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Biomaterials that promote cell-cell interactions enhance the paracrine function of MSCs

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ABSTRACT

Mesenchymal stromal cells (MSCs) secrete paracrine factors that play crucial roles during tissue regeneration. Whether this paracrine function is influenced by the properties of biomaterials in general, and those used for cell delivery in particular, largely remains unexplored. Here, we investigated if three-dimensional culture in distinct microenvironments - nanoporous hydrogels (mean pore size ~5 nm) and macroporous scaffolds (mean pore size ~120 μm) - affects the secretion pattern of MSCs, and consequently leads to differential paracrine effects on target progenitor cells such as myoblasts. We report that compared to MSCs encapsulated in hydrogels, scaffold seeded MSCs show an enhanced secretion profile and exert beneficial paracrine effects on various myoblast functions including migration and proliferation. Additionally, we show that the heightened paracrine effects of scaffold seeded cells can in part be attributed to N-cadherin mediated cell-cell interactions during culture. In hydrogels, this physical interaction between cells is prevented by the encapsulating matrix. Functionally blocking N-cadherin negatively affected the secretion profile and paracrine effects of MSCs on myoblasts, with stronger effects observed for scaffold seeded compared to hydrogel encapsulated cells. Together, these findings demonstrate that the therapeutic potency of MSCs can be enhanced by biomaterials that promote cell-cell interactions.

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1. Introduction

Inefficient natural healing of several debilitating musculoskeletal injuries such as volumetric tissue loss and severe muscle trauma has led to an unmet clinical demand for potent therapies. Strategies that embody the principles of tissue engineering and regenerative medicine, such as autologous and allogeneic stem cell therapies, have gained considerable traction in the past decade [1–3]. As witnessed by their extensive application in registered clinical trials, mesenchymal stromal cells (MSCs) in particular have attracted widespread interest as safe and effective candidates for the regeneration of injured tissues [4].

In addition to their well-known multi-lineage differentiation potential, an intriguing characteristic of MSCs is their ability to secrete a wide range of bioactive cytokines and growth factors that can influence nearby cells via paracrine signaling [5,6]. For instance, MSCs have been documented to be a generous source of bioactive factors including VEGF, FGF, HGF, IGF, PDGF, ILs, and MMPs [6–9]. These secreted factors can in turn orchestrate various biological processes that may be desirable for tissue regeneration such as angiogenesis, immune modulation, and cell recruitment and differentiation [10,11]. An increasing body of evidence suggests that the therapeutic benefits observed following MSC transplantation may largely be attributed to the paracrine and trophic function of these cells, rather than their differentiation potential [12]. For this reason, research efforts by a number of groups have focused on strategies that can enhance the secretory function of MSCs. These include preconditioning in hypoxic conditions, genetic manipulation to overexpress certain growth factors, and cytokine stimulation [13,14]. For example, in a previous study we reported that the paracrine effects of MSCs stimulated with soluble VEGF and IGF-1 enhanced myoblast migration, proliferation, and survival *in vitro*, and led to robust muscle regeneration *in vivo* [15]. Recent studies

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have highlighted the importance of cell-cell interactions in regulating the paracrine activity of MSCs by reporting that 3D multicellular spheroids show superior secretion profiles compared to similar number of MSCs in monolayer culture. For instance, Lee et al. reported that MSC spheroid formation via calcium dependent E-cadherin interactions led to improved paracrine activity and therapeutic efficacy in a rat myocardial infarction model [16]. Similar paracrine effects have been reported for spheroids formed using adipose derived [17], umbilical cord derived [18], and bone marrow derived MSCs [19].

Researchers have also explored the feasibility of applying MSC derived conditioned medium (MSC-CM) to stimulate tissue regeneration. For instance, Osugi et al. reported enhanced *in vivo* bone formation in rat calvarial defects after the application of MSC-CM that contained high quantities of IGF-1 and VEGF [20]. Others have used this cell-free approach in spinal cord and cardiac injury models [21,22]. While the transplantation of such a cytokine rich cocktail avoids the cost and labor intensive process of cell harvesting and subsequent expansion, these cytokines may quickly lose bioactivity *in vivo* due to short half-lives. Moreover, complete recovery from severe injuries may require a prolonged delivery of paracrine factors which necessitates MSC transplantation. In the vast majority of pre-clinical and clinical studies, MSCs are administered in a bolus manner either systemically (via the circulatory system) or locally at the site of injury [23,24]. A number of drawbacks are associated with these modes of cell delivery. These include rapid cell death [25], accumulation in non-targeted tissues [26], and diffusion of the cell suspension away from the site of injury due to anoikis [27]. By providing a temporary matrix that can facilitate cell attachment, viability, and growth, biomaterials can potentially be used as local delivery depots secreting paracrine signaling molecules at sites of tissue damage. Tuning the physical properties of such biomaterial depots may offer the possibility to elicit desirable MSC activity and thereby steer the regenerative function of target progenitor cells.

