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Human Platelet Lysate Enhances Wound Healing

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Background

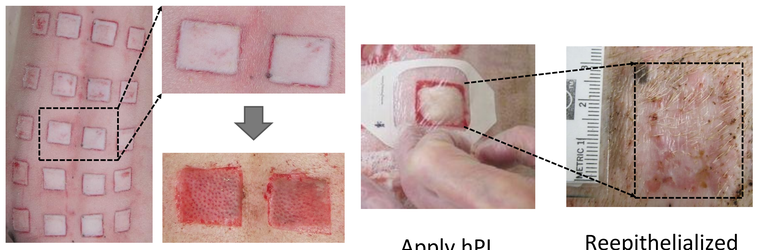
The preferential use of improvised explosive devices in modern battle has resulted in higher incidences of burn and acute wound injuries affecting increasing percentages of total body surface area (TBSA). The current standard of care for these injuries is immediate autografting or initial coverage with allografts followed by delayed autografting. This approach has not proven to be ideal due to the paucity of suitable donor sites, the high cost of hospitalization and materials throughout the process, and the offensive additional pain, scarring and risk of infection. New tissue engineering therapies offer an opportunity to overcome these challenges and to accelerate wound repair through the delivery of numerous growth factors (GFs) to the wound bed. The delivery of autologous platelet rich plasma (PRP) has shown enhanced wound healing, but its use is not always feasible in the battlefield or for patients with severe trauma**. Human platelet lysate (hPL) is a safe alternative source of concentrated human platelet-derived GFs that can be readily and easily applied to the wounds.** These GFs provide highly active biological cues which can provide signaling cues for improving tissue viability and initiating tissue repair for wound healing. Furthermore, the lyophilization of hPL enables its immediate use in austere and emergency settings. **The work presented herein evaluated the efficacy of hPL to accelerate skin wound healing.**

Methods and Materials

Preparation of hPL: Expired platelet units were acquired from FDA-registered AABB accredited blood banks and lysed using a freeze-thaw process. Cell debris and clotting factors were removed. Each lot was produced by pooling >100 donors.

Cell Culture: Routine cell culture techniques were utilized to assess the effects of hPL on human bone marrow derived mesenchymal stromal cell (hMSC), human adipose derived stem cells (hASCs), and normal human dermal fibroblast (NHDF) proliferation. Cells were cultured in medium supplemented with either 5.0% or 10% hPL, or 10% FBS. Proliferation of each cell type was quantified over increasing cell passages. The scratch assay was utilized to assess the effects of hPL on NHDF migration. Concentrations of PDGF-BB, VEGF, EGF, and bFGF in hPL, and pro-collagen and MMP-2 released from NHDFs, were quantified using human-specific ELISA kits.

Efficacy of pPL in vivo: Porcine PL (pPL) was produced from whole blood to evaluate the healing potential in a deep partial thickness (DPT) porcine burn wound model. The model created DPT wounds (1" x 1") on the back of one Yorkshire pig (n=4 burns per treatment group). The wounds were treated at every dressing change for two weeks with either triple antibiotic (standard of care) or pPL reconstituted in 3% alginate (w/v %). The rate of reepithelialization of wounds was evaluated over time on Days 7, 11, 14, 18, and 28. Scar depth and contraction was evaluated at Day 28.



DPT Burns Day 0 | Excise necrotic tissue Day 2 | Apply hPL for two weeks | Reepithelialized Wound Day 28

Figure 1: Overview of efficacy testing in the DPT porcine burn wound model

Results

Comparable Growth Factor Concentrations Across Multiple Lots of hPL

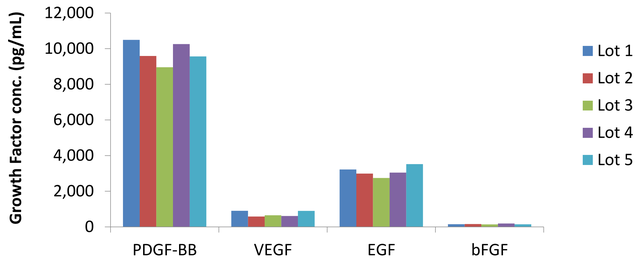


Figure 2: Individual concentrations of four important growth factors in five different lots of hPL, as quantified by ELISA. Growth factor levels are comparable for each lot.

