# XJENZA ONLINE

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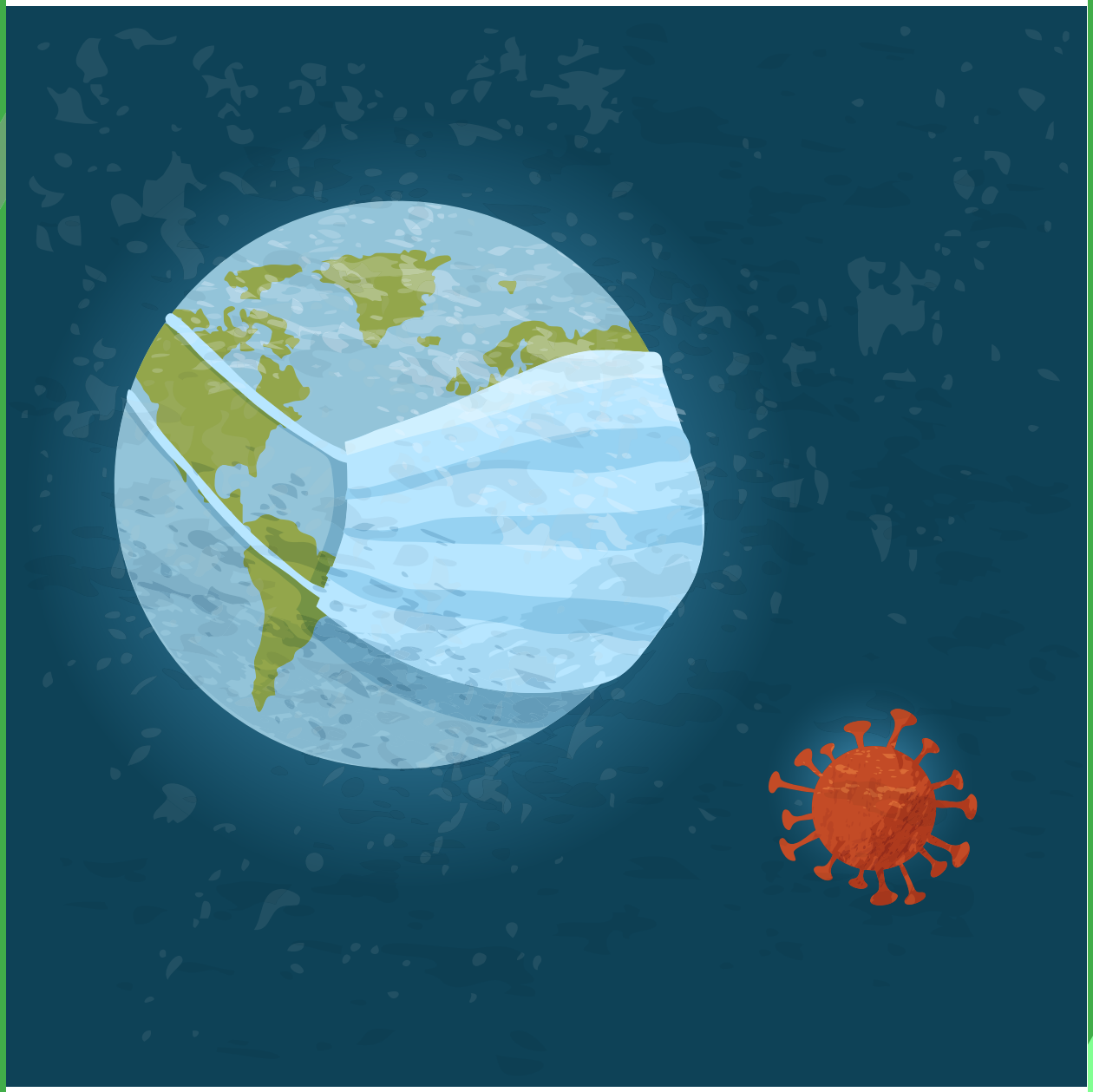

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Science Journal of the Malta Chamber of Scientists

Editor-in-Chief: Cristiana Sebu

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Xjenza Online Vol. 8 Iss. 2 - 2020
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Xjenza Online Vol. 7 Iss. 2 - 2019
Xjenza Online Vol. 7 Iss. 1 - 2019
Xjenza Online Vol. 6 Iss. 2 - 2018
Xjenza Online Vol. 6 Iss. 1 - 2018

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Xjenza Vol. 11 - 2006
Xjenza Vol. 10 - 2005
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# Scope of Journal

Xjenza Online is the Science Journal of the Malta Chamber of Scientists and is published in an electronic format. Xjenza Online is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of: mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of science.

The first printed issue of the journal was published in 1996 and the last (Vol. 12) in 2007. The publication of Xjenza was then ceased until 2013 when a new editorial board was formed with internationally recognised scientists, and Xjenza was relaunched as an online journal, with two issues being produced every year. One of the aims of Xjenza, besides highlighting the exciting research being performed nationally and internationally by Maltese scholars, is to provide a launching platform into scientific publishing for a wide scope of potential authors, including students and young researchers, into scientific publishing in a peer-reviewed environment.

# Instructions for Authors

Xjenza is the Science Journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website: http://www.mcs.org.mt/index.php/xjenza. Xjenza will consider manuscripts for publication on a wide variety of scientific topics in the following categories

1. Research Articles
2. Communications
3. Review Articles
4. Notes
5. Research Reports
6. Commentaries
7. News and Views
8. Invited Articles and Special Issues
9. Errata

**Research Articles** form the main category of scientific papers submitted to Xjenza. The same standards of scientific content and quality that applies to Communications also apply to Research Articles.

**Communications** are short peer-reviewed research articles (limited to three journal pages) that describe new important results meriting urgent publication. These are often followed by a full Research Article.

**Review Articles** describe work of interest to the wide community of readers of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and a critical discussion of the existing state of knowledge on a topic based on primary literature sources. Review Articles should not normally exceed 6000 words.Authors are strongly advised to contact the Editorial Board before writing a Review.

**Notes** are fully referenced, peer-reviewed short articles limited to three journal pages that describe new theories, concepts and developments made by the authors in any branch of science and technology. Notes need not contain results from experimental or simulation work.

**Research Reports** are extended reports describing research of interest to a wide scientific audience characteristic of Xjenza. Please contact the editor to discuss the suitability of topics for Research Reports.

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**Invited Articles and Special Issues:** Xjenza regularly publishes Invited Articles and Special Issues that consist of articles written at the invitation of the Editor or another member of the editorial board.

**Errata:** Xjenza also publishes errata, in which authors correct significant errors of substance in their published manuscripts. The title should read: Erratum: "Original title" by ***, Xjenza, vol. *** (year). Errata should be short and consistent for clarity.

# Submission of Manuscripts

Manuscripts should be sent according to the guidelines given hereafter to xjenza@mcs.org.mt.

**Referees** All manuscripts submitted to Xjenza are peer reviewed. Authors are requested to submit with their manuscript the names and addresses of three referees, preferably from overseas. Every effort will be made to use the recommended reviewers; however the editor reserves the right to also consult other competent reviewers.

**Conflict of Interest** Authors are expected to disclose any commercial or other types of associations that may pose a conflict of interest in connection to with the submitted manuscript. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page or at the end of the article.

**Policy and Ethics** The work presented in the submitted manuscript must have been carried out in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (`http://www.wma.net/en/30publications/10policies/b3/index.html`); EU Directive 2010/63/EU for animal experiments (`http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm`); Uniform Requirements for manuscripts submitted to Biomedical journals (`http://www.icmje.org`). This must be stated at an appropriate point in the article.

**Submission, Declaration and Verification** Author(s) must only submit work that has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that is not under consideration for publication elsewhere, that has been approved for publication by all authors, and tacitly or explicitly, by the responsible authorities where the work was carried out, and that, if accepted, will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder.

**Permissions** It is the responsibility of the corresponding author of a manuscript to ensure that there is no infringement of copyright when submitting material to Xjenza. In particular, when material is copied from other sources, a written statement is required from both the author and/or publisher giving permission for reproduction. Manuscripts in press, unpublished data and

personal communications are discouraged; however, corresponding authors are expected to obtain permission in writing from at least one author of such materials.

# Preparation of Manuscripts

Xjenza accepts submissions in MS Word, Libre Office Writer and LaTeX, the latter being the preferred option. Anyone submitting in LaTeX should use the journal template, the latest version of which can be found at `http://github.com/hicklin/Xjenza-Journal-Template`. All the necessary files to run the LaTeX document should be supplied together with the rendered PDF.

If a word processor is used the styling should be kept to a minimum. Bold face and italic fonts, as well as subscript and superscript text may be used as required by the context. Text should be in single-column format and the word processor options should not be used in order to justify text or hyphenate words. Alongside the native format of the word processer, a PDF file, generated by the word processor, must be provided. Furthermore, artwork should be in accordance with the artwork guidelines given below and must be submitted separately from the word processor file. Similarly, the bibliographic data of the cited material should be submitted separately as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BibTeX (*.bib) file.

## Article Structure

A manuscript for publication in Xjenza will typicall have the following components: Title page, Abstract, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusions, Appendices and References.

The manuscript will be divided into clearly defined and numbered sections. Each numbered subsection should have a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text, i.e. refer to the subsection by the section number.

### Title page

- The title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Indicate the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after each author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address.
- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, including post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and complete postal address. Contact details must be kept up to date by the corresponding author.
- Present/permanent address. If an author has changed the address since the work described, this can be indicated as a footnote to the author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

**Abstract** A concise and factual abstract is required of up to about 250 words. The abstract should state briefly the background and purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references and non-standard abbreviations should be avoided. If essential, these must be defined at first mention in the abstract itself.

**Abbreviations** Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote and should be used consistently throughout the text.

**Introduction** State the objectives of the work and provide an adequate background, avoid a detailed literature survey or a summary of the results.

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**Discussion** This should explore the significance of the results of the work, yet not repeat them. Avoid extensive citations and discussion of published literature. A combined section of Results and Discussion is often appropriate.

**Conclusions** The main conclusions based on results of the study may be presented in a short Conclusions section. This may stand alone or form a subsection of a Discussion or Results and Discussion section.

**Appendices** Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

**Acknowledgements** Collate acknowledgements in a separate section at the end of the article before the references. Do not include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided assistance during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

**Units** Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Anyone using LaTeX should use the package siunitx in all cases.

**Footnotes** Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes by a superscript number in the text and list the footnotes separately at the end of the article. Do not include footnotes in the Reference list.

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**Artwork** Electronic artwork general instructions:

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- JPEG or PNG: Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
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**Tables** Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be moderate with the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. Large tables should be submitted in CSV format.

**Citations and References** Reference and citation styles for manuscripts submitted to Xjenza should be in accordance to the APA v6 style.

**Citation in text** References to cited literature in the text should be given in the form of an author's surname and the year of publication of the paper with the addition of a letter for references to several publications of the author in the same year. For further information regarding multiple authors consult the APA v6 guidelines. Citations may be made directly

Kramer et al. (2010) have recently shown ...

or parenthetically

as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999).

Groups of references should be listed first alphabetically, then chronologically. When writing in LaTeX use `\textcite{}` and `\parencite{}` for the respective cases mentioned.

