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Title: A Standardized Ex Vivo Porcine Oromucosal Model for Evaluating Peptide Fluxes

Authors and Affiliations:

Muhammad Ijaz¹, Sandeep Karki¹, Sahil Malhotra¹, Estela Isabel Bini¹, Eoin D. O'Cearbhaill², David J. Brayden¹

¹UCD School of Veterinary Medicine and UCD Conway Institute, University College Dublin

²UCD School of Mechanical and Materials Engineering, University College Dublin

Corresponding Authors:

Muhammad Ijaz muhammad.ijaz@ucd.ie

David J. Brayden david.brayden@ucd.ie

Email Addresses for All Authors:

Sandeep Karki sandeep.karki@ucdconnect.ie

Sahil Malhotra sahil.malhotra@ucd.ie

Estela Isabel Bini estela.bini@ucd.ie

Eoin D. O'Cearbhaill eoin.ocearbhaill@ucd.ie

Muhammad Ijaz muhammad.ijaz@ucd.ie

David J. Brayden david.brayden@ucd.ie

Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**
If **Yes**, how far apart are the locations? 100-200m
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 21

Number of Shots: 46

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen.** Please answer in **stand-alone full sentence with sufficient context**

Answers are slightly edited in accordance with journal guidelines.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Muhammad Ijaz:** Our research developed a standardized ex vivo porcine buccal mucosa protocol to preserve tissue integrity and improve reliable measurement of peptide permeability over time.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Sandeep Karki:** This protocol is applicable in preclinical studies investigating peptide and small molecule permeation across porcine buccal and sublingual tissue models.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **David Brayden:** This protocol enables accurate measurement of drug permeation across mucosal tissues, supporting reliable preclinical absorption prediction and formulation development using tissue from large animal models.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Estela Bini:** The main challenge is maintaining tissue integrity throughout epithelial isolation and the entire experimental procedure to ensure accurate and reproducible permeability measurements.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Muhammad Ijaz:** Future studies can evaluate additional oromucosal peptide formulations using standardized cross-laboratory ex vivo protocols to improve correlation with human in vivo absorption.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JOVE article address?

1.6. **David Brayden, Professor:** (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

What value do you expect this video article to have for other researchers working in your field?

1.7. **Muhammad Ijaz, Research Scientist:** (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

How does the video format of your article help you to share your work with the broader scientific community?

1.8. **Sandeep Karki, PhD student:** (authors will present their testimonial statements live)

1.8.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Title Card

The pig cheeks used in this study were donated from a local abattoir; therefore, no ethical approval was required

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Isolation and Preparation of Porcine Buccal Epithelium

Demonstrators: Muhammad Ijaz, Sandeep Karki

Authors: During filming, please point out the organs or veins or parts before dissecting them.

- 2.1. To begin, obtain the pig cheek tissue in ice-cold PBS at pH 7.4 [2].
 - 2.1.1. WIDE: Talent placing the container with the collected pig cheek tissue + PBS on the work surface. ***Videographer:** Please take both WIDE and CU shot for this*
- 2.2. Using a scalpel, separate the buccal mucosae from the underlying tissue [1]. Rinse the tissue three times with PBS [2] and discard mucosae with visible surface damage [3].
 - 2.2.1. Talent using a scalpel to separate buccal mucosae from underlying tissue.
 - 2.2.2. Talent rinsing the tissue repeatedly with phosphate-buffered saline.
 - 2.2.3. Talent inspecting and discarding damaged mucosae.
- 2.3. Then, immerse the intact mucosae in PBS at 65 degrees Celsius for 3 minutes [1]. Using a size 5 watchmaker's forceps, separate the epithelium and underlying connective tissue from the smooth muscle [2].
 - 2.3.1. Talent placing intact mucosae into a container with PBS for incubation.
 - 2.3.2. Talent using a size 5 watchmaker's forceps to separate epithelium and connective tissue from smooth muscle.
- 2.4. Gently dry the dissected oromucosae on cellulose filter paper [1].
 - 2.4.1. Talent placing dissected oromucosae onto cellulose filter paper to dry.

