What is a good figure legend for this graph?

Immunohistochemical Analysis of Retention of Immune Cellularity in Ex Vivo Nasal Polyp Tissue Cell Cultures

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Abstract

Background: Chronic Rhinosinusitis with nasal polyps (CRSwNP) is characterized by a range of inflammatory cell infiltrates that play a role in underlying physiology. While prior studies have well characterized these cell types, studies of cellularity in whole tissue samples taken from patients subjected to cell culture development can offer a starting point for further analyzing disease treatment avenues.

Objective: To evaluate the preservation of cell types in surgically excised nasal polyp tissue subjected to cell cultures for various durations of time using immunohistochemical staining for leukocytes, mast cells, and neutrophils.

Methods: Polyp tissues from two patients with chronic rhinosinusitis were excised and fixed for a duration of 24, 48, and 72 hours. Using a protocol for immunohistochemistry (IHC) developed by Yuan et al., slides were prepared from paraffin blocks. Staining development utilized primary antibodies for leukocytes, mast cells, and neutrophils. Slide image analysis was done using ImageJ software for semi-automatic cell quantification

Results: Cellular presence for all cell types was demonstrated regardless of duration. The average mean cell count across all fixation times was 111 cells for mast cells, 162 for neutrophils, and 293 for leukocytes. Preliminary quantification revealed consistent detection of target cell types across samples.

Conclusions: Allowing the nasal polyp tissue to grow in cell cultures for up to 72 hours effectively preserves cellular and antigenic integrity in nasal polyp tissues. This supports the possibility of using these tissues for clinical and research use. IHC can be used to reliably identify immune cells within nasal polyp samples and offer robust histopathological analysis.

Introduction

Nasal polyps are benign, inflammatory lesions arising from the nasal mucosa that may be a result of dysregulation of the acute inflammatory response. These inflammatory environments contain a diverse array of inflammatory cell types. Lipid-derived molecules known as specialized pro-resolving mediators (SPMs) have been described as key components in the active inflammatory response. The molecule resolvin D2 may mitigate some of this inflammation and be a novel approach to CRS treatment. Developing a protocol to study how resolvin D2 works against immune cells in whole nasal polyp tissue ex vivo is the next step. Immunohistochemistry (IHC) allows for visualization and quantification of immune cells in whole nasal polyp tissues prepared in cell culture for further study.

Fig. 1: Illustration of polyps in the nasal cavity.

Rationale

- Whole nasal polyp tissue preservation is critical to examine the entire environment's cell survival for further study of treatment modalities.
- SPMs are thought to work on multiple cell types, and examining their effect on whole tissue environments has been difficult in the past.
- Optimization of cell culture for nasal polyp tissue remains unclear.
- This study examines cell preservation and types in nasal polyps obtained from two surgical patients with chronic rhinosinusitis with nasal polyps that were grown in cell culture media and stored at 4 °C for 24, 48, and 72 hours.
- With this technique, we can evaluate the preservation of immune cells in the sample and viability for using these samples for further studies.
- **Hypothesis:** Developing nasal polyp tissue in cell culture for up to 72 hours maintains antigenicity for accurate IHC-based cell quantification and offers proof of cellularity in nasal polyp tissue after exposure to cell culture media.

Sample Preparation and Immunohistochemical Staining

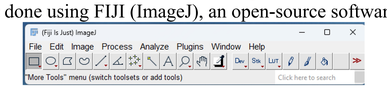
- Samples were surgically removed and exposed to the liquid culture media* for 24, 48, or 72 hours. Then paraffin-embedded and sectioned for immune staining.
- Immunostaining was performed over two days. Using three primary antibodies for targeting Leukocytes, Mast Cells, and Neutrophils.
- Protocol Overview
  - Slides were deparaffinized in xylene and rehydrated in graded ethanol.
  - Antigen retrieval was performed in a heated incubator using 1% Antigen Unmasking Solution and then rinsed with PBS.
  - A 5x animal-free blocking solution was applied and then rinsed from the sample.
  - Finally, samples were incubated in 4°C with the primary antibodies overnight.
  - The samples were rinsed in PBST bathes the following day and then incubated with the secondary antibody for 1 hour at room temperature.
  - Slides were then stained with DAB developer and counterstained with Mayers Hematoxylin solution and visualized under a Leica microscope after mounting.

Primary Antibody	Target Cell	Cat#	Vendor	Secondary Antibody	Cat#	Vendor
Myeloperoxidase (E1E7I) XP® Rabbit mAb	Neutrophils	14569T	Cell Signaling	Peroxidase-conjugated AffiniPure® Goat Anti-Rabbit IgG	111-035-144	Jackson ImmunoResearch Laboratories, Inc.
Tryptase (E7M2U) Rabbit mAb	Mast Cell	19523S	Cell Signaling	Peroxidase-conjugated AffiniPure® Goat Anti-Rabbit IgG	111-035-144	Jackson ImmunoResearch Laboratories, Inc.
CD68/SR-DI Antibody (KP1)	Macrophage	683AF647	Novus Biologics	Peroxidase-conjugated AffiniPure® Goat Anti-Rabbit IgG	111-035-144	Jackson ImmunoResearch Laboratories, Inc.