**The reference section** Every reference cited in the text should also be present in the reference list (and vice versa). The reference list should also be supplied as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. Consult the APA v6 guidelines for multiple authors. Below are some examples of referencing different bibliographic material.

### Reference to a Journal Publication:

Agree, E. M. and Freedman, V. A. (2011). A Quality-of-Life Scale for Assistive Technology: Results of a Pilot Study of Aging and Technology. *Phys. Ther.*, 91(12):1780–1788.

McCreadie, C. and Tinker, A. (2005). The acceptability of assistive technology to older people. *Ageing Soc.*, 25(1):91–110.

### Reference to a Book:

Brownsell, B. (2003). *Assistive Technology and Telecare: Forging Solutions for Independent Living.* Policy Press, Bristol.

Fisk, M. J. (2003). *Social Alarms to Telecare: Older People's Services in Transition.* Policy Press, Bristol, 1st edition.

### Reference to a Chapter in an Edited Book:

Brownsell, S. and Bradley, D. (2003). New Generations of Telecare Equipment. In *Assist. Technol. Telecare Forg. Solut. Indep. Living*, pages 39–50.

**Web references** The full URL should be given together with the date the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately or can be included in the reference list.

**References in a Special Issue** Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

**Journal Abbreviations** Journal names should be abbreviated according to:

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**Video data** Xjenza accepts video material and animation sequences to support and enhance the presentation of the scientific research. Authors who have video or animation files that they wish to submit with their article should send them as a separate file. Reference to the video material should be clearly made in text. This will the modified into a linked to the paper's supplementary information page. All submitted files should be properly labelled so that they directly relate to the video files content. This should be within a maximum size of 50 MB.

# Submission check list

The following list will be useful during the final checking of a manuscript prior to sending it to the journal for review. Please consult the Author Guidelines for further details of any item.

- One author has been designated as the corresponding author with contact details:
  - E-mail address.
  - Full postal address.
  - Telephone and fax numbers.
- All necessary files have been sent, and contain:
  - All figures are given separately in PDF, SVG, JPEG of PNG format.
  - Caption for figures is included at the end of the text.
  - All tables (including title, description, footnotes) are included in the text and large tables have been given separately as CSV.
  - The reference list has been given in XML, RIS, zotero.splite or BIB file format.
- Further considerations

- Abstract does not exceed about 250 words.
- Manuscript has been 'spell-checked' and 'grammar-checked'.
- References are in the required format.
- All references mentioned in the reference list are cited in the text, and vice versa.
- Bibliographic data for all cited material has been provided.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- A PDF document generated from the word processor used is submitted.

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*Editorial*

# Keeping up the Hope

**Cristiana Sebu***[1]
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Dear readers and authors of Xjenza Online, as Editor-in-Chief, I am very pleased to announce the release of the second issue of 2020 of Xjenza Online.

For the past year, the world has been through a terrible crisis which impacted on every aspect of our lives and work. In the higher education sector, in-person teaching, learning and research have faced severe disruptions or have been stopped altogether in order to curb the spread of COVID-19. Academic and student mobility has been reduced to the point that international research collaborations, conferences, visiting scholar and student exchange programmes were put on halt. Many research activities have also been threatened by the discontinuation of lab and field work. These interruptions have resulted not only in delayed or missed scholarship, but also in the loss of professional advancement and training for undergraduate, graduate, and postdoctoral researchers, not to mention the overall loss of human interaction from excess digitization. Nevertheless, this issue of Xjenza Online demonstrates once more the continuity of the scientific endeavour in the Maltese Islands, particularly the one drawn on the local research expertise.

The issue opens with an important and timely study by Martin, Desira and Zarb on the perceptions, beliefs and attitudes related to the use of face masks during the COVID-19 pandemic in the Maltese Islands. Quantitative and qualitative data were collected via an online survey with a sample made up of 990 respondents. For the vast majority of respondents, wearing of face masks while interacting in public spaces (both indoors and outdoors) leads to a sense of security, with increased sense of confidence in public safety measures. However, the study revealed that the knowledge of the population on face coverings is significantly associated with the level of education, and the common beliefs include the mistaken facts that a visor offers as much protection as a face mask and that wearing a face mask reduces the amount of oxygen available to breathe. Qualitative data also highlighted challenges linked to communication, heat, discomfort, anxiety about lack of oxygen, breathing difficulties, besides issues related to condensation on spectacles. Although compliance to public health directives was clearly dominant within the analyzed sample, the particular challenges highlighted within this study also identified areas of potential breakdown of safe practices where a more focused and science-informed public health communication strategy at national level would be beneficial.

The following review article by Anilal, Calleja-Agius and Felice explores the complex interplay of protein factors in embryonic development and in the control of gene expression.

The issue concludes with a manuscript by Emad Eddin Alzoubi et al., assessing the effectiveness of different tooth whitening products, highlighting any undesirable effects of whitening on the oral soft tissues, and evaluating if tooth whitening can serve as a motivational tool for patients to improve their oral hygiene.

To conclude, I wish you all to stay strong and safe, and continue to keep up the hope for better times. As always, Xjenza Online will continue to serve the local professional scientific community, to publish high-quality original findings in a peer-reviewed environment, and to help early-career researchers to advance their scientific discourse in the community.

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*Research Article*

# Mask-wearing during the Covid-19 pandemic in the Maltese context: attitudes, beliefs, perceptions and behaviour

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[3] *Department of Mathematics, University of Malta Junior College, Msida, Malta*

**Abstract.** The efficacy of any public health campaign is impacted in important ways by the level of public understanding and cooperation. The measures put in place to limit the spread of SARS-CoV-2 virus in Malta have had important influence on societal relations, with the use of face coverings arguably having the most impact. The aim of our study was to empirically explore the lived experience of wearing a mask - the perceptions, beliefs and attitudes related to the use of face- coverings during the COVID-19 pandemic in the Maltese Islands. A mixed methods research design was used to collect data via an online survey with a convenience, non-probability sample made up of 990 respondents. Quantitative data were collected via closed ended questions supplemented by qualitative data in open text boxes. Our data, collected before the wearing of face coverings was mandatory in all public places, showed how the vast majority of participants chose to wear them, with the predominant choice being facemasks. The vast majority of respondents claimed that the wearing of face masks while interacting in public spaces (both indoor and outdoor) leads to a sense of security, with increased sense of confidence in public safety measures. Data on beliefs and knowledge are significantly associated with level of education and include the mistaken belief that a visor offers as much protection as a facemask, and that wearing a facemask reduces the amount of oxygen available to breathe. Qualitative data highlighted challenges linked to communication, heat, discomfort, anxiety about lack of oxygen, and finding it harder to breathe, besides issues related to condensation on spectacles. The negative impact at work was also flagged, with increased level of irritability, reduced levels of concentration and reduced quality of service described in the data. Though compliance to public health directives was clearly dominant within our sample, the particular challenges highlighted within the study identify areas of potential breakdown of safe practices where focused science communication on a national level would be beneficial.

**Keywords:** Facemasks, Malta, Covid-19, pandemic, attitudes, beliefs, perceptions, behaviours

**Acknowledgements.** We are grateful to the reviewers for their constructive comments and suggestions. We also thank Dr Tanya Melillo from the Infectious Disease Prevention and Control Unit, Malta for her feedback on the questionnaire.

## 1 Introduction

On the 7th of March 2020 Malta recorded its first case of COVID-19, a 12-year-old girl who had returned to Malta from Italy on the 3rd of March developed symptoms and tested positive for SARS-CoV-2 (The Times of Malta, 2020, Mar 7). Within 5 days from the first case, on the 13th of March, all schools and educational facilities were closed by the Government in an effort to contain the spread of the virus. Just over a month later, Malta had its first Covid-19 victim with the first Covid-19 related death recorded on the 8th of April.

Some general population data within the Maltese context is necessary in order to put the rate of local spread of Covid-19 into perspective. Recent data show that the total population based on normal conditions of residence on the Maltese Islands was 514 564 at the end of 2019. The total land area for the islands is 315 kilometers squared, with a population density of 1633 persons per square kilometre (National Statistics Office, Malta, 2020). This makes Malta by far the most densely pop-

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ulated nation state within the EU where the average (EU27 from 2020) is 109 per square kilometre (Eurostat, 2020a). Added to this dense and vibrant predominantly urban local context is the impact of the highest tourism intensity in the EU, with 21 guest nights per inhabitant (Eurostat, 2020b).

During the first wave of SARS-CoV-2 infections (March-April-May), despite a palpable increase in anxiety levels (Grech et al., 2020), the general perception on the islands was that the Superintendence for Public Health was handling the pandemic adequately, considering that the country was recording below average active cases and deaths, when compared to the rest of Europe. There were various processes at play here, including the announcement of a public health emergency, the closing of our airport to nonessential flights, and the implementation and enforcement of strict social distancing measures which restricted people from meeting up in groups and also through the closure of most leisure, sports and recreational facilities including restaurants, bars, hairdressers, hotels, pools, open-air markets, churches and several health services such as physiotherapy and immunisation clinics. As the number of active cases became smaller, all of the services were allowed to reopen as announced on the 22nd of May 2020.

The Prime Minister, on the 14th of June, declared that the public health emergency was to be lifted and that by the 15th of July "all Legal Notices enacted in this regard will be removed" (The Malta Independent, 2020, Jun 14), however the Public Health preventive campaign continued. Although mask-wearing was not mandatory, the Superintendence of Public Health started to urge the public to wear masks. The Archdiocese of Malta made the wearing of face coverings in church mandatory as soon as churches were allowed to open to the public on the 13th of June 2020.

On the 1st of July, with a minimal number of active cases in Malta, all Covid-19 preventive measures were lifted. The Archdiocese of Malta also announced that the wearing of masks during mass shall not remain mandatory. On the 20th of July 2020 Malta had a mere four active cases, which is the smallest recorded number of active cases in one day since the start of the first wave of Covid-19 in Malta.

The relaxing of measures was quickly followed by an increase in cases, particularly after commercial flights to and from Malta resumed on the 1st of July, and the strict social distancing rules were abandoned, most pertinently in nightlife hotspots such as Paceville. Henceforth, by the beginning of August, the rates of new cases of COVID-19 started to increase, and public health restrictions followed suit. The country gradually started to make the use of masks mandatory, at first by obliging the public to wear masks when receiving or giving a service in enclosed spaces such as supermarkets, retail shops, shopping malls and hospitals. As of the 8th of August 2020, a fine of €50 was imposed on those who did not comply with mask-wearing regulations. On the same day during two separate press conferences, Prime Minister Abela, Health Minister Fearne and Superintendent Gauci all wore masks, with the latter two officials keeping it on whilst addressing the press. Although the legal notice with regards to face coverings solely referred to masks, the press raised a question to the spokesperson of the Ministry for Health about whether the wearing of visors without a mask was lawful or not. The spokesperson for the Ministry confirmed that Malta's Face Covering Legal Notice includes visors despite the legal notice only referring to masks. A few days later, on the 11th of August, the Superintendent of Public Health, Professor Charmaine Gauci confirmed, during her regular press meeting on national television, that the legal notice is to be amended to include visors as well as masks.

On the 19th of August, mask restrictions were further tightened as the wearing of masks became mandatory in all closed public spaces, with the exception of restaurants whilst seated. On the same day the operation of all bars and nightclubs was halted until further notice.

