



2025 Research Aid Award Dr. Jarlath McDonnell, University of Toronto

Biographical Statement

I began my dental training in the Dublin Dental Hospital in Trinity College Dublin, Ireland. During my program I was honored to be elected a Scholar of Trinity College and graduated with a gold medal. After graduating I worked for one year in the Dublin Dental Hospital in a GPR with a focus on pediatric dentistry, oral oncology, emergency dental care and treating patients with additional needs. I then did a one-year residency limited to oral and maxillofacial surgery in University Hospital Limerick.

During this time, I discovered that orthodontics was the aspect of dentistry that really excited me, in particular the areas of growth modification and temporomandibular joint dysfunction. I applied and was accepted to the University of Toronto to specialize in orthodontics. At the University of Toronto, I met Dr Limor Avivi-Arber, a leading expert in orofacial neurophysiology, and together we decided to pursue a project applying 3-dimensional analysis a whole cleared orofacial muscle. I felt that examining muscles in 3-dimensions was the best way to advance the research field in my principal interests of growth modification and temporomandibular joint dysfunction.

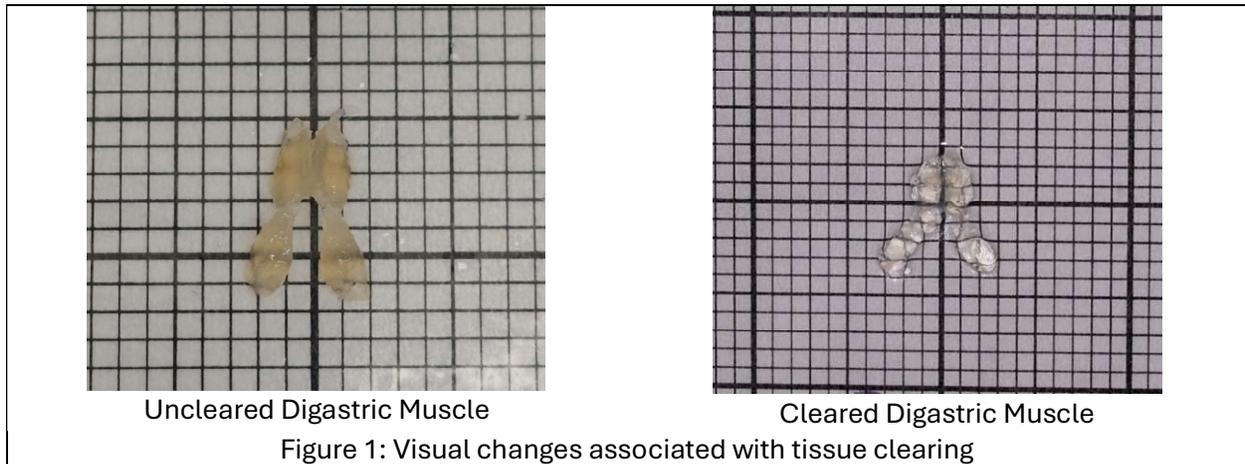
Outside of dentistry my hobbies include running, board games and music.

Brief Description of the Project

The CLARITY (Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/Immunostaining/ in situ-hybridization-compatible Tissue hYdrogel) technique was developed to render whole tissues transparent (i.e., see-through). This technique allows high-resolution imaging and molecular analysis of a whole tissue without sectioning. Our project attempts to develop and optimize, for the first time, a CLARITY-based protocol specifically for orofacial muscles (e.g. anterior digastric jaw opening muscle and masseter jaw closing muscle). Traditionally, 2-dimensional histological techniques have been used in orthodontics to investigate changes in tissue architecture and cellular organization associated with various orthodontic-related topics such as tooth movement (1), growth of mandibular condyles (2) and botulinum toxin administration to orofacial muscles (3). These applications have been fundamental in our understanding of orthodontic treatment; however, they have a limitation. Conventional methods are limited to 2-dimensional analysis, as tissues must be sectioned to allow for the transmission of light. Techniques have been described to reconstruct full tissues from sections, such as the Z-stack method, however they are exceptionally labor/time intensive and vulnerable to artefacts (4). Some structural features that may be evident in 3-dimensions could be missed by conventional 2-dimensional analysis.

Tissue clearing techniques attempt to circumvent this limitation by allowing the transmission of light through large sections of tissues, preserving architectural features. We are applying the CLARITY technique to murine anterior digastric and masseter muscles. By eliminating lipids and pigments from the tissues and harmonizing the refractive index incident light can pass through a

whole tissue, while a hydrogel scaffold maintains the tissue proteins and architecture intact. For example, see figure 1 below showing the tissue transparency following the tissue clearing stage at our laboratory.



One group has previously described the labelling of CLARITY prepared tissues using primary/secondary fluorescent antibodies (5) however we believe we are the first group to apply conjugated antibodies in this application. After labelling the tissues are imaged using a specialized microscope, either a confocal or lightsheet design, that allows for 3-dimensional image acquisition. For example, see table 2 below showing a recent staining trial from our laboratory.

Currently we are working on optimizing our staining protocol to demonstrate a robust, predictable and repeatable staining process. Using this protocol we then hope to investigate for baseline differences in orofacial muscles on a sex, genetic or age basis and eventually differences associated with orthodontic/prosthetic treatments.

