Reflections on Research Day 2024

Lois Rabinowitz Lamond

As I traveled to Research Day at UP Dental School, I bemoaned the fact that perhaps there would be no one present who remembered our parents; it had been many years. It turns out, I need not have worried.

At the lunch buffet, I got a wave from an older gentleman who remembered me from previous years. He introduced himself as Gary Cohen. I was so grateful for his welcoming gesture. He said that he had many great memories of Dad, laughing as he recalled the jokes that were always included in Dad's lectures. He whispered to me when he shared that Dad had gotten into some trouble for telling ethnic jokes. I nodded knowingly, "Yes, we tried to dissuade him from that on many occasions."

Gary clarified, "Your dad wasn't disparaging to any ethnicity, he just liked those jokes. One time José was reprimanded for telling ethnic jokes during a lecture. The next time he came to class, he began by carefully looking around the lecture hall. He then proceeded to tell a joke about two Albanians. After class, two students approached him sharing that they were Albanian." Gary laughed, "One time your dad said, 'Two Eskimos were on a train, one said to the other…' We all looked at him in warning and he proceeded, "So what are you doing for Passover?"

Then he said, "Rabinowitz is a very important name here at the Dental School. I don't mean that it's not an important name outside of the Dental School, but it is an important name here." He shared that Dad had helped create the Biochemistry Department at the Dental School. According to Dr. Cohen, in the late 1950s, all biochemistry was taught through the Medical School. Some of the other departments felt that their students were being short changed. First, the Veterinary School, then the Dental School each created their own Biochemistry Department. Gary went with the Veterinary School, and Dad and Julian Marsh created the department for the UP Dental School. He noticed that the department became very cohesive. "What I mean by that is that they all became close friends, they worked together and socialized together, they were very close. They had lunch together, they enjoyed each other's company, that doesn't happen often."

Claire H. Mitchell came over and sat at our table. She reminded me that she had come to our parents' home for Shiva. I told her that I remembered her from our conversation last year (she confided that she had expected to find that they lived in a mansion and was taken aback when realizing that the Rabinowitzes were of moderate income. She then realized the significance of the grant they had sponsored). She apologized if she had offended me in her comments (I assured her she had not). I did take the opportunity to ask how winning the Rabinowitz Award had impacted her as a researcher. She became animated, pulling up a link on her cell phone to the NIH website that listed the article she had published:

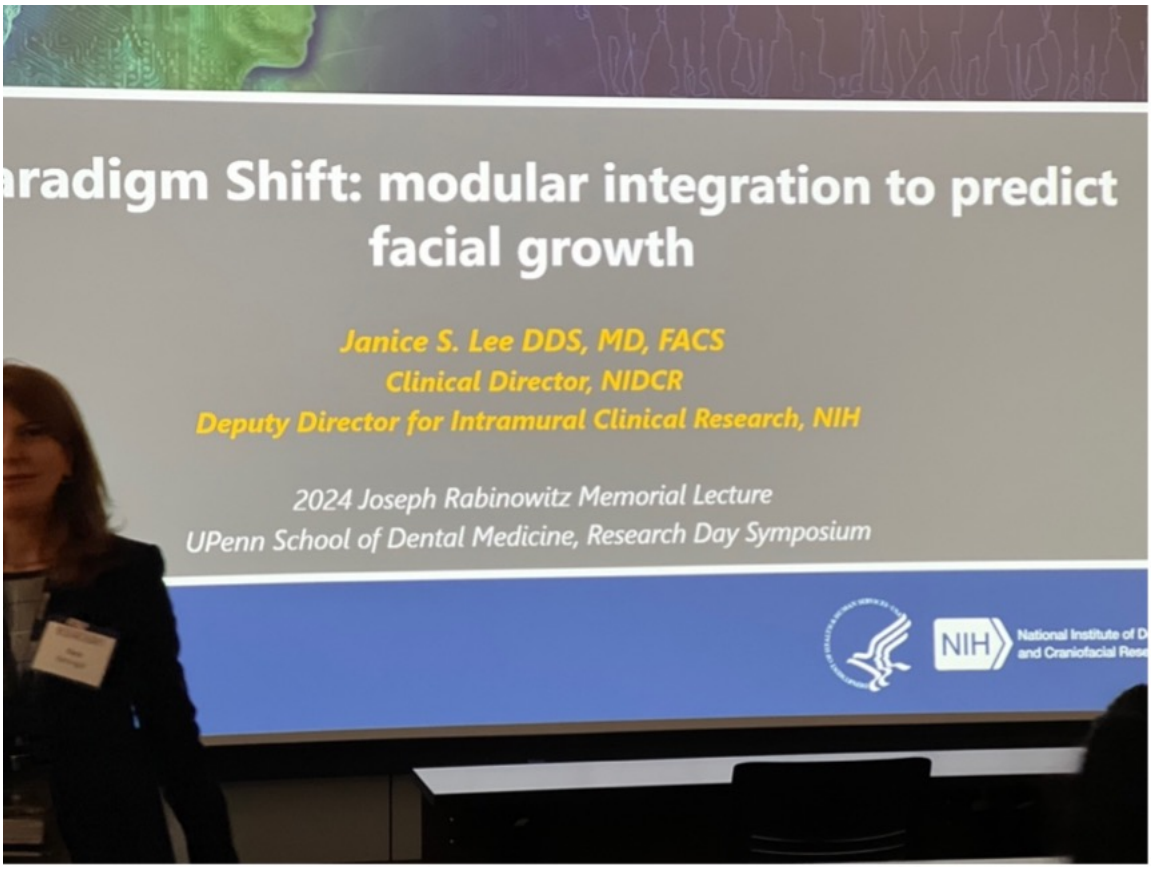
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6022788/

She said that she won the award with Elliott Hersh, who she classified as a *pain guy*. "I was focused on cell biology and he worked in neuroscience, pain management. There was a new drug that was being used in Europe, Articaine and there was a lot of discussion about whether it would be better than Lidocaine in both reducing pain and limiting the negative impact of the drug on cells (neurotoxicity).

Turns out the new drug was not as good, but you publish what you find. Before that research, I was much more focused on cell biology, now I think about pain management too."

That article included the following acknowledgement: *This work was supported by a grant from the Rabinowitz Foundation to Drs Mitchell and Hersh.*

I had to rush away from the lunch table to arrive in time for the Rabinowitz Lecture, a keynote address by Janice Lee, Deputy Director for Intramural Clinical Research at the National Institute of Health. The presentation, entitled *Paradigm Shift: modular integration to predict facial growth*, shared her research into Craniofacial Anomalies and Regeneration. (Her research developed a system for classifying anomalies and thereby more accurately predicting which patients will require surgery and which ones can be corrected with orthodontal intervention.) She dedicated the final 15 minutes of her time to encourage students to take advantage of opportunities for research at NIH and the importance of dentists applying for these positions. Talk about apt!



During the long break before the presentation of the Rabinowitz Award, I meandered through the poster presentations, primarily searching for the names of Rabinowitz Award winners from last year, hoping to see any published findings. I picked up several copies of the Research Day booklet, and Liz Kitterlinus, Vice Dean of Institutional Advancement, encouraged me to take a copy of the *Fall 2023 Penn Dental Medicine Journal.* She wanted us to know that the researcher on the cover, Karam Alyashooa, was a recipient of the **Laib and Rachel Rabinowitz Scholarship**. I mentioned to her that I was interested in hearing about how receiving the Rabinowitz Award had impacted the work of its recipients.