The rates of new cases continued to rise and, during the period of data collection for this research, a new record was recorded on the 16th of September, with 106 new cases (COVID-19 Public Health Response Team (Malta)). Breaching 100 new cases in a day was a significant milestone in the public imaginary.[1] On the 18th of September the Superintendent of Public Health encouraged the public to wear masks at all places and times, except when at home. The Malta Chamber of Commerce had proposed mandatory mask-wearing in all places outside homes on the previous day.

After data collection for this research had been finalised, the government of Malta made mask-wearing mandatory in all public spaces, indoor and outdoor, including offices. The government announced that this regulation would be enforced as of the 23rd of October 2020 and individuals who do not comply would have to face a fine of €50, or €100 if not paid immediately.

Once again, although the government constantly refers to masks, visors are not excluded from the law and thus members of the public who choose to wear a visor without a mask would not be breaking the law. Nonetheless, the local public health authorities have recommended the use of face-masks as opposed to visors, directly claiming that the latter type of face-covering

[1] Similar milestones occurred on 17th October 2020 with 204 new cases, and 2nd March 2021 with 336 new cases. Most significant was the highest number of new cases recorded in 24 hours to date of publication which was 510 on 10th April, 2021 (COVID-19 Public Health Response Team (Malta)).

is not as effective as the former. On Wednesday 11th November, Minister for Health Chris Fearne declared on national television that whilst masks are more effective than visors, the wearing of visors without a mask underneath remains lawful for the benefit of those who simply cannot cope with a mask on.

This dynamic flow of information and recommendations from Public Health and Government officials and institutions affects the general public in important ways, and has a direct impact on the effectiveness of public health campaigns. This paper sets out to highlight some societal elements at play. It draws on empirical data to offer exploratory engagement with the beliefs, attitudes and lived experiences related to the wearing of face coverings in the Maltese local context.

## 2 Literature Review

The World Health Organisation (WHO) initially emphasised the respiratory droplet infection route as the key mode of transmission of SARS-CoV-2 (World Health Organisation, 2020). The possibility of it also being airborne and spread via aerosols has been the source of much debate with early researchers claiming that experts can't agree on the matter (Lewis, 2020, pp. 1). A study by Lednicky et al. (2020), which was published in August, revealed that Sars-Cov-2 virus could be detected in the air up to 4.8 meters away from infected patients. The Lancet for Respiratory Medicine published an editorial in October 2020 highlighting the growing evidence for airborne infection, stating that "infective microdroplets are small enough to remain suspended in the air and expose individuals at distances beyond 2 m[eters] from an infected person" (The lancet respiratory medicine: Editorial, 2020). In tune with this new evidence, WHO updated their website in October 2020 to state that "aerosol transmission can occur in specific settings, particularly in indoor, crowded and inadequately ventilated spaces, where infected person(s) spend long periods of time with others, such as restaurants, choir practices, fitness classes, nightclubs, offices and/or places of worship" (World Health Organisation, 2020).

The beneficial impact of face coverings is clearly supported by research with one study concluding that "wearing a surgical mask or a KN95 respirator significantly reduced the outward number of particles emitted per second of breathing" (Asadi et al., 2020, pp. 5). Wearing masks helps with preventing the spread of Covid-19 because it drastically decreases the spread of droplets and viruses in exhaled breath (Leung et al., 2020). It is also apparent, however, that the science supporting the efficacy of face masks to control the spread of SARS-CoV-2 is not clear cut - not least because the wearing of facemasks is a behavioural variable. They are worn by people who choose different qualities of mask (surgical, fabric, KN95 etc), worn with different levels of adherence to best practice related to use, reuse, disposal and washing. Individuals' beliefs and attitudes are central to the process, indeed it has been claimed that "[h]uman behaviour is core to how well masks work in the real world" (Peeples, 2020, pp. 188).

Focusing on the societal context when exploring the wearing of face coverings has led to some interesting findings. It has been found that men are more likely to be non-compliant when it comes to face-covering regulations (Okten et al., 2020). Gender not only impacts behaviours but it also seems to impact perceptions towards mask-wearing. Men are more likely to perceive face-masks as a deprivation of their control and independence, whilst women are more likely to build their perceptions towards face-masks based levels of comfort, or the lack of it (Howard, 2020).

This finding resonates with pre-Covid-19 literature which shows that men have a tendency to be risk-takers with the aim of portraying a strong and courageous image. In fact, men are typically less likely to adopt preventive measures such as taking vaccines, but more likely to act dangerously through actions such as speed driving (Byrnes et al., 1999; Harris et al., 2006). A 2016 review declared that women were 50% more likely to engage in preventive measures, such as proper and frequent hand-washing, cleaning of surfaces and mask-wearing, in case of an epidemic such as influenza (Moran et al., 2016).

These findings suggest that mask-wearing campaigns would benefit from factoring in gender related behavioural differences. The importance of enforced regulations which leave everyone with no other option but to comply have also been flagged since the effectiveness of masks is largely dependent on compliance (Eikenberry et al., 2020). Research shows that policies which make the wearing of masks mandatory are a prerequisite to ensuring that the best outcomes are achieved (Betsch et al., 2020). Compliance is socially praised whereas not complying is perceived as deviant behaviour which is negatively sanctioned or "socially punished" (Betsch et al., 2020, pp. 2).

The efficacy of any public health campaign is impacted in important ways by the level of public understanding and cooperation. The measures put in place to limit the spread of SARS-CoV-2 virus in Malta have had important influence on societal relations, with the use of face coverings arguably having the most impact. The aim of our study is to empirically explore the lived experience of wearing a mask—the perceptions, beliefs and attitudes related to the use of face-coverings during the COVID-19 pandemic in the Maltese Islands.

## 3 Research Design

A mixed-methods research design was used, collecting quantitative and qualitative data via an online survey. The decision to collect quantitative and qualitative data via the same research tool, rather than sequentially using preliminary qualitative focus groups, was due to the urgency of the topic and time and human resources restrictions.

The research population for this study was the general public in the Maltese Islands. A convenience sample was created by inviting local Facebook users to participate via an active link to a google forms questionnaire. This was widely posted using personal contacts of the researchers and also (with permission) on popular closed Facebook groups. The urgency to collect data within a fluid and fast changing scenario led to this sampling strategy. Though it comes with intrinsic limitations related to the necessity of IT literacy and material (hardware) resources required to participate, framing the sample within the community of local Facebook users does offer a useful 'contingent window' (Candea, 2007) onto the situation in the local context.

The data collection for this research was carried out over a span of 11 days, from the 8th until the 18th of September 2020. A total of 990 responses were collected with a minimal amount of these responses being incomplete. Table 1 shows the profiles of the respondents. A self-assessment of vulnerability to COVID-19 resulted in 20.5% of respondents stating that they were in the 'vulnerable' category for COVID-19, while 66.7% said they were not, and 12.8% stated that they did not know.

It is important to flag that any claims to knowledge or understanding we make are limited to the population from which this sample was drawn—individuals with active Facebook accounts, resident in Malta. Tallies have been left unweighted with respect to gender, age or education as these, in themselves, are interesting data and reflect the gendered activity within the social media landscape and the social profile of individuals engaged in debates related to health and wellness on the web.

### 3.1 Survey Design

The survey was created and administered using google forms, in conformity with the University of Malta Research Ethics Committee (UREC) research ethics review procedure. All data are anonymous, with no identifiers collected. The introductory section on the survey offered a synopsis of the research aim, the institutional affiliation and contact details of the researchers, the rights of respondents, and a declaration that respondent was over 18 years of age.

The survey was organised under general themes of: Demographic data, Attitudes, Knowledge and Beliefs, Choices and behaviour. The survey was fielded in the English language, however respondents were given the option to use Maltese in the open-ended, qualitative responses. The vast majority of the questions were closed-ended, however participants had the option to add qualitative details in open-ended supplementary items. There was a good uptake in this respect.

The survey was piloted with a convenience sample of 13 to test for content validity, and it was also reviewed by a senior member of the Infectious Disease Prevention and Control Unit in order to identify any gaps in targeted data. Feedback led to further refinement and additions to the questionnaire, and the eventual final version had 45 questions, taking 15–20 minutes to complete.

Quantitative data were analysed using SPSS, whereas qualitative data were analysed thematically after manually identifying codes in the data which were colour coded and then sorted into overarching themes within Excel. The chi-squared test was the main statistical test used, because the main interest was in comparing responses across gender, age, and education level. A correction for multiple testing was not deemed necessary since we mainly considered each test individually. In all analysis, we consider a $p$-value of 0.05 or less to be significant. In all chi-squared tests used for comparing responses across gender, the expected values in each cell were in the accepted range so the results of the test are not considerably affected by the difference in numbers for males and females, even though a proportion more representative of the actual population would have been preferred.

This paper will highlight the key findings in the quantitative data while drawing on some of the key themes in the qualitative data.

## 4 Results and Discussion

### 4.1 Behaviour

When asked about wearing face coverings, even though not mandatory by law at time of data collection, almost all stated that they wear a face mask as directed by the Superintendent for Public Health: 96.7% of respondents replied they always do, 2.8% replied sometimes and 0.5% said they never did. This finding is to be interpreted within the limitations of the sample, and is not surprising as individuals taking up the invitation to participate in the survey would very probably be positively engaged in public health endeavours. Also expected is the finding that 99% of all respondents who self-identified as vulnerable to COVID-19 stated that they always wear masks.

The majority of respondents who sometimes or never wear masks as directed by Public Health opted to contribute qualitative data to expand on their response, with the majority of these (55%) flagging cynicism and

| | | No. of respondents | Percentage |
|---|---|---|---|
| Age | 18-24 | 65 | 6.6 |
| | 25-34 | 173 | 17.5 |
| | 35-44 | 265 | 26.8 |
| | 45-54 | 234 | 23.7 |
| | 55-64 | 190 | 19.2 |
| | 65-74 | 56 | 5.7 |
| | 75+ | 5 | .5 |
| Gender | Female | 777 | 78.7 |
| | Male | 210 | 21.3 |
| Level of Education | Post-secondary (sixth form) | 232 | 23.5 |
| | Primary/ Secondary | 135 | 13.7 |
| | Tertiary | 619 | 62.8 |

**Table 1:** Profile of respondents

disbelief in the usefulness of wearing masks, followed by 32% who claimed they were uncomfortable and that it was too hot to wear one. Other reasons flagged for not wearing a mask were the problems with communication that they cause, or the fact that "*they are horrible and take away freedom*". Interestingly, gender did not have a significant impact on these responses, however this is to be interpreted with caution and within the limitations of the opt-in recruitment technique for the survey.

### 4.2 Perceptions

When asked how they feel about people around them wearing face masks, 94% of respondents claimed they felt more comfortable when face masks are worn in enclosed public spaces, with this percentage going down to 79% in outdoor public spaces. There is a significant difference here, $\chi^2(1, N = 978) = 91.73$, $p < 0.001$.

Thematic analysis of the qualitative data gathered in this section led to three overarching themes : Feeling safer and protected; Respect for laws and regulations; Reciprocity in care. Out of the respondents who said that they feel more comfortable with people working in public space wearing masks, 82% of open ended responses mentioned feeling safer and more protected, believing there was less risk of infection, with participants describing how they "*feel reassured that the shop is implementing [safety] measures*" which leads them to "*feel[ing] secure and know[ing] that the place is following all protocols*". 10% mention that it is a reassuring sign of respect for law and regulations, with 8% flagging reciprocal care and social responsibility – "*because they would be respecting me just like I would be respecting them*", and "*I feel they show commitment to the well-being of the community*".