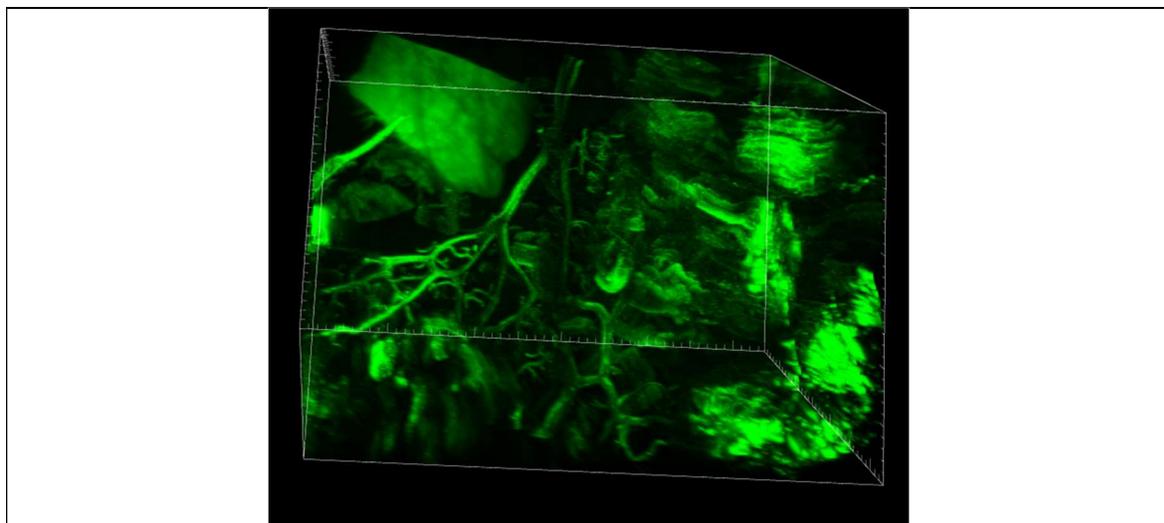


Figure 2: Immunolabelling of digastric muscle. Green label – Wheat Germ Agglutinin conjugated to Alexa Fluor 488, non-specific label for extracellular carbohydrates such as muscle fibers, neurons, blood vessels and connective tissues (7x magnification, 1:50 concentration, 3 weeks staining)

Statement of how Orthodontic Education will benefit from this award

Applications of 3-dimensional tissue analysis have only become feasible with the development of whole tissue clearing and sophisticated imaging technologies such as the light-sheet microscope and AI automated structure recognition software. We hope that this burgeoning field will create several benefits for orthodontic education.

Firstly, our methods can create detailed high resolution 3-dimensional reconstructions of orofacial muscles. These reconstructions can be used for both undergraduate and post-graduate anatomical training to help students better understand the structures of the muscles they study.

Next, we hope to develop a robust protocol that can be applied to experiments involving orofacial muscles. These muscles are integral to several intensely investigated but controversial areas in orthodontics, including growth modification, orthodontic relapse and even airway treatments. We hope that future investigations utilizing 3-dimensional analyses may be able to better identify changes associated with treatment or dysfunction and potentially develop better interventions.

Finally, it is known that there are sex and aging differences associated with a variety of orofacial conditions such as temporomandibular joint dysfunction (6,7). By investigating baseline differences between groups on a sex, age and genetic basis we hope to identify factors that may be contributing to these conditions and potentially identify novel treatment modalities.

Why the foundation is important for your project.

The funding provided by the AAOF has directly covered some of the costs associated with this project – including clearing reagents, conjugated antibodies and light-sheet microscope usage fees. Without the AAOF the project in its current conception would have been unfeasible with our current research budget.

We believe that our project also presents a unique area in dentistry – orofacial neurophysiology – which is shared by many different specialities. Our project represents a collaboration between orthodontics and prosthodontics to better understand the fundamental structures that affect function and dysfunction in the oral cavity. Both the International College of Prosthodontics and the American Association of Orthodontics Foundation have supported this project – and this highlights the multidisciplinary joint nature of orofacial neurophysiology. It also highlights key role that fundamental science research continues to play in the fields of orthodontics and prosthodontics.

Most importantly we appreciate the recognition of the importance of neurophysiological research by the foundation. Basic scientific neurophysiological research may seem quite separated from our day-to-day clinical practice; however, it is our strong belief that many of the most challenging situations we encounter as orthodontists; including maladaptation, relapse and temporomandibular joint dysfunctions, are manifestations of neural anomalies. By building on our understanding of these neural anomalies we hope to develop novel treatment modalities to address these challenging areas. We greatly appreciate that the foundation has recognized the importance of this research and will help to disseminate our research findings so clinicians can develop their understanding oral neurophysiology.

How Foundation funding is expected to or has benefitted your career.

The AAOF funding has been a great help to me starting my career in orthodontics. It has been a great honor to have our project recognized by the foundation for funding.

The AAOF followed a structured grant application model with structured critical feedback that helped by understand the importance of grant applications to research. This will help me in future applications for larger grants.

This project will also allow me to contribute to the orthodontic literature and demonstrate for future research/work applications the value I place in robust scientific research.

I also appreciate the opportunity that the AAOF has provided to present this research, and hope that by doing so I can encourage others to think about the fundamental neurophysiological processes that underlie our orthodontic treatments and about the benefits of research in this area.

Above all I value the vote of confidence that the AAOF has delivered to me and this project and look forward to updating you on its developments.

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