Dental Biofilm Disruption Using Plant-Made Enzymes with Chewing Gum Delivery

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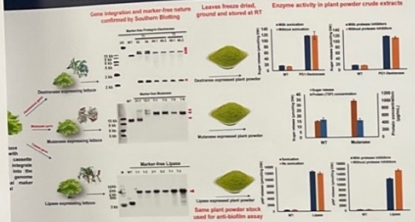
Introduction

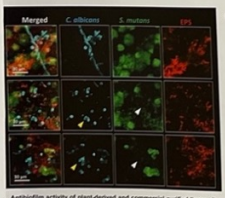
- Safe and affordable dental plaque biofilm treatment is an unmet need.
- Enzymatic biofilm disruption is a novel approach: enzymes produced in plants are stable & cost-effective.
- Due to complex structure of dental biofilm several enzymes are required.

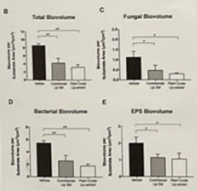
Material and Methods



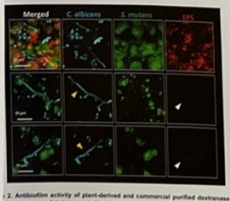
Results and Discussion

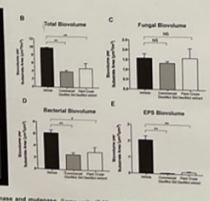
extranase or mutanase were expressed in plant cells, free of antibiotic resistance genes. Plant-derived lipase 5 better than purified commercial enzyme (equivalent enzyme unit) against mixed-kingdom biofilms (**Fig 1**), inhibiting phae formation (**Fig 1A, yellow arrow-heads**). Plant-derived dextranase/mutanase combination was highly effective ng EPS glucans (similar efficacy compared with the commercial purified enzymes), resulting in near abrogation of atrix in the biofilm (**Figure 2A, white arrow-heads**). Combination of lipase with dextranase and mutanase ed biofilm development by degrading the biofilm matrix, with concomitant reduction of bacterial and fungal tion (**Fig 3**). Moreover, the Total Biofilm Inhibition (TBI) index in the group treated with three-enzyme combination ficantly lower than that in groups treated with individual enzymes (**Fig 4A**). Most *C. albicans* cells (yeast form) in the ated with the three-enzyme combination were killed (**Fig 4B**). Chewing gum tablets were formulated with freeze- d cells (**Fig 5A**). The plant-made protein was efficiently released using a mechanical chewing simulator device (**Fig 5** in a time-dependent manner (**Fig 5D**). The plant-made protein in the chew gum is stable for up to 3 years at ambient ure (**Fig 5E**).





Antibiofilm activity of plant-derived and commercial purified lipase. Same unit (333.3 U/ml) of commercial purified lipase used as standard ole antibiofilm efficacy of plant crude extract against fungal-bacterial mixed biofilm. Confocal images showing the antibiofilm efficacy (A) and ve computational analysis of the confocal biofilm images (B-E) treated with commercial and plant-derived lipase. *C. albicans* cells (yeasts or are depicted in cyan; *S. mutans* cells are depicted in green; The EPS glucan matrix is depicted in red.





2: Antibiofilm activity of plant-derived and commercial purified dextranase and mutanase. Same units (7.08 and 0.84 U/ml) of commercial d dextranase and mutanase used as standards to evaluate antibiofilm efficacy of plant crude extract against fungal-bacterial mixed biofilm. Confocal e showing the antibiofilm efficacy (A) and Quantitative computational analysis of the confocal biofilm images (B-E) treated with commercial and plant- d enzymes. *C. albicans cells* (yeasts or hyphae) are depicted in cyan; *S. mutans* cells are depicted in green; The EPS glucan matrix is depicted in

