DESIGN OF A BIOCOMPATIBLE HYDROGEL CELL CAPSULE FOR USE IN SALIVARY GLAND TISSUE ENGINEERING APPLICATIONS

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ABSTRACT

Objective: Develop a functional, implantable salivary gland to restore salivary functions in patients with post-radiation xerostomia.

Study Design: Reconstruction of functional salivary glands builds on generation of cell-laden mini-modules (MMs) that reconstitute glandular secretory units when encased within a mechanically stable, yet biodegradable hydrogel capsule that persists long-term *in vivo*. Survival of such hydrogel implants requires stable vasculature. The ability to recruit and allow host blood vessel infiltration was evaluated in a rat model.

Methods: We developed a scaffold that combines thiolated and acrylated hyaluronate to produce a mechanically-stronger, porous, hydrogel than the encased, soft MMs used to house salivary structures. Test capsules were implanted in rat parotid resection model and stability and biocompatibility were evaluated over 5 weeks *in vivo* under conditions that permitted blood vessel infiltration.

Results: The test capsule, with an elastic modulus of 260Pa, provided a stiffer gel than the cell-containing MMs at 60Pa. Immunocompetent rats with implanted hydrogel capsules in their resected parotid bed showed no inflammation over 8 weeks during which implants were retained with minimal degradation. Blood vessels were seen around the implant at all time-points, but no infiltrating vessels were observed within hydrogels.

Conclusions: A stable, biocompatible and biodegradable hydrogel capsule for use in a modular hydrogel implant was identified. Ongoing studies included angiogenic growth factor-loaded hydrogel particles to encourage vascular infiltration into the capsule. The modular hydrogel culture system reported here will aid long-term survival and retention of salivary structures *in vivo*, and assist development of a tissue-engineered salivary gland.

INTRODUCTION

FIG. 1. The Salivary Glands.

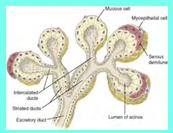


FIG. 2. Structure of the salivary gland.

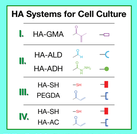


FIG. 3. HA-hydrogel systems

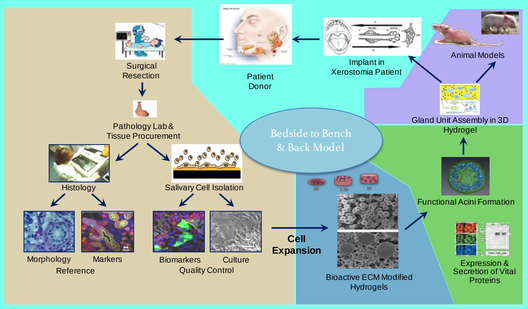
- Three-dimensional (3D) culture systems better mimic *in vivo* environments compared to traditional two-dimensional (2D) culture systems. 3D culture systems, therefore, are immensely useful when studying cell morphology and behavior for regenerative medicine applications.

- Hyaluronic Acid (HA), a major component of the ECM, is inherently angiogenic. 3D, porous and biodegradable hydrogels can be generated from conjugates of HA.

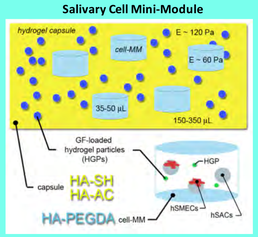
- A successful engineered model of the salivary glands will consist of hydrogels that support formation of salivary spheroids and complex cell-assemblies that can be maintained within the implant structure *in vivo,* long-enough to ensure integration with native structures.

- One of the current limitations of tissue engineering is its inability to provide sufficient blood supply in the initial phase after implantation. Insufficient vascularization can lead to improper cell integration or cell death in tissue-engineered constructs.

METHODS

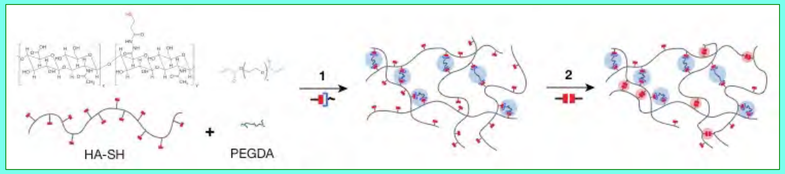


Salivary Cell Mini-Module



RESULTS

I. Generating cell-laden mini-modules (MMs): A 3D HA hydrogel culture system that leads to assembly of salivary gland cells into organized spheroid structures



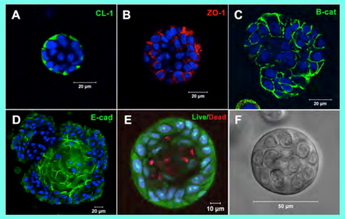


FIG. 4. Salivary gland cells form spheroids in 3D HA hydrogels. Spheroid structures express tight junction markers CL-1 (A), ZO-1 (B), E-cadherin (D) and adherens junction marker, β-catenin (C). Live/Dead staining shows Syto13 positive green cells and propidium iodide positive red cells (E). A representative phase image of an acinus-like structure is seen in F. Nuclei stain blue.

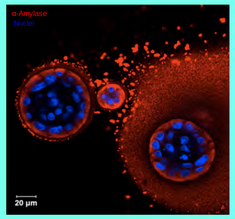


FIG. 5. Release of α-amylase by spheroid structures. The salivary enzyme, α-amylase is seen bursting out of the spheroid structures and gets entrapped into the hydrogel networks when cultured over time in the 3D HA hydrogels.