A wide range of biomaterials with varying structures, chemistries, and mechanical properties have been used for *in vivo* cell delivery [28,29]. From a clinical translation perspective, nanoporous hydrogels may be considered attractive materials for cell delivery because they can be injected near the site of injury in a minimally invasive manner and adapt to the size and shape of the defect [30,31]. Commonly used hydrogels such as alginate, poly ethylene glycol (PEG), and hyaluronic acid offer the advantage of tunable chemistries and mechanical properties, but prevent changes in the morphology of encapsulated cells. On the other hand, pre-formed 3D scaffolds fabricated via 3D printing, electrospinning, or freeze drying, may not be conveniently injectable but offer macroporous microenvironments that facilitate cell spreading, migration, and establishment of cell-cell contacts [32]. While a great deal of research effort has focused on using biomaterials to direct stem cell fate, there is a lack of sufficient knowledge on how biomaterial properties influence the paracrine function of MSCs.

We hypothesized that compared to nanoporous hydrogels that are used for cell encapsulation and have pores in the range of 5–70 nm [33,34], macroporous scaffolds with pore sizes in the range of 10–200 μm may facilitate both cell-cell and cell-matrix interactions and lead to enhanced paracrine effects of MSCs. To address this hypothesis, we evaluated the secretion profile of MSCs cultured in 3D alginate based substrates that had similar mechanical and chemical properties but distinct structural microenvironments. The secreted factors were characterized, and their potential implication for muscle regeneration was investigated by analyzing the behavior and function of myoblasts cultured in conditioned media. Functional blocking experiments were used to determine the role of substrate microenvironment mediated cell-cell interactions in regulating the paracrine effects of MSCs. Our data indicate that changes in biomaterial microenvironment can elicit strong paracrine responses from MSCs by promoting local cell-cell interactions.

2. Materials and methods

2.1. Alginate processing and substrate fabrication

Ultrapure low and high molecular weight alginates (LVG, MVG) were purchased from Pronova. A 1:1 (w/w) mixture of the alginates was reconstituted to a concentration of 1% w/v, and functionalized with the cell adhesive peptide motif G_4RGDSP (Peptide 2.0, USA) using standard carbodiimide chemistry to a final degree of substitution of 20, as previously described [35]. Briefly, NHS, EDC, and the peptide motifs were added sequentially to the reconstituted alginate, and allowed to react for 20 h. The reaction was stopped using hydroxyl amine hydrochloride, and the alginates were dialyzed (MWCO 10000, Spectra/Por®) over three days in distilled water with a decreasing salt concentration. The dialyzed alginate was mixed with activated charcoal, sterile filtered, frozen, and lyophilized until use.

Hydrogels containing encapsulated cells were prepared as follows. A 2.5% w/v solution of alginate was mixed with a cell suspension containing 2×10^6 freshly trypsinized MSCs and 4% v/v of calcium sulfate slurry (1.22 M in dH_2O) using two syringes connected with a Luer-lock syringe connector. The components were mixed rapidly to a yield a cell containing alginate mixture (conc. 2% w/v) and cast between two glass plates separated by a 1 mm spacer. The mixture was allowed to ionically crosslink for 45 min before gels were punched out (diameter: 8 mm) and placed in 24 well plates with full cell culture media. Scaffolds were prepared in a similar manner, but without mixing with cells. The punched out gels (~50 mm^3) were frozen at −80 °C and lyophilized to create a porous structure. The interconnected porous structure was confirmed with scanning electron microscopy, and pore size (short axis) was manually determined using ImageJ. Freshly trypsinized MSCs were then seeded onto the 3D porous scaffolds (4×10^6 cells/cm^3) and incubated for 20 min before adding full culture media.

2.2. Mechanical characterization

Hydrogel and scaffold stiffness was determined in the wet state using a bench-top PIUMA nanoindenter (Optics 11, Amsterdam). A spherical indentation tip (radius 48.5 μm) was used to probe samples immersed in PBS up to an indentation depth of 10 μm (sample thickness ~1000 μm). Matrix scans (3×3 points, step size in each direction = 100 μm) were carried out at several different locations on a number of hydrogel and scaffold samples. Using the Oliver and Pharr model, the PIUMA nanoindenter estimated the samples' Young's modulus from the slope of the unloading curve in the region 65%–85% of maximum load.

2.3. Cell culture

MSCs isolated from the tibiae of Sprague Dawley rats via bone marrow biopsies were used in this study. Cell culture was carried out in full culture media i.e. low glucose DMEM containing fetal calf serum (10%), penicillin/streptomycin (1%), and Glutamax™ (1%). Cells were passaged at approximately 70% confluency, and were not used beyond passage 5. C2C12 myoblasts (ATCC) were cultured in full culture media and were not used beyond passage 10. To induce myogenic differentiation, the serum concentration was reduced to 5%.

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