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Specific recruitment of circulating angiogenic cells using biomaterials as filters ☆

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ABSTRACT

Endogenous recruitment of circulating angiogenic cells (CACs) is an emerging strategy to induce angiogenesis within a defect site, and multiple recent strategies have deployed soluble protein releasing biomaterials for this purpose. However, the way in which the design of biomaterials affects CAC recruitment and invasion are poorly understood. Here we used an enhanced-throughput cell invasion assay to systematically examine the effects of biomaterial design on CAC recruitment. The screens co-optimized hydrogel presentation of a stromal-derived factor-1α (SDF-1α) gradient, hydrogel degradability, and hydrogel stiffness for maximal CAC invasion. We also examined the specificity of this invasion by assessing dermal fibroblast, mesenchymal stem cell, and lymphocyte invasion individually and in co-culture with CACs to identify hydrogels specific to CAC invasion. These screens suggested a subset of MMP-degradable hydrogels presenting a specific range of SDF-1α gradient slopes that induced specific invasion of CACs, and we posit that the design parameters of this subset of hydrogels may serve as instructive templates for the future design of biomaterials to specifically recruit CACs. We also posit that this design concept may be applied more broadly in that it may be possible to utilize these specific subsets of biomaterials as "filters" to control which types of cell populations invade into and populate the biomaterial.

Statement of Significance

The recruitment of specific cell types for cell-based therapies *in vivo* is of great interest to the regenerative medicine community. Circulating angiogenic cells (CACs), CD133+ cells derived from the blood stream, are of particular interest for induction of angiogenesis in ischemic tissues, and recent studies utilizing soluble-factor releasing biomaterials to recruit these cells *in vivo* show great promise. However, these studies are largely "proof of concept" and are not systematic in nature. Thus, little is currently known about how biomaterial design affects the recruitment of CACs.
In the present work, we use a high throughput cell invasion screening platform to systematically examine the effects of biomaterial design on circulating angiogenic cell (CAC) recruitment, and we successfully screened 263 conditions at 3 replicates each. Our results identify a particular subset of conditions that robustly recruit CACs. Additionally, we found that these conditions also specifically recruited CACs and excluded the other tested cells types of dermal fibroblasts, mesenchymal stem cells, and lymphocytes. This suggests an intriguing new role for biomaterials as "filters" to control the types of cells that invade and populate that biomaterial.

1. Introduction

The formation of functional vasculature is a key step during tissue regeneration [1]. Delivery of pro-angiogenic soluble factors, such as vascular endothelial growth factor (VEGF), is a widely explored approach to induce vascularization and promote tissue regeneration, but soluble VEGF delivery and similar approaches

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show little clinical benefit [1–3]. Thus, alternative approaches to induce tissue vascularization are needed to improve clinical tissue regeneration, and one alternative approaches is the use of pro-angiogenic cell types to stimulate angiogenesis.

This strategy potentially circumvents limitations associated with the delivery of a single soluble factor, because the delivered cells can secrete a wide range of the appropriate soluble factors and can respond to changes within the diseased tissue [4]. One particular pro-angiogenic cell type, blood derived CD133+ cells also known as circulating angiogenic cells (CACs), have been explored for use in such strategies because of their well-characterized roles in vascular biology including blood vessel maintenance, formation of new blood vessels, and the repair of damaged blood vessels [5–9]. Further, recent studies suggest that CACs more effectively promote angiogenesis than other commonly utilized cell types, such as mesenchymal stem cells [10,11].

CACs reside in the bone marrow with only a small number in circulation, but upon injury, the number in circulation increases due to systemic cues originating from the wound site [5–9]. Once at the wound site, CACs mediate angiogenesis [5–9], likely via paracrine mechanisms [5,6,8,9,12]. Strategies that augment CAC abundance in injured tissues in healthy animal models have shown markedly improved outcome at the wound site via increased levels of angiogenesis within diabetic wounds, ischemic cardiac muscle, critical limb ischemia, and bone defects [4,13–24]. However, typical therapeutic strategies involving harvest of CACs from the patient and re-delivering the CACs into the wound site. These strategies are hampered by the relatively low number of CACs present in patient blood and bone marrow [6], a lack of unique cell surface markers for CACs [5–9], and regulatory difficulties [25,26]. Thus, strategies to harness endogenous CACs *in vivo* may be particularly valuable [4,25].

Multiple studies of endogenous CAC recruitment in animal models of ischemic myocardium, diabetic wounds, and within subcutaneous sites have demonstrated the general efficacy of this type of approach [21,27–34]. A subset of these approaches utilize biomaterials to both deliver soluble recruitment factors and serve as a matrix for the recruited CACs [31,32,34]. However, little is known about how basic biomaterial design parameters such as stiffness, degradability, and encapsulated soluble factor content affect CAC invasion into the biomaterial. Additionally, previous biomaterial-based recruitment strategies are not specific to CAC recruitment and invasion, and thus simultaneous recruitment of multiple cell types occurs [32]. It is possible that these additional recruited cell types may be detrimental at the recruitment site by promoting increased inflammation from lymphocyte recruitment [35,36] or increased fibrosis from fibroblast recruitment [37].

