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**Positive clinical outcomes of cryopreserved human placenta  
membrane in treatment of diabetic foot ulcers**

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## **Abstract;**

The placenta has biological properties that are beneficial to the wound healing process of diabetic foot ulcers (DFU). It contains mesenchymal stem cells (MSCs). MSCs are 'multipotent', meaning they can produce several types of specialized cells, such as cartilage, bone, and fat cells that can repair damage from illness or injury. The aim of this report is to analyze the scientific evidence found in literature on the use of the placenta membrane to stimulate DFU healing.<sup>(1)</sup>

\*Method;

1-Sample collection and transportation: Stem cell isolation from placenta derived blood is done with the aid of a pump .

2-Isolation and analysis: From sample of placental blood, stem cells are isolated first. This is followed by chemical analysis where the cells are inspected to ensure their purity. Moreover chemical analysis results must assure that the isolated stem cells have the potential for treatment of conditions of illness.

3-Processing and storage (Cryopreservation): placental blood units are deprived of their erythrocytes (RBC's) by a process known as "red blood cell depletion".

Next, plasma is removed with the help of process called "volume reduction". The selected stem cells are preserved by keeping them at sub-zero temperatures in liquid Nitrogen".

This technique is known as "cryogenic preservation" which enhances shelf life of stem cells by inhibiting any microbial activity and maintaining ideal sterile conditions. The isolated stem cells are cryogenically preserved in a vapor phase nitrogen tank at very low temperatures of the order of 83 K.

They are then retrieved from the public or private banks to be used for implantation in the body of patients.

Firstly standard wound care procedures including wound cleaning are done before applying vCHPM weekly each case progression is documented for accuracy and completion.<sup>(2)</sup>

## **Introduction**

Diabetes is the inability of our body to produce or use insulin. Insulin is a hormone which allows your body to turn sugars and glucose to energy. So if a body has difficulty in metabolizing glucose and sugar there will be decrease in energy production, prevent oxygen and nutrients from energizing cells, prevent immune system from functioning properly and hence delay wound healing and ulcers. There are many other factors that contribute to delayed healing in such individuals like decreased or impaired growth factor (GF) production, angiogenic response, macrophage function, collagen accumulation and quantity of granulation tissue<sup>(3)</sup>. Many complications are associated with diabetic foot ulcers such as infections which may lead to hospitalization and increase risk of amputation due to significant tissue destruction and loss of normal foot function. Amputation will not only lead to physical disabilities but also to high risk of reamputation and psychiatric problems reduced quality of life and increase mortality rate. The 1 year post lower extremity amputation mortality rate has been reported between 10% and 50% and the 5 year post amputation mortality rate has been reported as high as 30-80%. Thus one of the most important goals of wound care center is to reduce the need for amputation and hospitalization and provide treatment that lead to fast and durable wound closure. In recent years a number of advanced wound care products have become available for management of (CDFU) one of them is the use viable cryopreserved human placental membrane<sup>(4)</sup>. These components are a structural matrix a native cocktail of GF and mesenchymal stem cells(MSC). Postpartum placenta

has a disk-shaped form 16–20 cm in diameter, weighing 500 g on average. Trophoblast cells, mesenchymal cells, and endothelial cells of vessels are the main cell types of the placenta. The placenta has several features that determine its function as well as the possibility of effective application in clinics and in biotechnology. Trophoblast cells are protected from the immune system, due to reduced expression of the major histocompatibility complex (MHC), apoptosis-inducing mechanisms, and the influence of hormones and growth factors on the cells of the immune system, thus making it difficult for the immune system to recognize these cells. Trophoblast cells synthesize a number of hormones, one of them is "estradiol" which causes proliferation of the endometrium and mammary glands, causes calcium retention, and antisclerotic effect this also explains the therapeutic efficiency of the components of the placenta in the treatment of respective pathologies. Extracts are obtained by lysing human placental tissues collected at full-term delivery. Therefore, the extracts do not contain cells but are rich in a wide range of proteins, minerals, amino acids, and steroid hormones. According to the data of various research groups, such extracts possess anti-inflammatory, analgesic antioxidant, cyto-, radioprotective and anti-allergic properties and express hormonal activity as well as stimulate proliferation and reparative processes "extracts isolated from the late gestation placenta possess the highest biological activity". Pronounced positive effects were received in the treatment of wounds, non-healing ulcers, and burns rate of epithelialization was significantly increased and a decrease of infiltration and reduction of the pain syndrome were observed. The extracts accelerate the wound healing in animals with the diabetes model, hence it was interpreted as a treatment for diabetic ulcers and angiopathy. By now, cells from, placental have been successfully isolated, phenotyped, and characterized. Isolation of the cells from placental villi requires specific protocol by application of DNase<sup>(1)</sup>.

