Investigating Olfactory Mucosa derived Mesenchymal Stem Cells (OM-MSCs) for Peripheral Nerve Repair

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Introduction: Schwann cells (SCs), the glia of the peripheral nervous system (PNS), play a crucial role in supporting axon regeneration after a peripheral nerve injury. In large gap injuries (>3cm), it has been reported that native SCs become stressed due to the burden of increased proliferation [1]. The delivery of additional SCs has been successful in facilitating a more conducive microenvironment to bridge large repairs [2]. However, retrieving additional autologous SCs requires an invasive surgery, damaging a separate nerve fiber [3]. Olfactory mucosa derived mesenchymal stem cells (OM-MSCs) are responsible for continued neurogenesis in adults and may be a great alternative to autologous SCs for synthetic repair solutions. These cells are easily accessible from patients through a non-invasive biopsy. They also exhibit an unusually high proliferation rate [4], overexpress many neural genes[5], and have a high likelihood to differentiate into neural cells [6]. Limited work has been conducted on driving these stem cells to PNS fates, but recent results have been promising [6]. This study aims to further characterize these cells and evaluate their potential use in peripheral nerve repair.

Methods: OM-MSCs, Dorsal Root Ganglia (DRG), and SCs will be isolated following previously established protocols [7]. Initial studies of primary OM-MSCs focus on better characterizing isolated OM-MSCs in different environments. Two weeks after isolation, cells were fixed and stained for a neural stem cell marker (Nestin) and astrocyte cell marker [glial fibrillary acidic protein (GFAP)]. After passaging the cells 3 times, the OM-MSCs were encapsulated in 10% and 20% (w/v) Gelatin methacrylol (GelMA) hydrogels, investigating changes in morphology that occur in a 3D environment with varying degrees of stiffness. After culturing for 5 days, morphology was observed OM-MSCs were also cultured for five days in spent media from SCs.

Planned experiments will further evaluate the potential of undifferentiated OM-MSCs as a support cell in peripheral nerve repair. An ELISA will be conducted to evaluate if OM-MSCs produce NGF in direct comparison to neo-natal SCs, and OM-MSCs that have been cultured in SC spent media for 2 weeks. DRG neurite outgrowth will be quantified when cultured with OM-MSCs, SCs, OM-MSCs + SCs (50:50), and a control group. Cell monolayers will be allowed to attach and proliferate for two days without the DRGs. After two days, the media will be changed and dissociated DRGs seeded. The DRGs will be cultured for 7 days, then fixed and stained for nestin, βIII, and DAPI.

Results: The expression of GFAP and Nestin was confirmed (**Fig 1**). Changes in morphology were seen when the OM-MSCs were cultured in 20% GelMA hydrogels under a stiffness of 100 kPa [8]. Cells were thin, elongated, and exhibited long extensions. Cells cultured in the 10% GelMA hydrogel (50 kPa [8]) were observed to have no change in morphology compared to 2D culture. After culturing OM-MSCs in SC spent media for five days, expression of Nestin was decreased in roughly 20% of cells. The expression of S-100 was also confirmed throughout the entire cell population.

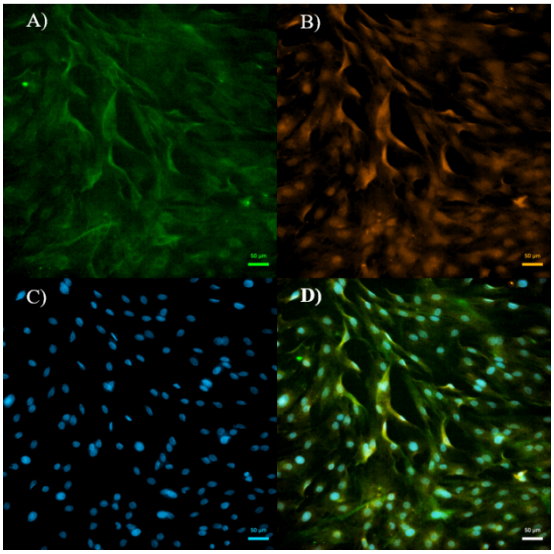

Figure 1: Representative image of OM-MSCs at 14 days of culture exhibiting robust expression of **A)** Nestin, Green **B)** GFAP, Orange **C)** DAPI, Blue, and **D)** Merged. (Scale bars = 50μm)

Discussion: Primary experiments demonstrated the expression of both central and peripheral glial markers, GFAP and S-100. Nestin, a neural stem cell marker was also expressed throughout the cell culture. Interestingly, the expression of Nestin was diminished after culturing in SC spent media, suggesting the onset of beginning stages of differentiation. Changes in morphology in 3D environments indicate that the material design will be important factor that may affect the proliferation and differentiation of the OM-MSCs similar to other stem cell types [9].

We hypothesize that the OM-MSCs will produce a similar amount of NGF as SCs, based on results from immunostaining and reports that these cells stain positive for P75, the cell receptor for NGF [6]. We also hypothesize that co-culturing DRGs with OM-MSCs will result in larger outgrowth compared to the control group and similar results compared to DRGs cultured with SCs.

Conclusion and Future Directions: In this report, we demonstrated preliminary data that support the potential use of OM-MSCs as support cells for PNS repair. In the future, we plan to terminally differentiate OM-MSCs into a SC-like phenotype based on results of differentiating mesenchymal cells derived from other tissue. We will also evaluate the inclusion of induced SCs in our recently developed adhesive and conduit biomaterials.

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