Herein, we use chemically well-defined, hydrogel biomaterials to systematically study the effects of biomaterial stiffness, degradability, and encapsulated stromal-derived factor-1α (SDF-1α) content on the magnitude and specificity of CAC invasion. We utilized a thiol-ene chemistry to rapidly polymerize our hydrogels within a plate-based format amenable to enhanced-throughput screening [38]. We employed 8-arm, poly(ethylene glycol) (PEG)-based hydrogels due to the ability to easily modulate cross-linker molecule identity, stiffness, and soluble factor incorporation within this hydrogel system [39,40]. We also utilized a combination of mathematical modeling and empirical measurements to determine that a range of SDF-1α concentration gradients could be presented within the hydrogels and that these gradients were largely independent of hydrogel formulation. Thus, we were able to efficiently multiplex the variables of soluble gradient presentation and hydrogel formulation in these screening studies. These studies identified a subset of hydrogel formulations that promoted robust and specific CAC invasion *in vitro*. Importantly, this identified subset of hydrogel formulations serves as the first specific design criteria presented for the design of biomaterials to specifically recruit CACs. Furthermore, we posit that such formulations may be a promising tool for local recruitment of CACs in future therapeutic approaches seeking to harness CACs for angiogenesis as part of an *in vivo* tissue regeneration strategy. While the present work only highlights the possibility of this concept, we posit that with additional investigation and robustness testing, specific hydrogel formulations may be further developed that serve as highly specific and robust cell "filters," which may be a promising new tool for tissue regeneration strategies.

2. Methods

2.1. PEGNB synthesis

Multi-arm, alcohol terminated, 8-arm PEG with a tripentaerythritol core (JenKem Technology USA) were functionalized with norbornene groups via reaction with norbornene anhydride, which is well-described elsewhere [39,40]. All other materials for this synthesis were obtained from Sigma-Aldrich. Briefly, norbornene acid at a 10x molar excess versus PEG alcohol groups and dicyclohexylcarbodiimide (DCC) at a 5x molar excess versus PEG alcohol groups were both dissolved in anhydrous dichloromethane (DCM). PEG, pyridine at a 5x molar excess versus PEG alcohol groups, and 4-(dimethylamino) pyridine at a 0.5x molar excess versus PEG alcohol groups were also dissolved within a separate DCM solution. Once the norbornene anhydride had formed (indicated by formation of a white precipitate), both DCM solutions were combined. This combined solution was stirred overnight, and the solution was then filtered to remove precipitates. PEG was recovered via precipitation in ethyl ether and was purified via dialysis against deionized (DI) water. Functionalization of the PEG polymer with norbornene groups (PEGNB) was confirmed via NMR.

2.2. Hydrogel formulation and photopolymerization

PEGNB hydrogels were polymerized via a thiol-ene reaction with dithiol cross-linkers in all cases. Precursor solutions contained PEGNB and dithiol cross-linkers, which were dissolved at desired concentrations in phosphate buffered saline (Fisher Scientific). In all cases, either a PEG-dithiol cross-linking molecule (3.4 kDa, Laysan Bio) or KCGGPQGIWGQGCK, a matrix metalloproteinase (MMP) degradable peptide [41], was utilized to cross-link hydrogels. CRGDS peptide (GenScript) was dissolved in these solutions at a concentration of 2 mM. When indicated, human, recombinant, stromal-derived factor-1α (R&D Systems) was also dissolved in these solutions by diluting from a 100 μg/mL stock solution containing 1 mg/mL bovine serum albumin as a carrier. Before polymerization, Irgacure 2959 photo-initiator (Ciba) was added to a concentration of 0.05 wt% via a 10-fold dilution from a 0.5 wt% stock solution. This reaction was commenced by sustained exposure to UV light (365 nm at 3.3 mW/cm^2 for 10 min unless otherwise specified). A complete list of all hydrogels used in the course of this work is included as Supplemental Table 1.

2.3. Culture of human circulating angiogenic cells

CD133+ cells collected from mobilized peripheral blood were purchased from AllCells, LLC. These cells were collected by AllCells, LLC from one donor (26 year old, African American, female) utilizing an IRB-approved protocol. For all experiments, these cells were cultured in StemSpan Serum Free Expansion Medium II (StemSpan SFEM II, StemCell Technologies) with CC100 supplement (StemCell Technologies) and 1% Penicillin/Streptomycin (Gibco). CD133+