1 and M2 markers in monocytes cultured on CTM or TCP. The activation of monocytes by culturing on CTM, TCP or GM-CSF was monitored by the expression of M1 macrophage markers (TNF-alpha, IL-1beta, IL-8) and M2 macrophage markers (CCL-18, CCL-22 and CD 206). The relative gene expression (Fold) was determined using qPCR and normalized to starting monocytes.

CONCLUSION

Based on the in vitro studies, utilization of the minimally manipulated human placental-based CTM in inflammatory and wound healing models, provided a natural, healthy ECM that supported transition from inflammatory to proliferative macrophage types, cell migration, adhesion, and proliferation—all key processes to progress wound healing:

❑ CTM, a natural ECM-based product, promotes adhesion and viability of cell types involved in wound healing.

❑ ECM molecules, including fibronectin and laminin, are present in the CTM and released in solution.

❑ Important cell types for wound healing and proper skin function, such as keratinocytes, fibroblasts, and endothelial cells adhere, spread and are viable on CTM.

❑ Conditioned media from fibroblasts cultured on CTM led to improved wound closure and angiogenesis in a tube-formation assay.

❑ Human monocytes cultured on CTM show increased adhesion and viability versus collage coated tissue culture plastic.

❑ Culturing monocytes on CTM induces macrophage differentiation of monocytes based on upregulation of early M1 macrophage markers IL-1beta and IL-8 after 24 hours, but falling off by day 6.

❑ CTM may be able to promote the immunological progression of wound healing in applications with integumental tissue defocots

We demonstrated that CTM contains key extracellular proteins important in cell adhesion including fibronectin and laminin. As such, human dermal fibroblasts (HDF), human epidermal keratinocytes (HEK) and human dermal vascular endothelial cells (HDMEC) adhere and proliferate on CTM, as demonstrated by Live/Dead staining and viability assays. The adhesion and proliferation of HEK was significantly enhanced when cultured on CTM compared to culturing on standard tissue culture surface (TCP). Attaching to CTM stimulated cells to release growth factors such as FGF. In the presence of the conditioned media collected from HDF cultured on CTM, HDMECs demonstrated a faster in vitro wound closure in a scratch assay. Human umbilical vein endothelial cells (HUVECs) showed improved angiogenic potential in a tube formation assay. These results suggested that CTM not only supported the proliferation but also enhanced the functions of key cell types involved in wound healing, such as cell migration. These data support our hypothesis that CTM may act as a scaffold which could replace abnormal ECM in a wound and interact with key endogenous cells to accelerate wound repair and closure. Furthermore, circulating immune cells, such as monocytes, play an important role in wound healing. We find that our CTM shows improved monocyte adhesion and viability over collagen coated tissue culture plastic (TCP). Markers of macrophage differentiation, such as IL-8 and IL-1beta, upregulated at early time points in monocytes cultured on CTM. This suggests that the use of CTM may be able to promote the immunological progression of wound healing.

REFERENCES

1. Eming SA et al. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol. 2007 Mar;127(3):514-25.
2. Demidova-Rice TN et al. Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. Adv Skin Wound Care. 2012 Jul;25(7):304-14.
3. Sindrilaru A et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest*. 2011 Mar;121(3):985-97.
4. Sorokin L et al. The impact of the extracellular matrix on inflammation. *Nat Rev Immunol*. 2010 Oct;10(10)
5. Xue J et al. Transcriptome-Based Network Analysis Reveals a Spectrum Model of Human Macrophage Activation. *Immunity*. 2014 Feb 20;40(2):274-88.