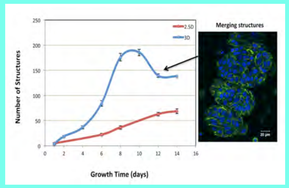


FIG. 6. Growth of spheroid structures in 2.5D (red) and 3D (blue) HA hydrogels. Each point represents the average of n=3 measurements. Error bars are +/- standard error.

II. Generating the cell-MM capsule: A mechanically strong 3D hydrogel system capable of encasing and supporting the soft cell-MMs over time *in vivo*

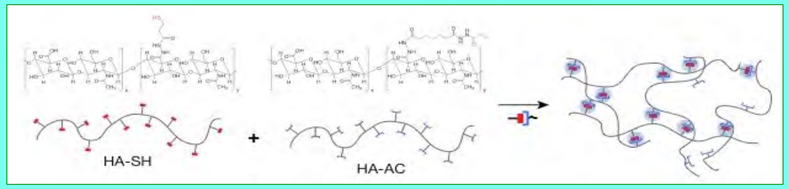


Table 1. Elastic moduli of HA-based hydrogels and human parotid tissue.

Sample	Elastic Modulus (Pa)
2% HAGMA Hydrogel	1490
3D HA-SH/PEGDA Hydrogel	60
3D HA-AC/HA-SH Hydrogel	260
Average Measurement for Tissue	1980 ± 295

III. Rodent parotid gland surgical resection model for evaluating engineered hydrogels

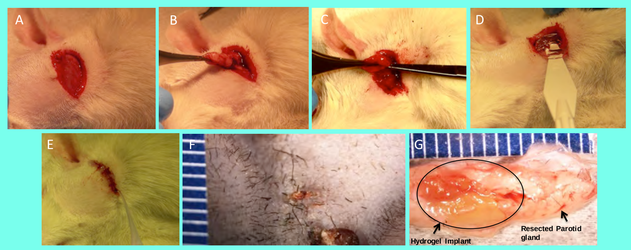


FIG. 7. Development of a parotid gland resection model to simulate acinar cell-loss. A 15mm incision caudal to the auricle (A) is made to access the parotid gland seen in panel (B). Three-fourths of the gland is resected (C) and the hydrogel implant is inserted into the cavity (D). The wound is sutured and surgical glue is applied (E). Panel (F) shows the site of implantation at one week. The partially vascularized hydrogel implant and the resected gland (G) are observed at the one week time point.

IV. Biocompatibility and biodegradability of hydrogel capsule (HA-SH/HA-AC) *in vivo*

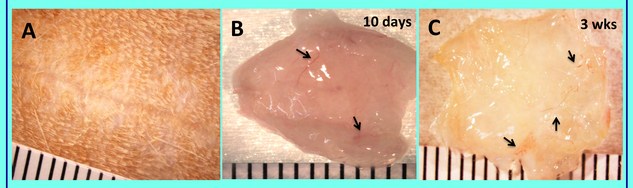


FIG. 8. Evaluating biocompatibility and biodegradability of hydrogel capsules *in vivo* via subcutaneous implantation in the back of Sprague Dawley rats. Hydrogel capsules (HA-SH/HA-AC) were implanted on the back of Sprague Dawley rats to ensure biocompatibility with host tissues and biodegradability over time. Site of implantation 3 weeks post-surgery showed no visible signs of inflammation (A). Extracted implant hydrogels show presence of a few blood vessels at day 10 (B) and at 3 weeks post-implantation (C).

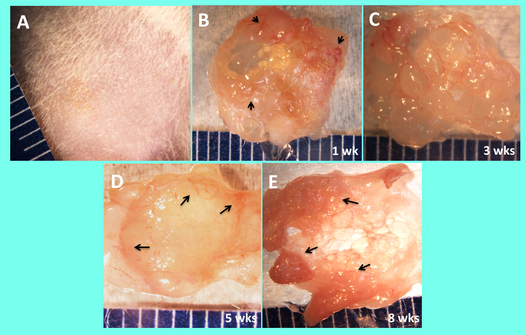


FIG. 9. Biocompatibility and biodegradability of hydrogel capsules implanted in a ¾ resected parotid bed. Hydrogel capsules were implanted in our parotid gland resection model and evaluated for up to 8 weeks. Capsules inserted in the ¾ resected parotid bed did not show visible signs of inflammation at the site of implantation (A). Hydrogel implants were analyzed at week 1 (B), week 3 (C), week 5 (D), and week 8 (E). Most of the hydrogels remained intact and showed minimal signs of degradation over 5 weeks. Some degradation was observed after 8 weeks *in vivo*. Blood vessels (black arrows) were observed on the periphery of the hydrogel implants.

CONCLUSIONS

- **Salivary gland cells form spheroid structures in soft 3D HA hydrogels (HA-SH/PEGDA) with an elastic modulus of ~60Pa.**
- **To surgically simulate acinar cell loss, a parotid gland resection model was developed.**
- **A ~260Pa hydrogel capsule (HA-SH/HA-AC) was maintained for over 8 weeks *in vivo* in a resected salivary bed, with minimal degradation.**
- **Implants developed vasculature in the periphery and on top of the hydrogels.**

FUTURE WORK

- **Encapsulate cell-MMs in capsule hydrogel to ensure survival and retention of salivary cells long-term, *in vivo*.**
- **Introduce VEGF/PDGF in hydrogels for long term *in vivo* studies.**
- **Analyze infiltration of blood vessels into hydrogels and determine the time needed for blood vessels to infiltrate through most of the hydrogel.**
- **Ensure formation of stable vasculature**

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