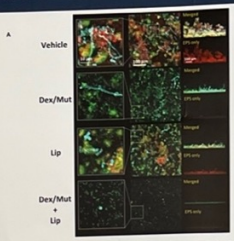


Figure 3. Prevention of fungal-bacterial mixed biofilm by topical sequential treatment of commercial Lipase and Dextranse/Mutanse combination. (A) Three-dimensional confocal images of the fungal-bacterial mixed biofilm formed after the topical sequential treatments. *C. albicans* cells (yeasts or hyphae) are depicted in cyan; *S. mutans* cells are depicted in green; The EPS glucan matrix is depicted in red. Representative merged biofilm images are displayed in the middle panel, while a magnified (close-up) view of each small box is positioned in the left panel. Lateral (side) views of each biofilm are displayed at the right panel (the merged image at the top and the EPS channel at the bottom). (B–E) Quantitative computational analysis of the confocal images. The title of each graph indicates the channel(s) used for individual analysis. *P < 0.05, **P < 0.01 (one-way analysis of variance with Tukey's multiple comparisons test).

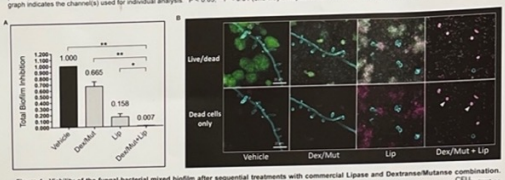


Figure 4. Viability of the fungal-bacterial mixed biofilm after sequential treatments with commercial Lipase and Dextranse/Mutanse combination. (A) Total Biofilm Inhibition (TBI) index of the treatments. TBI = $I_{fungal\ CFU} \times I_{bacterial\ CFU} \times I_{DW}$, where Inhibition of bacterial/fungal CFU or $I_{CFU} = \frac{CFU_{treatment}}{CFU_{control}} \times 100\%$, and Inhibition of Dry Weight or $I_{DW} = \frac{DW_{treatment}}{DW_{control}} \times 100\%$. *, p<0.05, **, p<0.01 (1-way analysis of variance with Tukey's multiple comparisons test). **(B)** Live/dead staining of the fungal-bacteria mixed biofilms. Live cells are depicted in green; Dead cells are depicted in magenta. *C. albicans* cell wall is depicted in cyan to indicate the location of fungal cells. The optimum activity units (U) were used for commercial purified lipase (1000 U/mL) and Dex/Mut (525/105 U/mL) in the experiments.

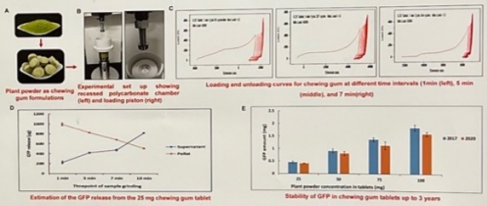


Figure 5. Plant-based chewing gum delivery system. Chewing gum formulation of plant powder (A), release of bioactive using mechanical device in human oral cavity simulated conditions (B-D) and stability assessment of bioactive in the chewing gum stored at ambient temperature (E).

Conclusions

- Lipase, dextranase and muatanse enzymes produced in marker-free lettuce are functionally active as crude extracts, without need for purification.
- Plant-made lipase facilitates antibiofilm efficacy mainly by targeting *C. albicans* transition from yeast to filamentous form while dextranase /mutanase effectively degrade EPS matrix of the biofilm.
- Combination of these three enzymes facilitates near-complete suppression of mixed-biofilm by degrading the biofilm matrix, with concomitant reduction of bacterial and fungal accumulation.
- Significantly lower TBI (Total biofilm inhibition) of combined treatment suggesting synergistic inhibitory effect, yeast form of *C. albicans* were dead in the treated biofilm.
- Time dependent release and prolonged stability at ambient temperature of chewing gum made with freeze dried plant cells reveals feasibility of chewing gum for topical delivery to treat the dental biofilm in an affordable manner.

Clinical Significance

We provide a conceptual framework for plant made biofilm-degrading enzymes and chewing gum-based protein delivery that may lead to an innovative and affordable approach of controlling dental biofilm. This strategy could help develop a low-cost technology for improvement of oral health, building upon the ACE2 chewing gum developed recently for decreasing SARS-CoV-2 infection and transmission. Chewing gum to deliver therapeutic enzymes proteins could serve as a new platform to treat oral pathogens, including bacteria, viral and fungal pathogens.