The majority of those who responded that they were indifferent expressed cynical beliefs on the efficacy of wearing masks, with some individuals questioning the very existence of the pandemic. 35% of these were cynical about the usefulness of wearing a mask in the first place. They pointed out that they were ineffective because people do not wear them correctly, don't change or wash them, with some going as far as questioning the need for them "*because I don't believe there is a pandemic*". Some describe feelings of frustration, and being "*fed up*" surrounded by people in masks: "*I don't like them they freak me out to wear them . . . because it keeps me and people safe. . . but really don't like them*". The respondents who expanded on why they feel less comfortable when people working in public areas wear masks described how "*it is just wrong*", "*ridiculous*", with one going as far as saying it is "*as if we are dogs that bite*". One key theme that emerged in the qualitative data is cynicism about the efficacy of wearing masks "*because I think it does more harm than good, it increases carbon dioxide which is harmful for the wearer*". Some refer to physical effects of wearing masks, claiming that they decrease the quality of communication ("*cannot hear voices*", "*cannot see half the face*").

When asked about how one feels about other persons wearing the mask incorrectly, i.e. under the nose, the responses in table 2 were obtained.

Interestingly, the main feeling is annoyed and this is consistent across gender, age and level of education with no significant differences between groups. When considering vulnerability there is a significant difference,

| | Frequency | Percentage |
|---|---|---|
| Annoyed — it shows lack of concern for others | 699 | 71.2 |
| Anxious — it puts me at risk as the mask is not effective worn that way | 229 | 23.3 |
| Fine — it works well worn that way | 9 | .9 |
| Indifferent — I don't care about they way people wear masks | 45 | 4.6 |

**Table 2:** Q43: How do you feel when people around you are wearing their mask under their nose?

$\chi^2(6, N = 975) = 22.75$, $p = 0.001$, and those who are vulnerable are more anxious, as expected, than the other two groups.

### 4.3 Beliefs

When asked about the protection offered by visors, the majority (68.7%) believe that it offers less protection than a mask, 13.4% believe it offers the same amount of protection, 15.3% were unsure while 2.5% believe the visor offers more protection. There is a significant difference, $\chi^2(3, N = 984) = 10.61$, $p = 0.014$, between male and female respondents here, with more men believing it offers less protection (70.3%), and more women being unsure (17% as opposed to 10% in males) The table below shows how the different age groups answered. Again the difference is significant, $\chi^2(18, N = 985) = 10.61$, $p = 0.013$.

In table 4, one can see that the highest percentage of those who believe the visor is less effective is in the 25–44 age bracket, while the lowest percentage is in the 65–74 age bracket, and in this age bracket 23.3% believe the visor offers the same amount of protection as a mask. It is important to note that, as previously mentioned, it was only towards the end of October (after data collection), that the health authorities publicly stated that masks are more effective than visors, and strongly recommended the use of masks rather than visors.

When asked about the effect of wearing a mask on the amount of oxygen breathed in, 47% think it has no effect, 24% answered maybe while 29% think it has an effect. Comparing the responses by gender, we see there is a significant difference, $\chi^2(2, N = 983) = 11.528$, $p = 0.003$, with 57% of men saying it has no effect as compared to 44% of women.

This gender difference is to be considered with caution and in tandem with data on education where there is also a significant difference, $\chi^2(4, N = 982) = 24.73$, $p < 0.001$, in responses when comparing levels of education. As one can see in table 5, the percentage of those who believe the mask has no impact on oxygen levels is much higher in the category of those who have tertiary education. It is important to flag that the level of education within our non-probability sample is not equal across genders, with 76% of male respondents having a tertiary level of education compared to 59% of female respondents.

### 4.4 Lived Experience of Wearing a Mask

Participants were also invited to reflect on their experience of day-to-day living with masks due to the pandemic. When asked whether they feel that it is harder to breathe with a mask on 39.3% of those who responded said yes, 35.8% said in certain situations, while 24.9% said no. Again there is a significant difference in responses when comparing gender, $\chi^2(2, N = 985) = 17.17$, $p < 0.001$, with 42% of women saying it is harder to breathe as compared to 31% of men. When comparing across ages within the female gender, we also see some interesting differences (table 6), $\chi^2(12, N = 986) = 27.92$, $p = 0.006$.

In the age group 55–64, a significantly larger percentage feel that it is harder to breathe with a mask compared to the other age groups.

Thematic analysis of the qualitative data offered in this section led to identification of 11 themes as itemised in table 7. The qualitative responses ($n = 416$) to Q30 were predominantly (39% of responses) focused on the issue of coping with the masks in excessive heat. This is unsurprising given the local climatic context at the time of data collection with peak daytime temperatures ranging from 24 to 27 degrees celsius.

The second most frequent situation flagged by respondents was that of exercise, with 26% of responses mentioning the difficulty they have in breathing with a mask during recreational exercise (walking, running, dancing), quotidian exertion (climbing stairs, walking fast), or work related physical activity. More interesting is the third situation flagged for creating challenges related to mask wearing. This is the reference to wearing masks in small, enclosed spaces, at 11% of responses for this question, with some participants specifically mentioning buses, planes, lifts and small rooms in their responses. The key issue in these cases, seems to be the feeling of "*lack of air*" due to poor ventilation, where "*oxygen is already limited as is*", where this feeling of "*not enough air*" makes it harder to breathe while wearing a mask. This finding is particularly interesting and relevant to understanding non-compliance with public

| | | Q43 | | | |
|---|---|---|---|---|---|
| | | Annoyed | Anxious | Fine | Indifferent |
| I am vulnerable to COVID-19 | Don't know | 69.4% | 28.2% | | 2.4% |
| | No | 73.5% | 19.6% | 1.2% | 5.7% |
| | Yes | 64.9% | 32.7% | 0.5% | 2.0% |

**Table 3:** Self-assessed vulnerability to COVID-19 cross-tabulated with Q43: "How do you feel when people around you are wearing their mask under their nose?"

| | | Q17 | | | | |
|---|---|---|---|---|---|---|
| | | No, a visor provides less protection | No, a visor provides more protection | Not sure | Yes, the same | Total |
| Age | 18–24 | 60.0% | 7.7% | 16.9% | 15.4% | 100.0% |
| | 25–34 | 62.8% | 3.5% | 16.9% | 16.9% | 100.0% |
| | 35–44 | 73.2% | 2.6% | 15.5% | 8.7% | 100.0% |
| | 45–54 | 74.7% | 1.7% | 14.2% | 9.4% | 100.0% |
| | 55–64 | 66.1% | 0.5% | 14.8% | 18.5% | 100.0% |
| | 65–74 | 58.9% | 3.6% | 14.3% | 23.2% | 100.0% |
| | 75+ | 80.0% | | 20.0% | | 100.0% |
| | Total | 68.7% | 2.5% | 15.3% | 13.4% | 100.0% |

**Table 4:** Q17: Do you believe that wearing a visor (without a mask) offers the same amount of protection TO THE PERSON WEARING IT as wearing a face mask?

| | | Q18 | | |
|---|---|---|---|---|
| | | Maybe | No | Yes |
| Level of education | Post-Secondary (6th form) | 26.0% | 39.0% | 35.1% |
| | Primary/ Secondary | 33.6% | 34.3% | 32.1% |
| | Tertiary | 20.9% | 52.7% | 26.4% |
| | Total | 23.8% | 46.9% | 29.2% |

**Table 5:** Level of Education cross-tabulated with Q18: Do you believe that wearing a mask has an impact on the amount of oxygen you breathe in?

| | | Q29 | | | |
|---|---|---|---|---|---|
| | | In certain situations | No | Yes | Total |
| Age | 18-24 | 33.3% | 28.2% | 38.5% | 100.0% |
| | 25-34 | 39.2% | 23.3% | 37.5% | 100.0% |
| | 35-44 | 42.5% | 18.4% | 39.2% | 100.0% |
| | 45-54 | 35.4% | 20.7% | 43.9% | 100.0% |
| | 55-64 | 28.5% | 21.5% | 50.0% | 100.0% |
| | 65-74 | 37.8% | 35.6% | 26.7% | 100.0% |
| | 75+ | | 66.7% | 33.3% | 100.0% |
| | Total | 36.4% | 22.1% | 41.5% | 100.0% |

**Table 6:** Female respondents by age cross tabulated with Q29: Do you feel that it is harder to breathe with a mask on?

| Q30 | Frequency of code | % |
|---|---|---|
| heat | 164 | 39.4 |
| exercise/ manual work/exertion (stairs) | 110 | 26.4 |
| enclosed areas | 45 | 10.8 |
| length of time | 40 | 9.6 |
| stress at work | 12 | 2.9 |
| other | 10 | 2.4 |
| inflamed sinuses /asthma | 10 | 2.4 |
| wearing specs | 8 | 1.9 |
| anxiety/dizzy | 6 | 1.4 |
| talking | 6 | 1.4 |
| hairdresser | 3 | 0.7 |
| hot flushes | 2 | 0.5 |
| Total | 416 | 100.00 |

**Table 7:** Qualitative data from Q30: In which situations is it harder to breathe with a mask on?

health directives on mask wearing, as this sense of lack of air in small enclosed spaces may pose particularly high risk for transmission of the Coronavirus COVID-19.

A further 10% of the responses to this question described how they felt it was harder to breathe with a mask on in situations when they had to wear them for long periods. Other reasons given for feeling it was harder to breathe with masks on included being stressed at work (3% of responses), health issues such as anxiety, asthma, inflamed sinuses (4%), hot flushes due to the menopause (0.5%), talking (1.4%) or sitting at the hairdresser (0.7%).

### 4.5 Communication

When asked about the effect of wearing a mask on communicating with others, 31% said it makes it harder to communicate, 59% said it sometimes makes it harder, while 10% said it doesn't make it more difficult. Here there is a significant difference in the response pattern when comparing across age groups, $\chi^2(12, N = 987) = 31.36$, $p = 0.002$, where those over 65 seemingly finding more difficulty (72% think it is always or sometimes more difficult to communicate with a mask on, while 28% think it is not a problem). Thematic analysis of qualitative data related to problems with communication due to use of facemasks resulted in the identification of seven key themes as itemised in table 8. The main problems with communication while using facemasks are related to the impact they have on vocal language communication. Some respondents drew attention to the end result with 13% highlighting the key frustration of not being understood or not understanding others. The majority (49%) of the responses to this question ($n = 953$) flagged issues related to the processes involved, with reduced voice projection and clarity of speech being the main problem with repeated reference to sound of voices being muffled and the problematic interference of other background noise.

Interesting detail was offered by seven respondents who explained that this was particularly problematic when they were speaking a language that was not their mother tongue — "*certain words come out mumbled*" and "*accents not being clear*" add to the general challenges in communicating while wearing facemasks.

Another dominant theme within the qualitative data offered in this section relates to the negative impact on effective communication linked to the inability to lip read. 16% of the descriptions referred to the way this reduces effective communication, especially when talking to elderly individuals who are hard of hearing. Interestingly, however, this was also flagged by respondents who described how "*you don't realise how much lip reading happens until your mouth is covered*", and how, when use of facemasks commenced they realised that they were not understanding because they "*lip read a lot out of habit*".