**\*Mechanism;**

The mechanism of action of placental extracts in the wound healing is by inhibiting the proinflammatory response and promoting increased expression of the anti-inflammatory factor IL-10, which is important during the early inflammatory stage and with the increase production of ;

**1-TGF- $\beta$**  in the early phase (TGF-beta stimulates the formation of granulation tissue which increases the rate of wound closure in full thickness skin wounds of regeneration)

**2-VEGF**(Vascular endothelial growth factor produced by cells that stimulates the formation of blood vessels "angiogenesis" ) in the late phase.

**3-Presence of FGF**(This protein functions as a modifier of endothelial cell migration and proliferation, as well as an angiogenic factor).

**4-Increase of expression of CD31**(Platelet endothelial cell adhesion molecule PECAM-1 makes up a large portion of endothelial cell intercellular junctions).<sup>(1)</sup>

**\*Benefits;**

- Naturally conforms to complex anatomies and may be used over exposed bone, tendon, joint capsule, and muscle.
- Designed for application directly to acute and chronic wounds.
- Flexible, conforming cover that adheres to complex anatomies.
- Immune neutral.
- Alternative to autologous skin grafting that eliminates the pain, comorbidities and procedure time associated with obtaining autologous grafts.
- No need for sutures.
- Available in multiple sizes to reduce wastage.<sup>(5)</sup>

## **Discussion;**

**Study1;** The first study was done in china on a 57 year female with type2 diabetes mellitus and severe foot ulcer. Conventional therapies had no effect on foot. She was treated with weekly applications of cryopreserved placenta

-Result; This patient foot was healed with no complications observed and no recurrence occurred in next 6 months.<sup>(6)</sup>

**Study2;**A multicenter, open-label, single arm trial of up to 17 week duration study was made in USA from Dec.2014 till Feb.2016 with a total of 31 patients whom were suffering from complex diabetic foot wounds <15cm that extend toward the dermis with evidence of exposed muscle or tendon. They were also suffering from significant comorbidities (80% were hypertensive, 60% smokers, 55% with heart disease, 45% with previous partial foot amputation , 3 of them were ESRD and were on hemodialysis ). There was a total of 28 men and 3 women with mean age of 63.5 years. They were treated with weekly application of cryopreserved human placenta membrane graft .

-Result; The primary end point of this study was 100% granulation of the wound by week 16. Secondary end point was 92.3% wound closure by week16(100% reepithilization of wound area). As determined by investigator here was complete wound closure by week 17 (100% reepithlization). <sup>(4)</sup>

**Study3;** 3 cases of chronic ulcerations - 1 arterial ulcer (AU), 1 pressure ulcer (PU), and 1 recurrence of a pyoderma gangrenosum ulcer (PGU) - that had failed previous courses of standard wound care for 4 weeks and were subsequently treated with vCHPM to determine if the treatment is an effective modality for treating wounds of these etiologies .They were treated with weekly applications of vCHPM. Each wound area was recorded and photographed on a weekly basis and wound area reduction also was charted weekly.

-Result ;The PU and AU both reached full closure in 4 and 5 weeks, respectively, without complication. The patient with the PGU achieved 64% closure after 9 applications of vCHPM. Hence Viable CHPM is an effective treatment modality for wounds of diverse etiologies and shows better results than have been previously published with standard of care.<sup>(7)</sup>

## **Conclusion;**

Weekly application of cryopreserved human placenta membrane is a valuable treatment option for chronic diabetic foot ulcers within a short period of time even in those with severe end stage diseases which indicate that it's a safe procedure with low or no side effects and reduces the risk of infections and amputation but works with less efficiency in cases of hyperactive immune diseases. It also opens a new gate as a source of potential treatments for wide range of diseases, many of which until now are incurable or have severely limited treatment options.

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