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Center for Innovation & Precision Dentistry
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❖ POTENTIAL APPLICATION OF GINGIVA-DERIVED MESENCHYMAL STEM CELLS IN TISSUE ENGINEERING AND REGENERATIVE THERAPY
❖ TUMOR MICROENVIRONMENT: A NEW ROUTE FOR TARGETED AND IMMUNOTHERAPY FOR HEAD & NECK TUMORS

Poster No.

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Lab Mission

Vision

- To build cutting-edge stem cell-based research programs that integrate expertise in stem cell biology and tissue engineering/regenerative medicine (TE/RM) aiming to foster a novel understanding of stem cells in tissue homeostasis and regeneration and accelerate the development of novel stem cell-based TE/RM products translatable to clinical application.

Missions

- To establish research programs that can integrate basic and translational research faculty and clinicians aiming to address the unmet clinical needs of patients who have encountered loss of both soft and hard tissues in the oral & maxillofacial regions.
- To foster the generation of novel scientific knowledge about the role of stem cells in tissue homeostasis, regeneration, and diseases that can be shared through publications, training, and educational activities.
- To accelerate the development of stem cell-based TE/RM products that can be translated to clinical application and patients' benefits.

Lab Info & Goals

The longstanding interest of our laboratory is to explore the potential translational application of human gingiva-derived mesenchymal stem cells (GMSCs) in tissue engineering and regenerative therapy of a variety of diseases. In this aspect, we are concentrating our efforts on the following three areas of study: 1) stem cell biology with a specific focus on addressing the immunomodulatory/anti-inflammatory functions involved in GMSC-mediated regenerative therapies; 2) development of functionalized GMSC-based tissue engineering/regenerative medicine (TE/RM) products applicable for reconstruction and regeneration of both soft and hard tissue defects in oral and maxillofacial regions. Our second area of focus is to understand how cross-talks between tumor cells, tumor stem cells, and stromal and immune cells in the tumor microenvironment contribute to the pathogenesis and progression of head & neck tumors. These three promising research focuses support the OMFS department's goal to develop state-of-the-art TE/RM products to meet the increasing clinical demands of patients with sustained oral and maxillofacial tissue loss.

Ongoing Projects

1. GMSC-based regenerative therapy

- Enhancing the NCSC-like property of GMSCs via nongenetic approaches
- Optimizing the condition to increase the production of GMSC-EVs
- Development GMSC-based TE/RM products
- Evaluating therapeutic potentials of GMSC-based TE/RM products in soft/hard tissue regeneration in preclinical animal models, e. g. the rat tongue defect model
- Exploring the mechanism of action (MOA) of GMSC-based regenerative therapy under different pathological settings

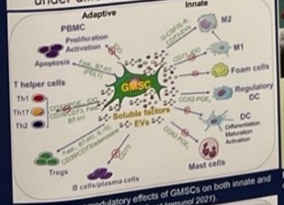

Figure 1. Immunomodulatory effects of GMSCs on both innate and adaptive immune cells (*Kim et al. Front Immunol 2021*).

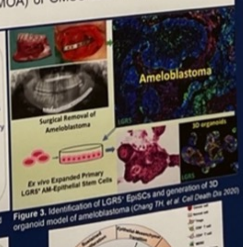

Figure 3. Identification of LGR5+ EpiSCs and generation of 3D organoid model of ameloblastoma (*Chang TH, et al. Cell Death Dis. 2020*)

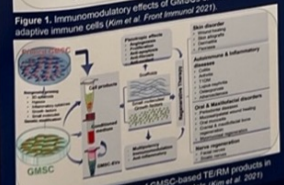

Figure 2. Potential application of GMSC-based TE/RM products in regenerative therapy for different disease models (*Kim et al. 2021*)

Figure 4. Contribution of tumor microenvironment (TME) to hallmarks of head & neck tumors (*Ghaur AA et al. Signal Transduct Target Ther 2021*)