Equally important in the data is the theme related to the reduced ability to read emotions and facial body language, with 17% of the reasons given in the responses describing how they found it harder to communicate when unable to read facial expressions "*you can never fully read the emotion on someone's reaction*".

A very small proportion of the respondents flagged issues related to wearing the mask itself, which physically interfered with their speech because of the heat and discomfort associated with it, or the misting up of spectacles. One described how they were impacted by the "*hot air whilst talking and mask sucks back into mouth*" or the "*mask moving in and out*". An equally small proportion described how their own state of anxiety linked to having to wear the mask impacts badly on communication, with one respondent describing how "*I don't communicate well when I wear a mask as it makes me nervous/frustrated*". The responses within this theme highlighted issues related to anxiety and breathlessness and a feeling of "*less oxygen, less breathing, less recognition of the partner or the situation you are in*".

### 4.6 Impact of Face Masks at Work

A total of 592 respondents (60% of sample) indicated that they wear a mask at work , with 65% of these saying they do so for long periods of time, or constantly. Of these, 302 respondents indicated that wearing a mask at work always, or sometimes, impacts on their performance. 286 individuals chose to offer qualitative details, out of which 6 respondents gave a positive answer, claiming that it makes them feel more secure and confident in doing their job, as they feel protected. The other 280 respondents claimed that the mask impacts their work in a negative way.

The most common response amongst those who answered this question was that the mask limits effective communication. A total of 143 respondents (50% of those who responded to this question) feel that they cannot do their job effectively whilst wearing a mask as they claim that it hinders communication. Difficulty with projecting one's voice, difficulty with properly hearing and understanding what colleagues, students, clients or customers say and the loss of nonverbal communication through facial expressions were the three key issues which participants flagged.

A few participants mentioned that the inability to communicate effectively impacts their mental wellbeing as they end up feeling isolated with one respondent claiming that "*it creates a sense of isolation, a restricted freedom*". Participants also mentioned that masks not only impede communication, but might also lead to miscommunication. Some participants mentioned that the masks restrict them from appearing warm in their customer-oriented jobs and they feel that they often can-

| Q32 | Frequency of code | % |
|---|---|---|
| Poor voice projection/clarity of speech | 468 | 49.1 |
| Can't see facial expressions/read emotions | 164 | 17.2 |
| Can't read lips/cant see mouth | 155 | 16.3 |
| Not being understood/understanding others | 125 | 13.1 |
| Out of breath/anxious/too hot | 14 | 1.5 |
| Discomfort of mask/steamed up specs | 13 | 1.4 |
| Problems when communicating in a foreign language | 7 | 0.7 |
| Other | 7 | 0.7 |
| Total | 953 | 100.00 |

**Table 8:** Qualitative data from Q32: What is the main problem with communication when wearing a mask?

not provide a satisfactory customer experience whilst wearing a mask, claiming that the mask "*makes [them] feel like [they] give a colder service*".

Several respondents who mentioned communication in their responses work in education and argued that the process of teaching and learning is not as effective when wearing masks particularly since masks cover facial expressions which are imperative to understanding the effectiveness of pedagogy. Furthermore, educators also mentioned that they feel uncomfortable about the fact that their students cannot read their expressions, as this makes the student-teacher relationship detached and less personal.

The second most common impact of mask-wearing on the experience of work as mentioned by the participants was the lack of concentration due to discomfort. 83 respondents (29% of those who answered the question) claimed that mask-wearing at their workplace creates discomfort leading to nervousness, irritability and thus to a decrease in concentration. A sense of suffocation, foggy glasses, heat and fatigue were the key discomforts mentioned by participants. Some participants (14) claimed that they feel that they inhale less oxygen whilst wearing a mask for a long duration and thus often end up feeling suffocated and develop other physical discomforts which limit concentration, such as dizziness, nausea, headache and sore or dry throat.

Another 14 participants mentioned that condensation is the main negative impact which the mask has on their work, as it leads to foggy glasses. "*Glasses get misty and can't see properly. [It is] not always comfortable and hence tend to take it off when on my own*".

It is concluded that a lack of effective communication and a lack of concentration caused by discomfort are the two main impacts which masks have on the participants' work.

## 5 Discussion and Conclusion

The practice of wearing facemasks is a core element in Malta's public health campaign to counter the local spread of SARS-CoV-2. Its level of efficacy is dependent on societal compliance and, though legal sanctions no doubt play an important part, individual and shared beliefs and attitudes related to the wearing of face coverings are also key drivers of behaviour in this regard.

Our data, collected before the wearing of face coverings was mandatory, show how the vast majority of participants within our non-probability sample chose to wear them, with the predominant choice being facemasks. The minority who declared they did not wear masks justified this by questioning the usefulness of wearing a mask in the first place, with a handful of individuals going as far as questioning the existence of a pandemic at all. Some pointed out that most of the time they are not worn as they should, and not washed as often as they should be, in order to be effective. This cynical perspective is also evident, though marginally so, when respondents described how they felt when people around them wore masks, with a very small minority claiming that they "*do more harm than good*" and make people look ridiculous.

Data from the overwhelming majority of respondents, however, strongly demonstrate that the wearing of face masks while interacting in public spaces (both indoor and outdoor) leads to a sense of security, with increased sense of confidence in public safety measures. More interesting from a relational perspective is the flagging of a sense of reciprocal respect and community solidarity that results from the wearing of masks in public. The obverse of this is also evident in our data with the vast majority (71%) of respondents flagging the way they are annoyed by individuals wearing their mask incorrectly. 23% went as far as saying that this increased their level

of anxiety, and, as would be expected, this was highest in the respondents who self identified as vulnerable to COVID-19.

There are two areas of interest that emerged in the data related to knowledge and beliefs. The first is the mistaken belief that a visor offers as much protection as a facemask, with this ironically being most evident in the vulnerable 65-74 age group. The evidence that use of visors without a mask does not offer efficient protection has since become well established (Salimnia et al., 2021; Verma et al., 2020). The mistaken belief that emerged in our data may now be less of a concern locally as public health messages that have been launched intending to correct this have probably taken effect. The second area of interest, however, is more predominant and potentially leads to non-compliance in the wearing of a facemask, or anxiety when indeed wearing one. This is the evidently strongly ingrained belief that wearing a facemask reduces the amount of oxygen available to breathe. Our data show that 53% of participants believe that this is, or may be, true. The fact that our data show more accurate beliefs are related to higher levels of education is not an unexpected finding, however this, in itself, flags the need for more effective science communication in the public domain.

Our data related to the lived experience of wearing a mask strongly indicate a negative impact on everyday experience. The fact that data were collected when the average for peak daytime temperature was 26 degrees celsius makes all the qualitative data on hardships related to excessive heat very predictable. The same applies to the problems flagged with wearing masks during exercise, or the physical irritations due to the tight covering over the mouth, and consequent issues of misting up on spectacle lenses. More interesting are the data about individuals feeling that it is harder to breathe with a mask on, with more women than men claiming this to be the case, with it being up as high as 50% of all women in the 55–64 age group. There are qualitative data which offer a possible explanation for this, with participants highlighting the problematic impact of menopausal hot flushes in this scenario, a finding that dovetails with the quantitative data from the 55–64 year old female respondents described above. Another finding with possible important implications on non-compliance to public health recommendations and increased risk of SARS-CoV-2 transmission, is the fact that 11% of respondents who offered qualitative detail on situations where they feel it is harder to breathe with a mask on ($n = 416$) mentioned enclosed, small spaces such as lifts, cars, buses etc.—all areas where risk of transmission by droplet infection is high. This is another scenario that warrants careful and effective science communication to redress.

Both quantitative and qualitative data presented above highlight the negative effect on quality of everyday social interaction and communication that the use of face masks entails. Besides this issue being raised by the elderly and individuals with hearing impairments in the sample, it is also interesting to flag how people with no such disability also reported that they had difficulty comprehending individuals speaking while wearing a mask, with frequent remarks in the qualitative data about being surprised at how much they relied on lip reading in normal everyday situations. The input from individuals describing how the challenges related to speaking a foreign language from behind a mask are also important to flag, where they described increasing problems because of their pronunciation and decreased opportunity for using facial expressions.

These challenges related to communication, heat, discomfort, anxiety about lack of oxygen, finding it harder to breathe, besides the more mundane issue related to condensation on spectacles, all have a negative impact on the experiences of participants who wear masks at work. Half of respondents offering data in this section ($n = 286$) flagged barriers to communication as the key problematic impact of wearing face masks at work, with others (29%) describing how it increased their level of irritability and reduced their level of concentration and the quality of the service they offered.

These findings, based on data drawn from a convenience, non-probability sample ($n = 990$) in the Maltese context are not intended to flag any generalisable conclusions. Though overall compliance to public health directives is clearly dominant within our sample, our data offer a better understanding of the social processes impacting, and impacted by, the wearing of face coverings in the COVID-19 pandemic scenario. The findings, in particular those highlighting lay epidemiology rooted in mistaken beliefs, barriers in communication, and the subjective description of anxiety levels and discomfort, may serve as a contingent lens to identify areas of potential breakdown of safe practices where focused science communication would be beneficial in the general arena.

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*Research Article*

# The control of developmental global gene expression

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**Abstract.** The complex interplay of a myriad of protein factors in embryonic development encapsulates the importance of accuracy in the control of gene expression, regulation and physical factors including cell-environment contact. C. *elegans* has an extremely similar gene interplay and hence its study has paved way a greater understanding. This review will explore cell lineage specification, mutual regulation, the consequences of mutations, and how gene regulatory networks utilise spatio-temporal triggers.

**Keywords:** C. *elegans*, Regulatory network, lineage, spatio-temporal

## 1 Introduction

### 1.1 Lineage and Tissue segregation

Two established theories regarding the triggering of the cell differentiation from the fertilised zygote play in synchrony—beginning a complex cascade of events.

### 1.2 Mosaic (determinate) theory

This theory involves the premise that the ovum contains morphogenic determinants or factors in the cytoplasm which then get distributed to different extents with subsequent cleavage divisions once fertilised by sperm, and hence the resultant blastomeres inherit specific compositions of mRNAs and proteins that enable them to specialise accordingly (Jeffery, 1988).

### 1.3 Regulative (indeterminate) theory

This theory encompasses cell-to-cell induction via physical contact and this intertwines with the mosaic theory as induction would be directed according to the inherited cytoplasmic determinants (Jeffery, 1988). The regulative induction hence is dependent on the cell surroundings; if isolated from a group of cells the other cells are as a result affected and in fact compensate, for example in the case of the formation of identical twins, through regulation (Gilbert, 2000a). As a result, the eventual variation in gene expression for differentiation is triggered by both mechanisms via the 'commitment' of phenotypically similar cells to a particular fate (Gilbert, 2000a).

### 1.4 Cell Division

The initial mitotic divisions are triggered by mitosis promoting factors (MPFs) and constitute a decrease in cell size and hence cytoplasm via bypassing the growth phases and this cleavage produces an 8-cell group of totipotent blastomeres that compacts via alterations in cell-cell contact (Gilbert, 2000b). Additional divisions produce a morula of 12-15 blastomeres and with subsequent divisions a cavitation or blastocoel forms in its centre. The reduction in ovum cytoplasm with divisions is a trigger for the temporal gene expression and cleavage halts once "a new balance between nucleus and cytoplasm" is established (Gilbert, 2000b).

