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A novel platelet lysate hydrogel for endothelial cell and mesenchymal stem cell-directed neovascularization

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ABSTRACT

Mesenchymal stem cells (MSC) hold promise in promoting vascular regeneration of ischemic tissue in conditions like critical limb ischemia of the leg. However, this approach has been limited in part by poor cell retention and survival after delivery. New biomaterials offer an opportunity to localize cells to the desired tissue after delivery, but also to improve cell survival after delivery. Here we characterize the mechanical and microstructural properties of a novel hydrogel composed of pooled human platelet lysate (PL) and test its ability to promote MSC angiogenic activity using clinically relevant *in vitro* and *in vivo* models. This PL hydrogel had comparable storage and loss modulus and behaved as a viscoelastic solid similar to fibrin hydrogels despite having 1/4–1/10th the fibrin content of standard fibrin gels. Additionally, PL hydrogels enabled sustained release of endogenous PDGF-BB for up to 20 days and were resistant to protease degradation. PL hydrogel stimulated pro-angiogenic activity by promoting human MSC growth and invasion in a 3D environment, and enhancing endothelial cell sprouting alone and in co-culture with MSCs. When delivered *in vivo*, the combination of PL and human MSCs improved local tissue perfusion after 8 days compared to controls when assessed with laser Doppler perfusion imaging in a murine model of hind limb ischemia. These results support the use of a PL hydrogel as a scaffold for MSC delivery to promote vascular regeneration.

Statement of Significance

Innovative strategies for improved retention and viability of mesenchymal stem cells (MSCs) are needed for cellular therapies. Human platelet lysate is a potent serum supplement that improves the expansion of MSCs. Here we characterize our novel PL hydrogel's desirable structural and biologic properties for human MSCs and endothelial cells. PL hydrogel can localize cells for retention in the desired tissue, improves cell viability, and augments MSCs' angiogenic activity. As a result of these unique traits, PL hydrogel is ideally suited to serve as a cell delivery vehicle for MSCs injected into ischemic tissues to promote vascular regeneration, as demonstrated here in a murine model of hindlimb ischemia.

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1. Introduction

Regenerative therapies hold great promise for improving the treatment of patients with disabling chronic diseases from tissue ischemia, such as critical limb ischemia (CLI). CLI is the most severe form of peripheral arterial disease (PAD), whereby the tissues of the leg do not have sufficient perfusion to meet resting tissue demands. This causes rest pain, tissue loss, or gangrene. These patients require timely revascularization in order to limit their risk of major amputation [1–4]. However, approximately half of CLI patients do not have traditional revascularization options [5]. Thus novel approaches to promote vascular regeneration, such as cellular therapy, are critically needed [6]. Mesenchymal stem cells (MSCs) are a particularly attractive cell to stimulate vascular regeneration as they promote neovascularization [7,8] and have

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robust paracrine activity on surrounding cells, including endothelial cells (EC) [9].

Despite generally optimistic summaries of cellular and regenerative therapy trials in PAD [10,11], the effectiveness of cellular therapies in preventing amputations remains to be proven [11]. This is likely due in part to poor cellular retention after intravascular delivery (~1% 2 h after arterial injection) [12] or direct injection into the muscular tissue (11% 1 h after injection into pig hearts) [13]. Limited retention of cells after delivery provides opportunities for the use of biomaterials to help localize these cells and retain them in the desired tissue. Advancing technologies enable biomaterials that may be designed to promote cell survival and engraftment into these tissues for sustained function.

In order to meet the clinical needs of cellular therapy with biomaterial solutions, we have developed a novel hydrogel from human platelet lysate (PL). PL is a promising non-xenogenic serum supplement designed for the expansion of human MSCs that replaces fetal bovine serum [14]. We have shown that PL significantly increases human MSC expansion capacity compared to fetal bovine serum and can re-stimulate senescing MSCs [15]. In addition to PL's nutritive effect on MSCs, PL is rich in a variety of endogenous growth factors [16], including platelet derived growth factor-BB (PDGF-BB) [17]. PDGF-BB has recently been shown to improve MSC engraftment into tissues [18], therefore PL is a nearly ideal supplement that when incorporated into a gel could encourage MSC retention, viability, and sustained neovascular activity.

The objective of this study was to develop and characterize the mechanical properties of a PL scaffold and assess the biologic effect of PL gel on MSCs and ECs *in vitro* and *in vivo*. Here we test the hypotheses that PL gel has desirable mechanical properties for cell delivery and supports MSC ingrowth. Additionally we examine the ability of PL to enhance the pro-angiogenic activity of MSCs on ECs in 3D culture. Finally, we test whether MSCs in PL gel will promote neovascularization *in vivo* in an immunocompromised murine model of hind limb ischemia (HLI).