3. Ex Vivo Permeation Study Using Franz Diffusion Cells

- 3.1. Mount the dissected mucosal tissue in Franz diffusion cells with an exposed diffusion area of 1.2 square centimeters [1]. Equilibrate the tissue for 30 minutes with 1 milliliter of PBS at pH 6.8 in the donor compartment and 10 milliliters of PBS at pH 7.4 in the receptor compartment [2].
 - 3.1.1. Talent mounting dissected tissue mucosae in Franz diffusion cell.
 - 3.1.2. Talent adding PBS solutions into donor and receiver compartments for equilibration.
- 3.2. After equilibration, replace the donor compartment's PBS solution with 1 milliliter of PBS containing a 1 to 1 ratio of GLP-1RA and GDC, each at 25 milligrams per milliliter [1-TXT].
 - 3.2.1. Talent removing the donor solution and adding the prepared GLP-1RA and GDC mixture. **TXT: GLP-1RA: Glucagon-Like Peptide-1 Receptor Agonist; GDC: Glycodeoxycholate**
- 3.3. Continuously stir the receptor compartment at 300 revolutions per minute while maintaining 10 milliliters of PBS at pH 7.4 and 37 degrees Celsius [1]. Sample 1 milliliter from the receiver compartment every 60 minutes during the 180-minute-long experiment [2]. Take triplicate samples from the donor compartment at the start and end of the experiment [3]. Replenish the chambers with 1 milliliter of PBS after each sampling [4].
 - 3.3.1. Talent placing the chamber on a stirring setup for incubation.
 - 3.3.2. Talent withdrawing sample from the receiver compartment.
 - 3.3.3. Talent collecting sample from the donor compartment
 - 3.3.4. Talent adding PBS back into one of the chambers.
- 3.4. At the end of the experiment, store the tissue in 10 percent formalin for histology [1] or homogenize the tissue to quantify the GLP-1RA peptide remaining in the tissue [2].
 - 3.4.1. Talent placing tissue into a container with formalin.
 - 3.4.2. Talent homogenizing the tissue sample.
- 3.5. To confirm the permeation-enhancing effect of GDC before film formulation, perform GLP-1RA permeability studies using Hilltop chambers loaded with the peptide-enhancer solution [1].

3.5.1. Talent placing the Hilltop chamber onto the mounted porcine buccal tissue and loading the GLP-1RA:GDC solution.

3.6. After confirming enhancer activity using Hilltop chambers, assess ex vivo permeability from GLP-1RA and GDC-loaded mucoadhesive bilayer films mounted on porcine buccal tissues in Franz diffusion cells or use 3D inserts [1].

3.6.1. Talent placing a GLP-1RA:GDC-loaded bilayer film onto the apical surface of tissue mounted in a Franz diffusion cell.

3.7. For assessing epithelial barrier integrity, add fluorescein isothiocyanate dextran 4000, or FD-4, at 0.05 millimolar to the donor compartment [1]. Collect samples from the receiver compartment over 3 hours to assess tissue leakage [2].

3.7.1. Talent pipetting FD-4 into the apical donor compartment.

3.7.2. Talent collecting samples from the receiver compartment for leakage assessment.

3.8. After the 3-hour permeation experiment, perform ultra-high-performance liquid chromatography to quantify cumulative GLP-1RA flux in samples collected from the basolateral compartment [1].

3.8.1. Talent loading samples into the ultra-high-performance liquid chromatography system.

4. Three-Dimensional Insert-Based Ex Vivo Permeation Assay

4.1. To improve peptide detection sensitivity during film permeation studies, use pre-prepared GLP-1RA and GDC-loaded buccal bilayer films [1]. Obtain a 3D insert using an ultraviolet-curable resin and mount the tissue into the insert [2].

4.1.1. Talent walking towards the work station with the loaded films and showing the loaded pre-prepared buccal bilayer films.

4.1.2. Talent showing the ultraviolet-curable resin 3D insert and mounting the tissue into it.

- 4.2. Position the porcine buccal mucosae across a window area of 1.4 square centimeters between the apical and basolateral chamber [1]. Place films of area 1 by 1 square centimeters on top of the tissue [2].
 - 4.2.1. Talent placing 3D inserts with porcine buccal mucosae within the Transwells.
 - 4.2.2. Talent placing films onto the tissue surface.

- 4.3. To confirm porcine buccal and sublingual epithelial tissue integrity, use FD-4 at 0.05 millimolar as a fluorescent marker [1].
 - 4.3.1. Talent adding fluorescein isothiocyanate dextran 4000 solution to the apical donor side of the tissue.

- 4.4. After placing the film, pipette 0.5 milliliters of PBS at pH 6.8 into the apical chamber [1] and then pipette 1.5 milliliters of PBS at pH 7.4 into the basolateral chamber [2].
 - 4.4.1. Talent pipetting PBS into the apical chamber.
 - 4.4.2. Talent pipetting PBS into the basolateral chamber.

- 4.5. Collect 0.2 milliliter samples from the receiver side at 1-hour intervals over 3 hours [1] and replace with equal volumes of fresh buffer after each sampling [2].
 - 4.5.1. Talent withdrawing sample from the receiver compartment.
 - 4.5.2. Talent adding fresh buffer to replenish the chamber.