This review aims to incorporate what we know about gene regulatory networks to produce a logical timeline of the process and factors involved in differential gene expression for lineage specification from day 0.

## 2 Mammalian embryo development

Asymmetric distributions of cytoplasmic molecules and proteins and the "establishment and transduction" of cell asymmetry is a ubiquitous mechanism for morphogenesis in all organisms. PAR proteins are an evolutionarily conserved trigger (Johnston et al., 2010).

### 2.1 Blastomere polarisation

At the 8-cell stage, blastomeres undergo calcium-dependent compaction—the first true morphologic step which triggers significant steps such as initiating the differentiative division into the trophectoderm and an inner cell mass (ICM). Compaction is thought to be

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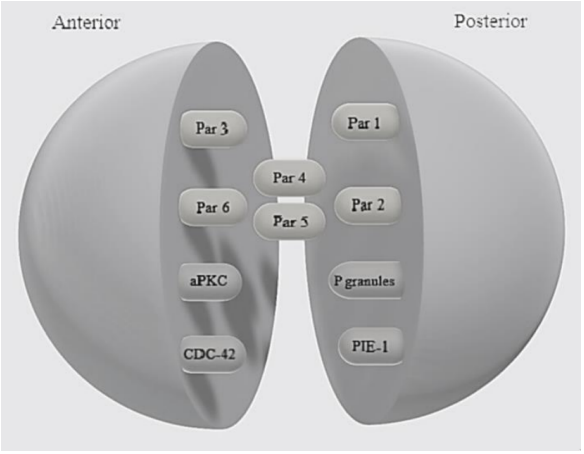


**Figure 1:** The localisations depicted by illustration of PAR proteins with associated P granules, PIE-1, aPKC and CDC-42

induced by a "minimization of the surface energy of cells" and consequently it utilises a cell-to-cell interface adhesion molecule E-cadherin – Cdh1, which partakes in the energy differences between the cells versus the cell and the external environment (Maître et al., 2015). Oscillating contractility produces compaction through the rearrangement of actin filaments mediated by Cdh-1 (Maître et al., 2015) and this is the 'first step' for blastomere polarisation (Zhu et al., 2017).

Actin rearrangement is also involved in polarising blastomere organelles and enables precursors of the trophectoderm cells (outer cells) and ICM at the 8-cell stage to form 16 cells: providing the first means of directing cell fate (Humięcka et al., 2017). Cytoplasmic components that are localised in the apical portion include PAR proteins which omits actin and produces a "mature apical cap" (Zhu et al., 2017).

### 2.2 PAR proteins

PAR3 and PAR6 interact with atypical protein kinase C (aPKC) via their association with actin (Zhu et al., 2017). Actomyosin accumulates apically because before fertilisation, the sperm pronuclei enters and hence establishes the 'posterior' pole via inducing the "local loss of the uniform NMY-2 network" (via downregulation of RHO-1 proteins and accumulation of PPK-1) that was previously ubiquitous around the whole ovum cortex (Johnston et al., 2010; Nance et al., 2011). RHO-1 downregulation alongside areas of RHO-1 upregulation controls the distribution of contractility, directing actomyosin fibre contraction away from the posterior pole hence constituents are directed to and accumulate in the apical pole (Johnston et al., 2010). Experiments highlight the effects of PAR protein loss, where maternal par gene mutant screens resulted in the "first embryonic cleavage to be symmetric" (Nance et al., 2011).

As illustrated in figure 1 apical localisation of PAR3, PAR6 and aPKC contrasts to PAR1 and PAR2 localisation posteriorly whereas PAR4 and PAR5 are unaffected in its distribution due to the anteriorly localised PAR proteins which interact with the other PAR proteins via "inhibitory interactions" (Nance et al., 2011). There is differential accumulation due to a variety of mechanisms such as PAR-3 association with NMY-2 (depleted posteriorly) as shown by experiments utilising dysfunctional F-actin and thus leading to lack of PAR-3 apically (Nance et al., 2011). However, asymmetry of both actomyosin and the PAR proteins is not definitively or strictly linked since experimental induction of actomyosin asymmetry early (in a 4-cell embryo of C. elegans) does not independently lead to PAR protein apical localisation and hence there must be other components interacting with actomyosin in order to 'carry' PAR proteins apically i.e. intermediate factors that supposedly physically links the two (Zhu et al., 2017).

PAR proteins are kinases which once directed, initiate a cascade of effects reinforcing cell asymmetry via phosphorylation, however "only a few direct targets for these kinases are currently known" (Johnston et al., 2010). Asymmetry of PAR proteins enables the subsequent "asymmetries in mRNA and protein" mainly via intermediates MEX-5 and MEX-6 and therefore affect the localisation of developmental lineage determinants (Nance et al., 2011; Tenlen et al., 2008).

Ezrin, actin, PAR3/6 and aPKC all contribute to the apical cap. Phosphorylated ezrin becomes localised apically after compaction uses actin to aid the formation of the apical microtubule cap by linking actin to the cell membrane (Humięcka et al., 2017).

## 3 Translational control

Spatially and temporally controlled translation of maternal mRNAs via maternal RNA binding proteins (RBPs) enables cell specification (Oldenbroek et al., 2013). The maternal RBPs for the maternal mRNA zif-1 include "OMA-1, OMA-2, POS-1, SPN-4, MEX-3, MEX-5 and MEX-6" where different RBPs have different roles; such as MEX-3 mediated inhibition of translation in 1 or 2-cell embryos (Oldenbroek et al., 2013).

### 3.1 MEX-5 MEX-6

MEX-5 is a zinc finger protein that becomes apically localised as a result of PAR proteins—from being distributed evenly in the ovum, it is dependent on PAR 1 proteins that have become localised posteriorly, and also PAR 4 proteins which phosphorylate the C terminal Ser 458 facilitating mobility and therefore localisation to the apically localised actomyosin filaments (Daniels et al., 2010; Nance et al., 2011; Tenlen et al., 2008). MEX 5 is the first "somatic determinant" defining its lineage progression and experiments have indicated that it ant-

agonises the expression of any germline proteins where it localises anteriorly (Daniels et al., 2010). MEX-5 binds to the RNAs glp-1 and nos-2 leading to the inhibition of expression of proteins "SKN-1, PIE-1, NEX-1, POS-1 and PAL-1" alongside expression of the proteins "GLP-1 and MEX-3" and specific destruction of some zinc finger proteins (Pagano et al., 2007).

MEX-6 is a very similar zinc finger protein localised apically, working concurrently with MEX-5 to produce localisation of germline proteins at the posterior pole (Johnston et al., 2010).

### 3.2 PIE-1

PIE-1 is (mainly) a nuclear protein and the first "germline determinant" also localised by PAR proteins in the nucleus posteriorly, where it inhibits gene expression in germline-fated cells (Daniels et al., 2010). The dependency between PIE-1 and PAR protein localisation was demonstrated by par mutants which caused a lack of MEX5/6 and PIE-1 polarisation yet PIE-1 mutants did not affect the other two proteins, i.e localisation of PIE-1 (and MEX5/6) is a consequence of PAR protein asymmetry, however, MEX5/6 was also identified to be affecting the polarisation of PAR proteins and hence provides a mutual interaction for asymmetry (Johnston et al., 2010). In addition, some PIE-1 proteins are bound to "large RNA/protein granules" found posteriorly known as P granules, which are directed posteriorly through MEX5/6 mediated antagonism anteriorly (MEX5/6 mutants led to the lack of posterior localisation of P granules and hence also of PIE-1 indirectly) (Johnston et al., 2010). MEX-5/6 localising anteriorly also antagonises other germline lineage proteins such as POS-1 and MEX-1 which like PIE-1 distributes posteriorly (Daniels et al., 2010). PIE-1 prevents the action of transcription factors and hence the transcription of mRNAs leading to somatic lineage such as SKN-1, protecting totipotency of germline lineage blastomeres (Tenlen et al., 2008).

### 3.3 Other germline determinants: POS-1 and MEX-1

POS-1 and MEX-1 are also germline lineage proteins but are located in the cytoplasm and promotes the translation of mRNAs of germline-fated daughter cells (Daniels et al., 2010). POS-1 mutants alone cause blastomeres to switch to a somatic-fated cell, yet abnormalities differ from MEX-1 and PIE-1 mutants, indicating "distinct roles in the specification of germline blastomeres" (Tabara et al., 1999). Furthermore, MEX-1 and POS-1 proteins also associate with P granules (Tabara et al., 1999).

### 3.4 Other somatic line determinants: PLK-1 and CDC-25

These provide an insight into the intertwining of cell cycle progression and asymmetry since MEX-5/6 binds to PLK-1 and hence aids its anterior apical cytoplasmic localisation and so is present in somatic (AB), versus germline (P), where it triggers "earlier mitotic entry" (Noatynska et al., 2013). To prevent MEX-5/6 binding (directly) to PLK-1 before it itself has been apically localised, it is inactive until it is phosphorylated — at the end of meiosis II, Cyclin-Dependent Kinase 1 (CDK-1) phosphorylates MBK-2, which in turn phosphorylates MEX-5 (Noatynska et al., 2013).

CDC-25, a cell division cycle kinase (cyclin dependent) accumulates anteriorly in the nucleus due to PLK-1 to "promote differences" in the timing of cell cycles (Noatynska et al., 2013). An increased concentration of PLK-1 anteriorly (cytoplasm) results in an increase in localised "nuclear CDC-25 in the AB cell compared to the P1 cell" (Johnston et al., 2010).

## 4 Cell Division for Differentiation

Germline blastomeres are totipotent and after a few divisions from the 1-cell stage, a point is reached where further divisions only produce germline daughter cells; possibly due to temporal differentiation differences since there is a link between the inherited ovum mRNAs and posteriorly localised factors with totipotent characteristics in germline blastomeres (Tabara et al., 1999). Moreover, gene expression varies between germline and somatic blastomeres since at the 4-cell stage when gene transcription begins, "mRNA transcripts are detected only in the somatic blastomeres" yet inhibited in germline blastomeres, which are therefore unaffected by evenly distributed maternal transcription factors, which subsequently define gene expression in somatic lineage blastomeres after the 4-cell stage (Tabara et al., 1999).

## 5 ICM and Trophectoderm development

After 1–2 weeks the morula forms a blastocyst consisting of outer specified cells contributing to the trophectoderm and an inner totipotent group of cells termed the inner cell mass surrounded mostly by a fluid cavity termed the blastocoel.