Ongoing Projects

2. Tumor microenvironment (TME) of head & neck tumors

- Identifying novel markers for tumor stem-like cells in both benign and malignant head & neck tumors, e. g. ameloblastoma and oral squamous cell carcinoma
- Determining the critical role of mesenchymal stromal cells and macrophages in regulating proliferation, epithelial-mesenchymal transition (EMT), and tumor stem cell properties in head & neck tumors.
- Identifying the critical metabolic and signaling pathways involved in the cross-talk between TME, particularly, MSCs and macrophages, and head & neck tumor cells.
- Exploring the therapeutic potential of tumor-associated macrophage (TAM)-targeted immunotherapy alone or in combination with immune checkpoint blocking (ICB) or chemotherapy in the treatment of oral squamous cell carcinoma in allogeneic murine or humanized OSCC models

Highlighted Project

- Reprogramming of *ex vivo* expanded GMSCs into neural crest stem-like (NCSC) or Schwann-like cells (GiSCs) via nongenetic approaches, e. g. by optimizing cell culture conditions and leveraging the substrate stiffness of 2D scaffolds
- Generation of functionalized nerve guidance conduit or protector laden with GiSCs to facilitate peripheral nerve regeneration after injury
- Optimizing the condition to increase the production and improve the therapeutic potential of GMSC-derived extracellular vesicles (EVs) in peripheral nerve and myomucosal regeneration
- Exploring the critical role of tumor-associated macrophages (TAMs) in regulating proliferation, epithelial-mesenchymal transition (EMT), and tumor stem cell properties in OSCC.
- Determining the dual roles of polyamine/eIF5A hypusination in regulating TAM activation and proliferation/stem cell property in OSCC cells.
- Targeted inhibition of polyamine/eIF5A hypusination for the treatment of OSCC in allogeneic murine or humanized OSCC models
- Combination of TAM-targeted immunotherapy and immune checkpoint blockade (ICB) therapy for the treatment of OSCC

Key advancements

- Characterization of GMSCs with potent immunomodulatory and anti-inflammatory functions *in vitro* and in several inflammatory disease models
- Establishment of nongenetic approaches to reprogram *ex vivo* expanded GMSCs into NCSC-like or Schwann-like cells (GiSCs) with enhanced therapeutic potentials in peripheral nerve regeneration
- 3D bio-printed nerve constructs and generation of functionalized nerve guidance conduits laden with GiSCs that can foster rat facial nerve regeneration
- Identification of LGR5 as a unique marker for epithelial stem-like cells (EpiSCs) in ameloblastoma
- Implantation of SIS-ECM membrane laden with GMSC or GMSC-derived NCSC to promote myomucosal regeneration in a critical-sized tongue defect model in rats

Future Directions

- Determine the mechanism of action of GMSCs and their derivative EVs in immunomodulation and regenerative therapy in different preclinical animal models
- Evaluate the therapeutic effects of functionalized nerve guidance conduits laden with GiSCs in a facial nerve defect model in minipigs
- Explore TAM-targeted immunotherapy for oral squamous cell carcinoma