- 4.6. Measure the transepithelial electrical resistance using the chopstick electrodes placed in the donor and receiver sides of the inserts [1] and calculate the transepithelial electrical resistance using Ohm's Law [2 or 3].
 - 4.6.1. Talent placing chopstick electrodes into the donor and receiver compartments of the insert.
 - 4.6.2. Talent recording values and writing in a notebook.
 - 4.6.3. SCREEN: TEER calculations.mp4 00:05-00:14

- 4.7. After the 3-hour experiment, quantify GLP-1RA permeation and residual tissue content using ultra-high performance liquid chromatography [1]. Repeat all permeation experiments three times and quantify the cumulative flux of GLP-1RA [2 and 3].
 - 4.7.1. Talent loading the samples into ultra-high performance liquid chromatography column.
 - 4.7.2. Talent analyzing data using a calibration curve on the computer.
 - 4.7.3. SCREEN: TEER calculations.mp4 00:25-00:35

5. Histological Assessment of Porcine Oromucosal Tissue Integrity

- 5.1. Fix the tissues in 10 percent formalin for 48 hours after the completion of the experiment [1] and then embed the fixed tissues in paraffin [2].
 - 5.1.1. Talent placing tissue samples into a container with formalin for fixation.
 - 5.1.2. Talent embedding the fixed tissue in paraffin.
- 5.2. Section the tissues at a thickness of 5 micrometers using a microtome [1].
 - 5.2.1. Talent sectioning paraffin-embedded tissues using a microtome.
- 5.3. Next, stain the tissue sections with hematoxylin and eosin [1] to examine their histological integrity using light microscopy at 4x magnification [2].
 - 5.3.1. Talent adding stain to the tissue sections.
 - 5.3.2. Talent placing the sample under a light microscope.
- 5.4. Finally, image the slides and analyze them using a suitable image analysis software [2].
 - 5.4.1. SCREEN: Histology image capture_25052026.mp4 00:07-00:17.

Results

6. Results

6.1. GLP-1RA permeation increased when GDC was included at a 1 to 1 ratio [1] as compared to controls without GDC [2].

6.1.1. LAB MEDIA: Figure 5. *Video editor: Highlight the black bars*

6.1.2. LAB MEDIA: Figure 5. *Video editor: Highlight the red bars/data points*

6.2. When the GLP-1RA and GDC combination was incorporated into bilayer films, GLP-1RA permeation across isolated porcine buccal tissues was not quantifiable using the conventional Franz diffusion cell setup [1].

6.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the purple dots near zero in the FDC group.*

6.3. Using 3D inserts, cumulative GLP-1RA permeation from buccal films over 3 hours was measurable and reached approximately 1.8% [1].

6.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the purple bar in the 3D Inserts group.*

6.4. TEER values declined over time in the GDC-added treatment groups and corresponded with increased GLP-1RA permeation [1].

6.4.1. LAB MEDIA: Supplementary Figure 3. *Video editor: Highlight the red and green lines decreasing from 0 to 180 minutes.*

6.5. PBS control-treated tissues showed no TEER changes, indicating that GDC was the primary contributor to the permeation-enhancing effect [1].

6.5.1. LAB MEDIA: Supplementary Figure 3. *Video editor: Highlight the blue line that stays near the top of the graph.*

6.6. Histological assessment indicated that the buccal mucosae exposed to either an aqueous mixture or a bilayer film containing GLP-1RA and GDC maintained epithelial integrity [1]. Only mild edema [2] and superficial epithelial erosion were observed [3].

- 6.6.1. LAB MEDIA: Figure 6v2. *Video editor: Show panel B and C*
- 6.6.2. LAB MEDIA: Figure 6v2. *Video editor: Show panel B and C and highlight the region pointed by the blue arrow*
- 6.6.3. LAB MEDIA: Figure 6v2. *Video editor: Show panel B and C and highlight the region pointed by the red arrows*

- 6.7. Exposure to blank film did not produce visible tissue damage [1].
 - 6.7.1. LAB MEDIA: Figure 6v2. *Video editor: Show panel A*

- 1. Buccal
Pronunciation link: <https://www.merriam-webster.com/dictionary/buccal>
IPA: /'bʌkəl/
Phonetic Spelling: buh·kuhl
- 2. Epithelium
Pronunciation link: <https://www.merriam-webster.com/dictionary/epithelium>
IPA: /,epə'thi:liəm/
Phonetic Spelling: eh·puh·thee·lee·uhm
- 3. Mucosae
Pronunciation link: <https://www.merriam-webster.com/dictionary/mucosa>
IPA: /mju:'koʊsi:/
Phonetic Spelling: myoo·koh·see
- 4. Oromucosae
Pronunciation link: No confirmed link found
IPA: /,ɔ:roʊmju:'koʊsi:/
Phonetic Spelling: or·oh·myoo·koh·see
- 5. Cellulose
Pronunciation link: <https://www.merriam-webster.com/dictionary/cellulose>
IPA: /'seljə,looʊs/
Phonetic Spelling: sel·yuh·lohs
- 6. Permeation
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeation>
IPA: /,pɜ:mi'eɪʃən/
Phonetic Spelling: pur·mee·ay·shuhn
- 7. Equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ɪ'kwɪlə,breɪt/
Phonetic Spelling: ih·kwil·uh·brayt
- 8. Compartment
Pronunciation link: <https://www.merriam-webster.com/dictionary/compartment>