Cells are 'committed' to either by differentiative division where the apical (polar) cell contributes to trophectoderm and the basal (apolar) cell to the ICM; similarly to stem cells, whereas conservative division enables both daughter cells to be part of the same cell population as it distributes cell constituents equally (Humięcka et al., 2016). Differentiative cell division involves differentiative contractility if "differences in surface contractility. . . exceeds a predictable threshold" cells become in-

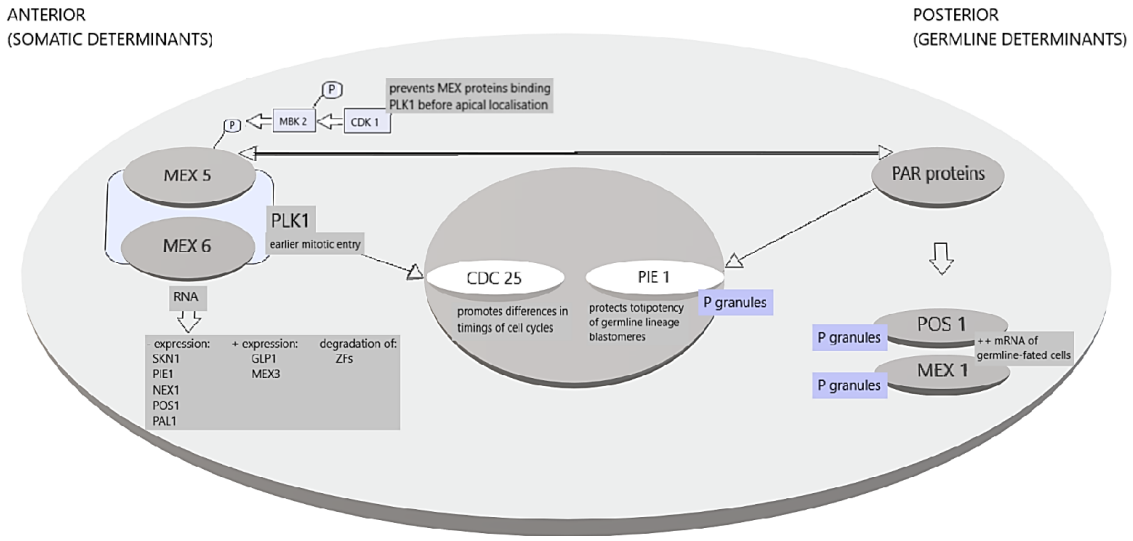


**Figure 2:** A summary of the effects of apical localisation of MEX 5 and MEX 6 somatic determinants (AB), alongside posteriorly localised PAR proteins and P granules as germline determinants that protect blastomere totipotency (P)

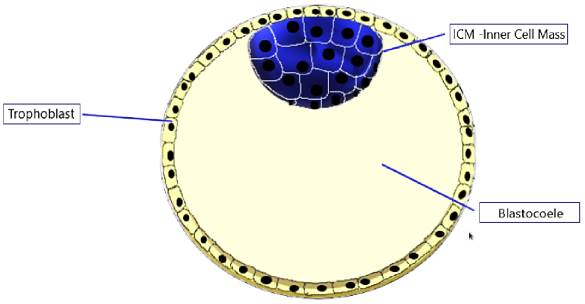


**Figure 3:** The morula at 1–2 weeks initially consists of a totipotent mass of cells—the inner cell mass—derived from apolar cells, and the outer trophectoderm consisting of trophoblasts derived from apical cells. The blastocoel is a fluid filled cavity or 'blastocyst cavity'

ternalised for this first lineage specification (Humięcka et al., 2016).

### 5.1 Trophectoderm

Cdx2, a caudal-related homeobox transcription factor, is the initial transcription factor only expressed by the trophectoderm and hence enables implantation where abolition in experiments utilising RNAi of Cdx-2 mRNA results in the "failure of embryos to. . . implant" (Wu et al., 2010). Cdx2 also activates the progressive expression of Hox genes to establish an anterior-posterior axis (Wu et al., 2010). The Cdx-2 target Eomesodermin is a transcription factor that enables the specialisation of cells becoming part of the polar or mural trophectoderm and another transcription factor Elf5 enables specification of polar trophectoderm cells determined to be extra-embryonic ectoderm (EEE) or cells producing the ectoplacental cone (Degrelle et al., 2005).

The trophectoderm contains trophoblast stem cells—TS cells, maintained by internal trophoblastic cells (the 'polar trophectoderm', versus the external 'mural trophectoderm') which are closer to the ICM and hence receiving of the ICM paracrine factor FGF4 (Marikawa et al., 2009). FGF4 stimulates the trophectoderm to form the EEE which in turn produces BMP4 which aids in the differentiation of the ICM by directing its patterning while the EEE itself eventually develops to form the embryonic side of the placenta—the chorion (Marikawa et al., 2009).

Cdx-2 null embryos have highlighted the role of Cdx-2 in late blastomeres where the formed blastocoel starts to fail due to a possible increase in apoptotic activity alongside the failure of "epithelial integrity" of external trophectoderm, however there is still an increase in Oct4 expression, a protein specifically expressed in wild-type ICM cells (Marikawa et al., 2009). Cdx-2 has been identified to compartmentalise the expression and hence effects of Oct-4 to the ICM; consequently the specification of first lineages arises from intertwining mechanistic effects of stimulatory and inhibitory regulation in contrast to a linear sequence of sequential gene expression, providing a means of mutual antagonism and hence restricted differential specialisation (Marikawa et al., 2009).

### 5.2 Interaction between trophectoderm and ICM (Yap / HIPPO pathway)

It is apparent that Oct4 protects the totipotent and pluripotent cells of the ICM from differentiating into trophectoderm and its two major targets has been identified as the FGF-4 and Nanog genes (Marikawa et al., 2009). Oct-4 expression in the ICM essentially provides

the polar trophectoderm with its co-expressed signal, FGF-4 to enable its stability and development and additionally through targeting the Nanog gene, it preserves the plasticity of embryonic stem cells by inhibiting its differentiation into primitive endoderm and the pluripotency of the ICM (Marikawa et al., 2009).

Tead4 is a transcription factor expressed in both the ICM and the trophectoderm at the 8-cell stage and studies using Tead-4 null embryos displayed normal ICM progression yet failures in trophectoderm specification and development such as the lack of blastocoel formation (in comparison to the formation then failure of the blastocoel with Cdx-2 null embryos) and hence it can be deciphered that Tead-4 may act on the gene encoding Cdx-2 as well as other targets that caused a greater subsequent effect than that of just Cdx-2 null embryos alone (Kaneko et al., 2013; Marikawa et al., 2009). Since the gene for Tead-4 is "genetically upstream" relative to the gene encoding Cdx-2, this indicates that it may not be required for the differentiation of stem cells into the trophectoderm lineage (Nishioka et al., 2009). On the other hand, it was also decided that the maintained potential of TS cells was not "fully substituted" by Cdx-2 alone in Tead-4 null embryos and subsequently, Tead-4 is more important than Cdx-2 in determining the trophectoderm lineage (Nishioka et al., 2009). Furthermore, even though Tead-4 was "genetically upstream", Cdx-2 null embryos failed to produce TS cells and hence it is also essential for cells of the trophoblastic lineage but in a way in which "Tead-4 promotes trophoblast fate through both Cdx-2-dependent and independent pathways,... while Cdx-2 is a major mediator of Tead-4 dependent changes in trophoblast gene expression" (Nishioka et al., 2009).

The mechanism in which Tead-4 null embryos lead to blastocoel failure was linked to an increase in ROS production since the development of the blastocoel involves "increased oxidative phosphorylation" and hence ROS (Kaneko et al., 2013). It is worth noting that Tead-4 is not vital for the lineage determination itself of trophoblastic cells since "once the trophectoderm was specified, Tead-4 was not essential for either proliferation or differentiation of trophoblast cells in culture", but that Tead-4 is vital in "maintaining energy homeostasis" during early embryonic development prior to complete invasion of the mural trophectoderm into the uterus (Kaneko et al., 2013).

Tead-4 is not localised to the cytoplasm, but its selective action relies on the transcriptional co-activator YAP1 which is found specifically in the nuclei trophectoderm cells (Nishioka et al., 2009). The Hippo pathway is a cascade that directs the distribution of YAP1 in a way that enables its binding transcription factor Tead-4 to be functional in the trophoblastic lineage cell populations.

The differential activation (in contrast to the usual mechanism of differential expression) of Tead-4 determines trophoblastic lineage since it is found distributed evenly throughout the blastomeres. YAP1 found in the nuclei of trophectoderm cells from the 8-cell stage is localised through the action of a Hippo signalling pathway factor called Lats2 which is a kinase that phosphorylates and removes YAP1 from the nuclei of cells in the ICM and hence prevents the transcription of Tead-4 in the ICM (Sozen et al., 2014). Yap1 defective embryos still developed functional trophectoderm and so although Tead-4 functions with and without Cdx-2, there must be another way of transcribing Tead-4 initially and the trigger was found to be similar in structure to YAP1 — the cofactor WWTR1 (Nishioka et al., 2009; Sozen et al., 2014).

Similarly to compaction triggering the first steps towards cell specification, cell-cell contacts also play a role in Hippo signalling to be active in the ICM and not the trophectoderm in order to localise Tead-4 appropriately and hence trophectoderm commitment in these outer cells (Sozen et al., 2014). This is achieved by the extensive cell-cell contacts between cells of the ICM and was demonstrated by an experiment looking at the effects of a lack of cell where "differentiation towards TE, possibly as a result of the inactive Hippo pathway, decreased cell polarity and increased levels of Cdx2" (Sozen et al., 2014). The Hippo pathway is specific to the internal, high cell-cell contacted cells of the ICM because of the specification mechanisms utilising cell positioning and cell asymmetry—demonstrated when the apical cap complex was targeted in early embryos, YAP-1 was reduced in the outer (polar) cells (Hirate et al., 2013).

Asymmetric distribution of proteins Amot and Amotl2 have been highlighted as essential for ICM linked Hippo pathway activity by binding to actin in adherens junctions between these cells and through the high cell-cell contact, activating the Hippo signalling pathway in these non-polar cells, meanwhile in outer cells, as Amot is apically localised via the PAR-aPKC complex, because the basal adherens junctions are not devoid of Amot they fail to activate Lats2 and therefore YAP1 / WWTR1 and therefore Tead-4 (Hirate et al., 2013).

## 6 ICM second lineage segregation

Prior to the implantation of the embryo there is further differentiation of the ICM, approximately 24 hours after the formation of the trophectoderm, into a pluripotent epiblast which eventually forms the embryo itself, and an endoderm (primitive endoderm – PE) which forms extra-embryonic endoderm (Humięcka et al., 2016). Secondary lineage commitment is under the influence of

various "lineage-specific transcription factors" that are favoured in a regulatory manner (Sozen et al., 2014).

Mutual antagonism between the two lineages within the ICM — particularly the establishing markers Gata 6 in the primitive endoderm with Nanog in the epiblast, is partially maintained by FGF4 and FGFr2 which are initially ubiquitous in the ICM until the 64-cell stage when FGFr2 becomes upregulated in the primitive endoderm lineage cells and FGF4 in the epiblast lineage cells (Sozen et al., 2014). FGF4 is also upregulated in the primitive endoderm and it potentiates the action of FGFr2 in maintaining the expression of Gata 4/6 and Sox-17 thus there is potentiated antagonism of Nanog via the increase in Gata 6 concentration (Sozen et al., 2014). Although FGF4 is also upregulated in the epiblast lineage cells and so both lineages are dependent on FGF4, FGFr2 is downregulated and so are its subsequent effects (Sozen et al., 2014).