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Acknowledgements

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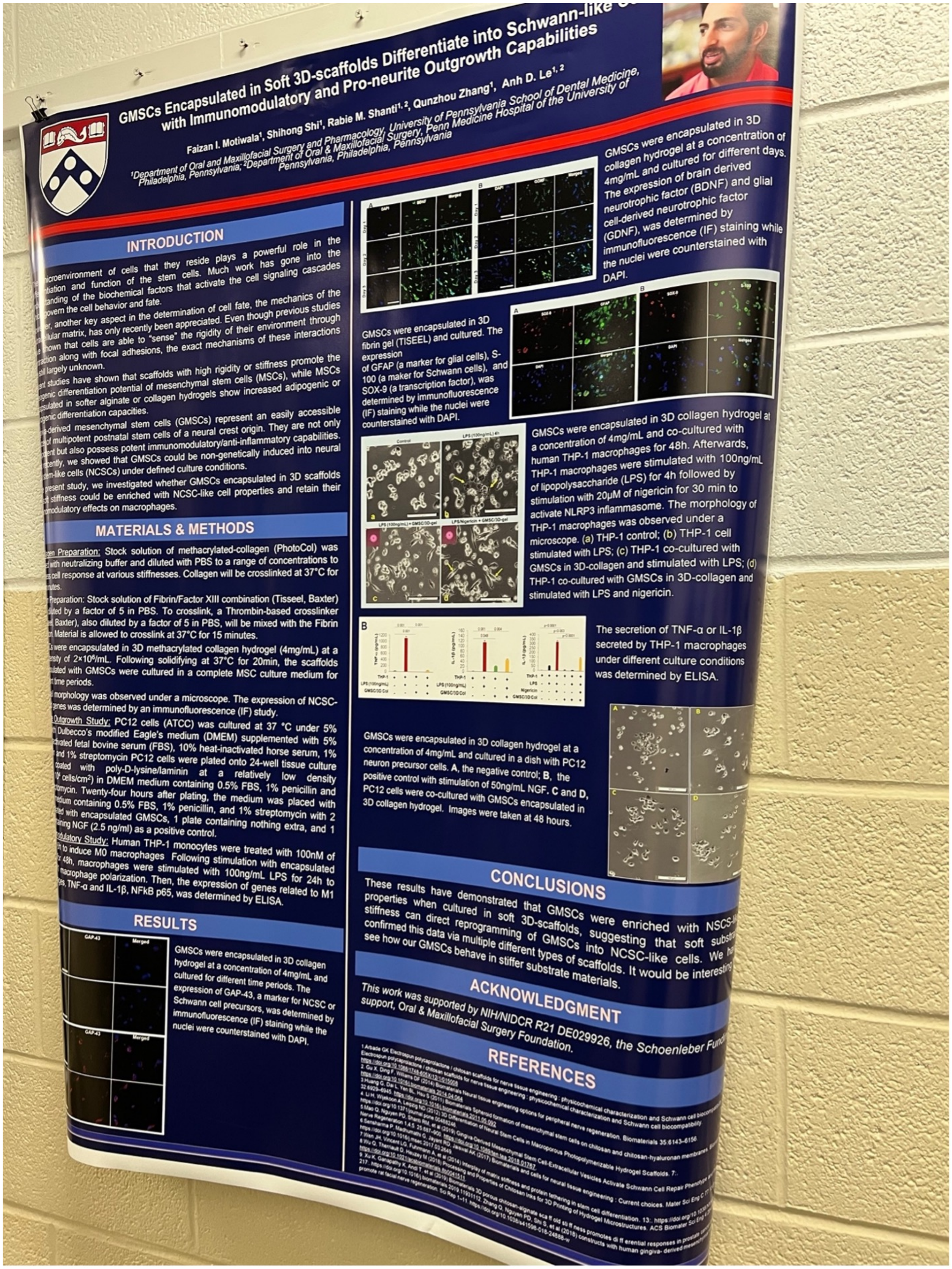
GMSCs Encapsulated in Soft 3D-scaffolds Differentiate into Schwann-like
with Immunomodulatory and Pro-neurite Outgrowth Capabilities
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INTRODUCTION
MATERIALS & METHODS
RESULTS
GMSCs were encapsulated in 3D collagen hydrogel at a concentration of 4mg/mL and cultured for different time periods. The expression of GAP-43, a marker for NCSC or Schwann cell precursors, was determined by immunofluorescence (IF) staining while the nuclei were counterstained with DAPI.
GMSCs were encapsulated in 3D collagen hydrogel at a concentration of 4mg/mL and cultured for different days. The expression of brain derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF), was determined by immunofluorescence (IF) staining while the nuclei were counterstained with DAPI.
GMSCs were encapsulated in 3D fibrin gel (TISEEL) and cultured. The expression of GFAP (a marker for glial cells), S-100 (a maker for Schwann cells), and SOX-9 (a transcription factor), was determined by immunofluorescence (IF) staining while the nuclei were counterstained with DAPI.
GMSCs were encapsulated in 3D collagen hydrogel at a concentration of 4mg/mL and co-cultured with human THP-1 macrophages for 48h. Afterwards, THP-1 macrophages were stimulated with 100ng/mL of lipopolysaccharide (LPS) for 4h followed by stimulation with 20µM of nigericin for 30 min to activate NLRP3 inflammasome. The morphology of THP-1 macrophages was observed under a microscope. (a) THP-1 control; (b) THP-1 cell stimulated with LPS; (c) THP-1 co-cultured with GMSCs in 3D-collagen and stimulated with LPS; (d) THP-1 co-cultured with GMSCs in 3D-collagen and stimulated with LPS and nigericin.
The secretion of TNF-α or IL-1β secreted by THP-1 macrophages under different culture conditions was determined by ELISA.
GMSCs were encapsulated in 3D collagen hydrogel at a concentration of 4mg/mL and cultured in a dish with PC12 neuron precursor cells. A, the negative control; B, the positive control with stimulation of 50ng/mL NGF. C and D, PC12 cells were co-cultured with GMSCs encapsulated in 3D collagen hydrogel. Images were taken at 48 hours.
CONCLUSIONS
These results have demonstrated that GMSCs were enriched with NCSC-like properties when cultured in soft 3D-scaffolds, suggesting that soft substrate stiffness can direct reprogramming of GMSCs into NCSC-like cells. We have confirmed this data via multiple different types of scaffolds. It would be interesting to see how our GMSCs behave in stiffer substrate materials.
ACKNOWLEDGMENT
This work was supported by NIH/NIDCR R21 DE029926, the Schoenleber Fund support, Oral & Maxillofacial Surgery Foundation.
REFERENCES