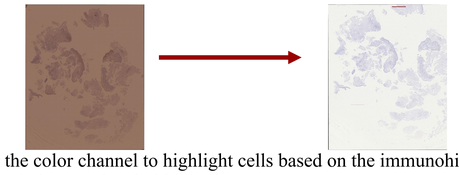
***** Cell Culture Media: Ham's F12 (750ml), Dulbecco's Modified Eagle Medium (250ml), adenine 924 mg/ml), Cholera toxin (1mg/ml), epithelial growth factor (28 ug/ml), Insulin (5mg/ml), hydrocortisone (3.62 mg/ml), Y-276 20 mM, and gentamicin (30 ug/ml)

Image Analysis

- Slide image analysis was done using FIJI (ImageJ), an open-source software for scientific image processing.



- Images were optimized and set to pixel scale based on the microscope specifications.



- The software filters the color channel to highlight cells based on the immunohistochemical staining chosen, and a standard pigment threshold is set.
- The parameters of the approximate cell size and shape help to semi-automatically capture cells.
- A summary of cell count, total area, average cell size, and % area stained is provided.

Fig. 2a: Image J tool bar. **Fig. 2b.** Optimization of cell staining slides using Photoshop for brightness, contrast, and scale bar.

Results

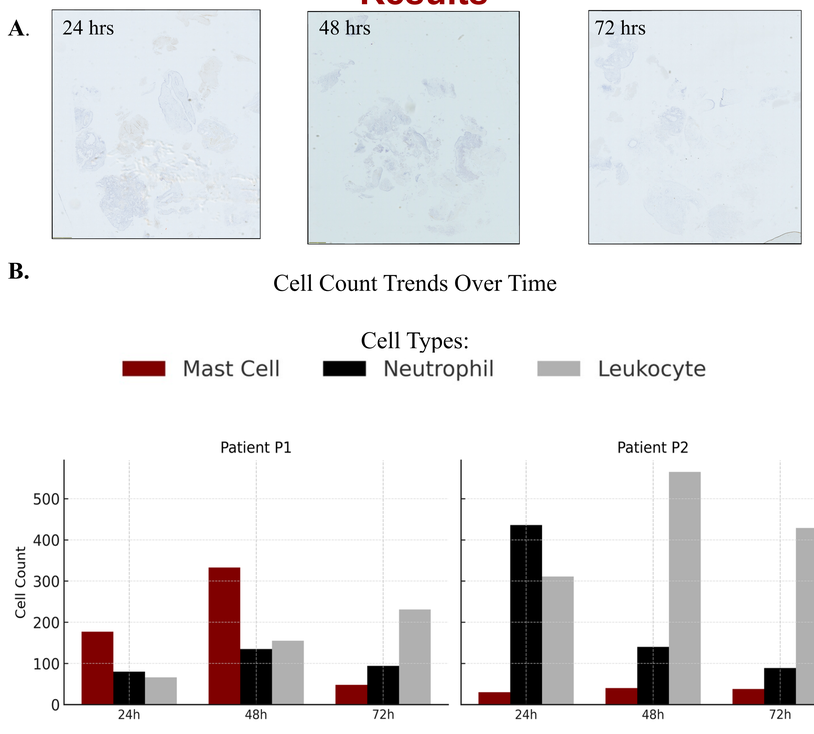


Fig. 3: Results. A. Examples of slide imaging for neutrophil staining at 24, 48, and 72 hours. **B. Quantification of immune cells in nasal polyp tissue.** Report of cell count by cell type (mast cell, neutrophil, and leukocyte) for semi-automatically quantification using ImageJ analysis of nasal polyp tissue samples from two patients (P1 and P2). Reported for fixation at each time period (24, 48, and 72 hours).

Conclusions

- Nasal polyp tissue grown in cell culture media for up to 72 hours effectively preserves cellularity and antigenicity.
- Consistent detection of all cell types (Leukocytes, Mast Cells, and Neutrophils) supports staining protocol reliability.
- ImageJ software is an accessible tool for semi-automated cell quantification and analysis.
- Future work can expand to include additional cell culture media that uses a combination of medias that promote both immune cell and epithelial cell growth, as well as possibly examine longer growth time points.
- Flow cytometry may also offer insights into how these cells are functioning as well.

References

Hopkins C. Chronic Rhinosinusitis with nasal polyps. *N Engl J Med*. 2019;381(1):55-63. doi:10.1056/NEJMcp1800215

Gelardi M, Giancaspro R, Cassano M, Ribatti D. Nasal cytology and histology in CRSwNP: Two sides of the same coin. *Front Med (Lausanne)*. 2023 Mar 9;10:1143351.doi:10.3389/fmed.2023.1143351. PMID:36968832.

Robinson PZ, Delgado C, Khan K, Frank DN, Ramakrishnan VR. Mediation of LPS-induced inflammation with pro-resolving treatment in nasal polyps: A pilot study. *Int Forum Allergy Rhinol.* 2025 Jun ; 15 (6) :645-647. doi :10.1002/alr/23543. Epub 2025 Feb 10. PMID: 39920908.