Ex vivo Expansion of NHDF with hPL: Decreased Doubling Time = More Cells, Faster

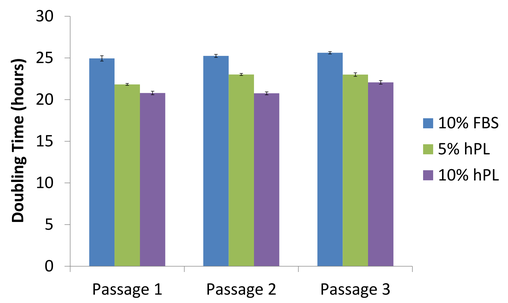


Figure 3: Data represents the average doubling time NHDF (n=3 donors) over multiple passages in αMEM supplemented with different concentrations of hPL, or FBS. Culture of NHDFs with hPL resulted in decreased doubling times.

Ex vivo Culture of NHDF with hPL: Protein Production is Enhanced

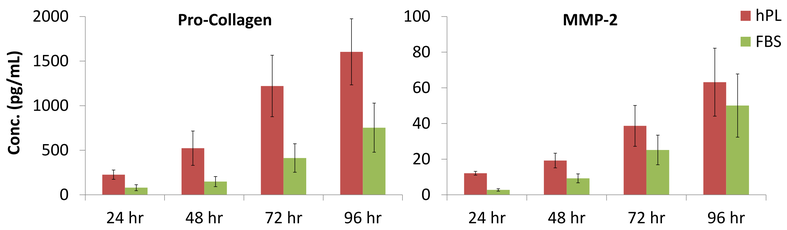


Figure 4: Data represents the average concentration of released collagen and MMP-2 following culture of NHDF (n=3 donors) with hPL or FBS. Culture with hPL yielded increased protein production.

Ex vivo Expansion of hMSCs and hASCs with hPL: Decreased Doubling Time = More Cells, Faster

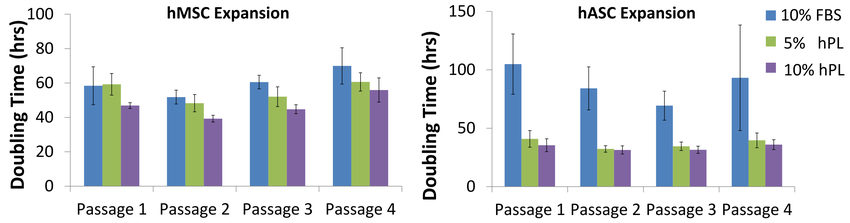


Figure 5: Data represents the average doubling time for hMSCs (top, n= 9 donors) and hASCs (bottom, n = 6 donors) over multiple passages in αMEM supplemented with different concentrations of hPL, or FBS. Culture of hMSCs and hASCs with hPL resulted in decreased doubling times.

Ex vivo Culture of NHDF with hPL: Fourfold Increase in NHDF Migration at 72h

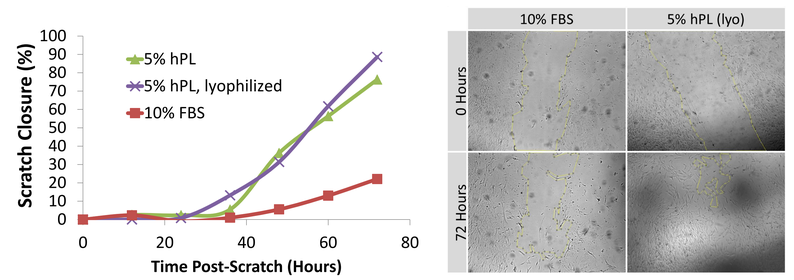


Figure 6: Data represents the percentage scratch closure for NHDF over 72 after culture in media supplemented with hPL, lyophilized hPL, or FBS. The images used in the calculation for FBS and lyophilized hPL are shown in the image panels. The migration promoting activity of hPL is both unaffected by the lyophilization process and is favored over culture with FBS.

Administration of hPL to DPT Burn Wounds: Faster Reepithelialization and Decreased Scarring

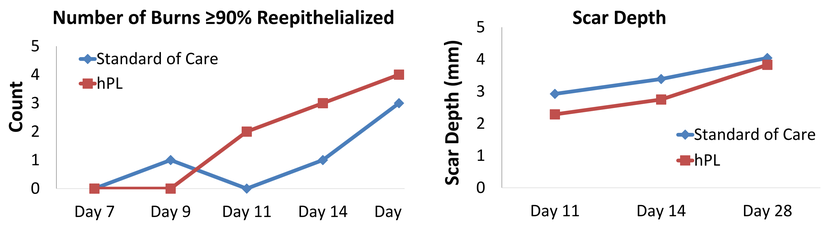


Figure 7: Data represents the number of burns that were ≥ 90% reepithelialized over time (left panel) and the average scar depth (right panel) for DPT burns treated with either hPL or triple antibiotic ointment. Increased reepithelialization and decreased scar depth was observed for DPT burn wounds treated with hPL.

Applicability to the Warfighter

Lyophilized hPL can be carried and reconstituted by our warfighters for the immediate treatment of soft tissue injuries.

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