While I wandered through the poster exhibit, a man introduced himself to me as a Rabinowitz Award winner from 2020 (Rahul Singh). I asked if he had been able to acquire additional funding for his research as a result of the award. He said that he had not, but that because of the award, he received a promotion which would put him into a position to apply for new grant funding.

I took the elevator to the fifth floor, hoping to peek into the offices where Dad had worked. The hallways were lined with posters and I found one from Rahul Singh. I noticed that along with his name on the poster was the name Qunzhou Zhang, Rabinowitz Award winner in 2023. I had a list of the winners from previous years along with the titles of their research proposals, and wondered if these posters were for that same research.

A woman came into the hallway; I think she wanted to know why a stranger was browsing these halls. When I explained, she got Dr. Zhang to come out of his office to explain his research to me. (As was the case with many of my conversations last year, my limited dental science literacy and the foreign accent of the award recipient impacted my comprehension of the content of this conversation.)

The hallway circled the building, and I eventually found my way to the Biochemistry office. Kathy Battaglia was there, and we chatted for a short while. She told me that she was very impressed with the 2024 research proposals (three winners this year). One of the winners was creating a longitudinal study of how chewing changes teeth. She mentioned that she had won the Rabinowitz Award in 2011. When doing that research, a graduate student had assisted her in the lab. That student is now conducting further research on the topic.

She then took me to the conference room and showed me this:

She also shared this photo from her desk:

Research Day ended with photo ops for “Investigator Poster” winners and the formal presentation of the 2024 winner of the **Joseph and Josephine Rabinowitz Award for Excellence in Research**. The three winners (all assistant professors) were in attendance. They seemed genuinely grateful for their grants. As I attempted to slip out from the back of the courtyard, all three of the new winners approached me again to thank me. I asked them each to please include an acknowledgement of the Rabinowitz Award when they publish, and shared that I wanted to hear about their findings when I come to Research Day next year. One of recipients asked how to contact me so he could notify me of other publications that might come from this award.