2. Materials and methods

2.1. Production of fibrinogen-rich platelet lysate hydrogels

Two units of expired human platelets were obtained from the Emory University blood bank through an IRB approved research protocol. The platelets were pooled and exposed to two sequential freeze/thaw cycles [freezing at −80 °C for 48 h, rapidly thawing at 37 °C for 8 h] followed by centrifugation at 1500*g* for 10 min. The supernatant was collected and stored at −20 °C. Prior to use, the platelet lysate was thawed at 37 °C, centrifuged at 10,000*g* for 10 min in 1.5 mL microcentrifuge tubes, and sequentially filtered through 0.45 and 0.2-μm syringe tip filters. Fibrinogen content was determined using an ELISA kit for human fibrinogen (Molecular Innovations) [17].

For hydrogel production, an activating solution was prepared containing αMEM (Corning), bovine thrombin (Sigma), and $CaCl_2$ (Sigma). Cells were suspended in αMEM at pre-specified concentrations and added to the activating solution. Hydrogels were polymerized by adding PL to the activating solution in a 1:1 ratio with a final concentration of $CaCl_2$ and thrombin at 5 mM and 2 U/mL in a 50% PL gel, respectively. For the control fibrin hydrogel, fibrinogen from human plasma (Sigma) was dissolved in αMEM then mixed with activating solution [final fibrinogen concentration was 2.5 mg/mL and 1.0 mg/mL for high and low concentration fibrin gels, respectively]. The 2.5 mg/mL fibrin-only hydrogel was chosen as a control because it represents a physiologically relevant concentration of fibrinogen that is equivalent to that found in human plasma. Additionally, the use of 2.5 mg/ml fibrin gels has been used extensively in the hydrogel invasion assay described below [19]. The 1.0 mg/mL fibrin gels were selected as an additional control to more closely represent the concentration of fibrinogen found in PL solution. The 1.0 mg/ml fibrin hydrogels were mechanically equivalent to the 50% PL hydrogels, and hydrogels containing less than 1.0 mg/mL fibrinogen either would not form hydrogels or formed hydrogels that lacked the durability to be utilized in our assays. The addition of $CaCl_2$ to fibrin-only hydrogels caused precipitation of calcium phosphate, so for generation of fibrin-only hydrogels an activating solution was prepared containing only thrombin in αMEM (which contains of $CaCl_2$ at a concentration of 1.80 mM).

2.2. Rheology

A Physica MCR 501 cone and plate rheometer (Anton-Paar, Graz, Austria) was used (2.014° cone angle and 24.960 mm tool diameter) to measure the viscoelastic properties of fibrin and PL gels. Gels were formulated as described above. Strain sweeps from 0.1 to 100% strain at 1 Hz were performed to establish the linear regime. Frequency sweeps from 0.01 to 1 Hz in the linear regime at 0.5% strain were then performed to obtain the frequency-dependence of the storage modulus (G′) and loss modulus (G″). Average storage moduli were calculated from the average value over the frequency range for three different gel constructs.

2.3. Confocal imaging

PL and fibrin hydrogels were formed as described above at a volume of 0.5 mL and polymerized in 24-well 1.5 glass bottom tissue culture plates [20,21]. Laser scanning confocal microscopy (LSM 700, Carl Zeiss, Inc.) with a 63X oil immersion objective was performed for visualization of the fibrin network using 5% FITC-labeled fibrinogen (Sigma). At least 3 gels per group were imaged at a distance at least 25 μm above the glass interface to avoid heterogeneities in the network near contact with the glass surface.

2.4. Scanning electron microscopy

40 μL hydrogels were formed as described above and allowed to polymerize at 37 °C for 1 h, then fixed overnight in 2% glutaraldehyde. After fixation, samples were dehydrated with an ethanol gradient, critically point dried, and sputter coated with gold–palladium. SEM images were acquired at 20,000X magnification using a Topcon DS-130F Field Emission Scanning Electron Microscope.

2.5. FITC-dextran diffusion

Solute diffusion through PL and fibrin hydrogels was assessed as previously described [22]. Briefly, 0.5 mg/mL 70 kDa FITC-dextran (Sigma) was added to 0.5 mL hydrogels in microcentrifuge tubes prior to polymerization. The hydrogels were then allowed to polymerize and covered with 0.5 mL of phosphate buffered saline (PBS, pH 7.4) with or without 10 μg/mL of aprotinin. The PBS was collected off of each hydrogel and replaced with fresh PBS at predetermined time points for up to 20 days. Each time point was performed in triplicate. The concentration of FITC-dextran released into the supernatant was determined by comparing sample fluorescence to a standard curve using a Synergy-HT microplate leader with 485/20 Ex, 528/20 Em filter set.

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