- IPA: /kəm'pɑ:rtmənt/
Phonetic Spelling: kuhm·part·muhnt
9. Glucagon
Pronunciation link: <https://www.merriam-webster.com/dictionary/glucagon>
IPA: /'glu:kə,gɑ:n/
Phonetic Spelling: gloo·kuh·gon
 10. Agonist
Pronunciation link: <https://www.merriam-webster.com/dictionary/agonist>
IPA: /'ægənɪst/
Phonetic Spelling: ag·uh·nist
 11. Glycodeoxycholate
Pronunciation link: <https://www.howtopronounce.com/glycodeoxycholate>
IPA: /,glɑ:kouði:,pksi'koʊleɪt/
Phonetic Spelling: gly·koh·dee·ok·see·koh·layt
 12. Histology
Pronunciation link: <https://www.merriam-webster.com/dictionary/histology>
IPA: /hɪ'stɑ:lədʒi/
Phonetic Spelling: hih·staa·luh·jee
 13. Homogenize
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /hə'mɑ:dʒə,naɪz/
Phonetic Spelling: huh·maa·juh·nyze
 14. Mucoadhesive
Pronunciation link: <https://www.howtopronounce.com/mucoadhesive>
IPA: /,mjʊ:koʊəd'hi:sɪv/
Phonetic Spelling: myoo·koh·ad·hee·siv
 15. Apical
Pronunciation link: <https://www.merriam-webster.com/dictionary/apical>
IPA: /'eɪpɪkəl/
Phonetic Spelling: ay·pih·kuhl
 16. Basolateral
Pronunciation link: <https://www.howtopronounce.com/basolateral>
IPA: /,bəsoʊ'lætərəl/
Phonetic Spelling: bay·soh·lat·uh·ruhl
 17. Fluorescein
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescein>
IPA: /flʊ'resi:n/
Phonetic Spelling: floor·eh·seen
 18. Isothiocyanate
Pronunciation link: <https://www.merriam-webster.com/dictionary/isothiocyanate>
IPA: /,aɪsoʊ,θaɪoʊ'saɪə,neɪt/
Phonetic Spelling: eye·soh·thy·oh·sy·uh·nayt
 19. Dextran
Pronunciation link: <https://www.merriam-webster.com/dictionary/dextran>

- IPA: /'dekstræn/
Phonetic Spelling: deks·tran
20. Chromatography
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>
IPA: /,kroʊmə'tɑ:grəfi/
Phonetic Spelling: kroh·muh·taa·gruh·fee
21. Transepithelial
Pronunciation link: <https://www.howtopronounce.com/transepithelial>
IPA: /,trænz,ɛpə'θi:liəl/
Phonetic Spelling: tranz·eh·puh·thee·lee·uhl
22. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /,ʌltrə'vaɪələt/
Phonetic Spelling: ul·truh·vy·uh·luht
23. Formalin
Pronunciation link: <https://www.merriam-webster.com/dictionary/formalin>
IPA: /'fɔ:rməlɪn/
Phonetic Spelling: for·muh·lin
24. Microtome
Pronunciation link: <https://www.merriam-webster.com/dictionary/microtome>
IPA: /'maɪkrə'toʊm/
Phonetic Spelling: my·kruh·tohm
25. Hematoxylin
Pronunciation link: <https://www.merriam-webster.com/dictionary/hematoxylin>
IPA: /,hi:mə'tɑ:ksəlɪn/
Phonetic Spelling: hee·muh·tok·suh·lin
26. Eosin
Pronunciation link: <https://www.merriam-webster.com/dictionary/eosin>
IPA: /'i:əsɪn/
Phonetic Spelling: ee·uh·sin
27. Magnification
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnification>
IPA: /,mægnəfə'keɪʃən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn
28. Permeability
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeability>
IPA: /,pɜ:miə'bɪləti/
Phonetic Spelling: pur·mee·uh·bil·uh·tee
29. Porcine
Pronunciation link: <https://www.merriam-webster.com/dictionary/porcine>
IPA: /'pɔ:rsam/, /'pɔ:rsɪn/
Phonetic Spelling: por·syne, por·sin
30. Edema
Pronunciation link: <https://www.merriam-webster.com/dictionary/edema>

IPA: /ɪ'di:mə/

Phonetic Spelling: ih·dee·muh