Cell-cell Contacts Mediated by Microsphere Formation Promotes Viability of Hydrogel-Encapsulated Submandibular Gland Cells

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Statement of Purpose: Every year, 40,000 patients in the U.S. are diagnosed with head and neck cancers. Most of these patients will undergo radiation therapy, which severely damages the salivary gland and results in permanent xerostomia or dry mouth [1]. Currently, there is no treatment that can fully restore function to salivary gland tissue destroyed by radiation [1]. We propose the use of poly(ethylene glycol) (PEG) hydrogels to transplant salivary gland cells to promote regeneration of functional gland tissues. PEG hydrogels are synthetic and bio-inert with controllable biological and physical properties. Therefore, PEG provides a "bottom up" approach to tissue regeneration. However, primary salivary gland cells isolated directly from mouse submandibular glands (SMG) exhibit poor viability within PEG hydrogels [2]. As cell-cell interactions are critical for survival and function of glandular cell types [3], we examined how salivary gland microsphere formation and encapsulation affected SMG viability and proliferation within PEG hydrogels.

Methods: All cells were isolated from submandibular glands of 2-6 month old female C57BL/6 mice according to a previously published protocol [4]. Microspheres were formed by 48 hour suspension culture of freshly-isolated SMG cells at a density of 1.2 * 10^6 cells/mL within untreated petri dishes. To compare cell viability of microspheres to single cells, microspheres were disassociated by trypsinization and filtration through a 40 µm strainer. Single cell suspensions or microspheres were encapsulated in 30 µL (concentration: 5*10^6 cells/mL) of 4 wt% PEG hydrogels, composed of norbornene-functionalized 4-arm PEG (MW~20,000, JenKem) and di-thiol PEG (MW~ 3,400, Laysan Bio) formed using standard photopolymerization methods [5]. Cell viability and microsphere sizes were imaged for 14 days using Live/Dead staining (Life Technologies) and confocal microscopy (Olympus, FV1000) at 0, 4, 7, and 14 days after encapsulation. Using ImageJ, microspheres were classified into small spheres (300-3000 µm^2), medium spheres (3000-10,000 µm^2), large spheres (10,000-30,000 µm^2), and cysts (>30,000 µm^2). To quantitate viability, the CellTiter-Glo (Promega) kit was utilized.

Results: Encapsulated microspheres maintained consistent and high viability (~80%) over 14 days of in vitro culture. This level of viability is comparable to non-encapsulated control cells immediately prior to encapsulation. Of the viable cells, 80% of cells were in small and medium spheres at day 0 (Fig. 1A). At day 7 (Fig. 1B), 70% of cells resided in large spheres and cysts. The number of small spheres decreased significantly ($p < 0.05$) from ~500 to ~150 per field of view at day 7. Conversely, the number of cysts increased significantly ($p < 0.05$) through day 7 from 0 to 5. The number of large and medium spheres was statistically similar at all time points. These results suggest that larger spheres have preferential survival and that cells within the spheres proliferate substantially. Measurement of ATP levels of encapsulated microspheres support this conclusion and show a statistically significant increase of 298% from days 0-4. Disruption of microspheres into single cells prior to encapsulation causes a statistically significant drop in ATP levels of 40% between days 0-4 (Fig. 1C). These results demonstrate the importance of cell-cell contacts to hydrogel-encapsulated SMG cell viability.

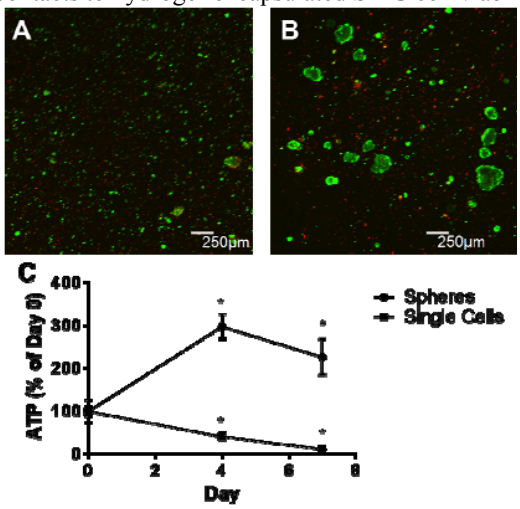

Figure 1: Encapsulated Microspheres Maintain Viability and Grow in PEG Hydrogels. Representative Live/Dead z-stacks of microspheres at day 0 (A) and day 7 (B). ATP values of hydrogel-encapsulated SMG cells normalized to day 0 (C) error bars +/- std dev, *= $p < 0.05$ versus day 0.

Conclusions: Microspheres formed from suspension cultures of submandibular gland cells provide cell-cell interactions that maintained encapsulated SMG cell viability. In the absence of cell-cell interactions, encapsulated SMG cell populations exhibit a steady decrease in cell number, while encapsulated microspheres more than double in cell number over 7 days in culture. Image analysis suggests that spheres larger >3000 µm^2 at day 0 are more likely to survive within hydrogels, with cells proliferating within spheres resulting in sphere expansion to >30,000 µm^2 at day 7. Experiments are underway using rotary culture [6] as a means to control sphere size, reduce heterogeneity, and directly relate cell viability and proliferation to varying microsphere sizes. Due to their high viability and potential for growth, microspheres represent a promising cell population for hydrogel-mediated transplantation to regenerate damaged salivary glands.

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