Although there are two lineages forming in the ICM, they are scattered, and this heterogeneity is a result of different transcripts since signalling rather than cell position triggers specification. A 'cell sorting model' was proposed which encompasses organisation via "waves of asymmetric divisions" that results in relative amounts of each lineage type to change—that of primitive endoderm increases and vice versa for that of the epiblast, and how during the formation of the blastocoel, movement of cells towards the outside or inside can occur but is regulated by the transcription markers on their cell surface (Artus et al., 2010; Sozen et al., 2014). The presence of proteins such as DAB2 and ECM LAMC1 have also been found to play a role in cell sorting Artus et al. (2010). Cells with transcription markers indicating epiblastic lineage i.e. expression of a larger amount of Gata-6, tend to have less cell-cell adhesion and so tend to move inwards and those expressing large amounts of Nanog tend to have higher cell-cell adhesion and move externally (Sozen et al., 2014).

## 7 Conclusion

Calcium-dependent compaction, as the first morphological change, is in essence the most important factor controlling early developmental gene expression since it enables the subsequent chain of events establishing networks of gene expression through changes in cell surface energy; which results in the formation of an apical cap and therefore the polarisation of blastomeres. This apical polarisation complex is the means by which cytoplasmic determinants, maternal mRNAs and proteins are differentially localised and hence subsequently cause compartmentalised gene expression for the progression into specific developmental lineages.

Recurring methods of control are evident throughout early development — such as cell-cell contact being a regulator initially in compaction, but it is also a method involved in Hippo signalling and again later in cell sorting after transcriptional heterogeneity.

## Declaration of Interest

There are no conflicts of interest.

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*Research Article*

# The Effectiveness of tooth whitening products in the Maltese market: A Clinical Study

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**Abstract. Background:** Tooth whitening has gained popularity in recent years, with many products emerging on the market.
**Aim:** To assess the effectiveness of different tooth whitening products, highlight any undesirable effects of whitening on the oral soft tissues, and evaluate if tooth whitening can serve as a motivational tool for patients to improve their oral hygiene.
**Method:** 127 participants were invited to join the study and 77 were enrolled in the study according to the selection criteria. They were randomly divided into 8 groups, each group receiving a different tooth-bleaching product. Data collection was performed at 4 different time-points.
**Results:** 39% of participants were excluded due to suboptimal oral health, thus emphasizing the need for a routine check-up before treatment. Only professional tooth whitening provided by dental professionals showed significant tooth shade improvements (Kruskal–Wallis tests $p < 0.05$). Tooth whitening had no significant impact on oral soft tissues (Kruskal–Wallis test $p \geqslant 0.05$). Tooth whitening can serve as a motivating tool to improve patients' oral health.
**Conclusions:** Tooth whitening procedures should be carried out by dental professionals. Only non-over the counter (OTC) products showed significant colorimetric shade improvement. Whitening treatment had no significant impact on oral soft tissues. The achieved tooth whitening directly improved oral health.

**Keywords:** tooth whitening, tooth shade, oral health

## 1 Introduction

Society has moved into an era of high influence by media and all associated with aesthetics. The dental profession and manufacturing industry have not been immune to this shift with the dental patient as consumer directing the profession, the dental industry and dental literature. Dental cosmetic procedures have seen an increase over the past years together with the worth of the dental industry and an increasing commercial culture within dentistry (Doughty et al., 2016). Initially factors associated with the request for cosmetic procedures included female gender, young age, a lower level of education and social influence (Vallittu et al., 1996), however the ageing consumerist population is also presenting high aesthetic demands as a sign of 'successful aging' (Wulfman et al., 2010).

One such dental cosmetic procedure is that of tooth whitening. An increasing number of oral health care products now target tooth discoloration and the need for a whiter and brighter smile (Epple et al., 2019). Tooth discolorations are a result of light reflection or adsorption by tooth surfaces and may be classified as either intrinsic or extrinsic (Rodríguez-Martínez et al., 2019). Extrinsic discolorations are those due to direct or indirect staining by food products, tobacco or cetylpyridinium chloride products. Intrinsic stains are caused by metabolic disorders, medical treatments, inherited disorders, idiopatic disorders or as an effect of dental trauma. Idiopathic disorders such as molar incisor hypomineralisation are the reason for the requests for tooth whitening even by the paediatric/adolescent dental patient population. The American Academy of Pediatric Dentistry recognises this need and regulates the practice (American Academy of Pediatric Dentistry Council on Clinical, 2019). Such practice in persons under the age of 18 is however restricted in EU countries ("COUNCIL DIRECTIVE 2011/84/EU of 20 September 2011 amending Directive 76/768/EEC, concerning cosmetic products, for the purpose of adapting Annex III thereto to technical progress", 2011; Monteiro et al.,

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2019).

Tooth whitening systems are several and may be classified by various parameters including the whitening agent used, the method of application or the concentration of the oxidant agent. This study adopts the classification which defines the products as In-Office or At Home systems. The In-Office systems are those applied by dental health care professionals utilising higher concentrations of oxidant agent over a shorter period than At-Home systems. At-Home systems are further divided into those that are professionally supervised and those available as Over The Counter (OTC) products. The latter are freely available for purchase for use and potentially misuse by all ages. Due to the oxidative free radicals they contain, whitening products may have undesirable effects. These include tooth hypersentivity, root resorption, modificatons of surface morphology, gingival irritation and effects on restorative materials (Rodríguez-Martínez et al., 2019).

For countries belonging to the European Union the provision of whitening/bleaching products is regulated by directive 2011/84/EU ("COUNCIL DIRECTIVE 2011/84/EU of 20 September 2011 amending Directive 76/768/EEC, concerning cosmetic products, for the purpose of adapting Annex III thereto to technical progress", 2011). This states "that a maximum concentration of 0.1% of hydrogen peroxide present in oral products or released from other compounds or mixtures in those products is safe. It should therefore be possible to continue to use hydrogen peroxide in that concentration in oral products, including tooth whitening or bleaching products". Furthermore, "tooth whitening products containing or releasing between 0.1% and 6% hydrogen peroxide can only be sold to dental practitioners". Directive 2005/36/EC further defines a dental practitioner. Despite these regulations, studies have shown that select dental practitioners within the EU were unaware of these directives (52%), or unaware of the directives regulating their use in paediatric patients, or aware of the regulations but were still providing the treatment (Monteiro et al., 2019). A further cross-sectional survey of 179 dental practices and 76 beauty salons in the UK found that most were applying products containing a greater concentration of hydrogen peroxide permissible by current regulations (Doughty et al., 2016). This highlights that both oral health practitioners and the public may be misusing and abusing whitening products. This can be a concern especially in overzealous teenagers (Croll et al., 2014).

Reports of the clinical outcomes of the various whitening products on the market are divergent. Such conflicting reports may be due to differences in protocols, concentrations used (Rodríguez-Martínez et al., 2019) and lack of adherence to manufacturer instructions.

The aim of this study is to assess the clinical effectiveness of the various tooth-whitening products available locally, highlight any undesirable effects of whitening procedures on the oral soft tissues, and evaluate whether tooth whitening can serve as a motivational tool for patients to improve their oral hygiene. This information will serve as a guide to the general dental practitioner.

## 2 Materials and Methods

The prospective cohort study was carried out at the University of Malta, Faculty of Dental Surgery Teaching Clinic over a period of two months. Ethical approval for the research project was obtained (UREC-DP 1801010DSG).

### 2.1 Sample

Participation was voluntary following a social media posting and all eligible subjects were selected based on inclusion and exclusion criteria (table 1). Participants were randomly allocated to one of the eight 'Tooth Whitening Product' groups as outlined in table 2. This was carried out by a Senior Dental Nurse who was blinded to the type of treatment they were prescribed.

### 2.2 Study Design

All patients received a clinical examination. This included charting of the dentition according to the ICDAS (Gugnani et al., 2011; ICDAS, n.d.) a periodontal examination, and an examination of the mucosa, gingival tissue and gingival condition to assess for any lesions. The oral health profile was recorded utilising the following indices: Greene-Vermillion index for soft deposits - plaque index (Greene et al., 1964) and the Löe –Silness gingival index (H., 1967). Additionally, the patient's oral hygiene habits (brushing method, use of interdental brushes, type of toothpaste used) were recorded. Subsequently, subjects received prophylaxis to remove any staining and received oral health instructions. A pretreatment questionnaire was completed.

Impressions were taken for those patients allocated to the products requiring customised whitening trays for home use. Each whitening treatment was carried out according to the manufacturer's instructions. A one-use demonstration, following the manufacturer's directions, was given to the participants allocated the home kit. All participants received oral hygiene instructions and whitening maintenance advice based on the manufacturers' direction. The patients underwent tooth shade measurements using the VITA Easyshade® V digital spectrophotometer (VITA Zahnfabrik, Germany). The measurements were done on teeth (13) upper canine (coded 1), (11) upper first central incisor (coded 2) and (31) lower left central incisor (coded 3). The VITA Easyshade® device displays tooth shade measurement results in three different modes — The VITA

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Adults ≤18 years of age | Medically compromised patients |
| Presence of 20 natural teeth — no prosthesis | Requiring dental treatment due to caries and poor oral hygiene |
| Willing to participate in post-whitening phase | Smoking Habits |
| Presence of all maxillary and mandibular teeth | Oral pathology requiring immediate care |
| No restorations on Anterior teeth | Previous stains due to Tetracycline |
| Absence of hypersensitivity | Pregnancy or lactating mother |

**Table 1:** Inclusion and Exclusion Criteria to participate in the study

| Group | No. of Participants | Intervention |
|---|---|---|
| Group 1 | 10 | Philips Zoom Speed In Office; 6% HP |
| Group 2 | 9 | Beyond Osmo In Office; 6% HP |
| Group 3 | 10 | Philips Zoom Home-kit (daywear); 6% HP |
| Group 4 | 9 | Beyond Corewhite Home-kit (daywear); 6% HP |
| Group 5 | 10 | Ultradent Opalescence PF Home-kit (night wear); 18% CP |
| Group 6 | 10 | Ultradent Opalescence GO Home-kit (day wear); 6% HP |
| Group 7 | 9 | PearlSmile Standard Treatment In Office; <0.1% HP |
| Group 8 | 10 | Pearl Light Home-Kit; <0.1% HP |

**Table 2:** Treatment Groups of Different Whitening agents

SYSTEM 3D-MASTER (29 shades) mode, the VITA classical A1–D4 (16 shades) tooth shade system, or as a Bleach Index. The numerical value output decreases as the tooth shade whitens.

Shade measurements were carried out 1 month before the treatment (T0), on the day of treatment after bleaching (T1), 2 weeks after the treatment (T2) and 1 month after the treatment (T3). Sequential shade readings were compared. The participants were asked to complete a post-treatment questionnaire. At the one-month visit (T3) measurements for the plaque index, gingival index and oral hygiene index were repeated. During this visit the oral mucosa and gingival tissue were examined.

### 2.3 Statistical Analyses

The results were tabulated and analysed with computer software (SPSS software IL, USA). Data derived from the history and examination of each patient were analysed per group, to assess the effectiveness of the product. The In-Office kits (Groups 1, 2, and 7) three shade readings (VITA SYSTEM 3D-MASTER, VITA classical and Bleach Index) and Home kit (Groups 3, 4, 5, 6, and 8) three shade readings were compared separately. Furthermore, all groups were then compared together. The Kruskal-Wallis H Test allowed between groups analysis of the non-parametric continuous scores derived from the various groups. In addition, analysis of before and after scores of oral hygiene indexes and oral mucosa data was carried out. Statistical significance was set at $p < 0.05$.

## 3 Results

One hundred twenty-seven subjects agreed to participate in the study. Following dental examination, 77 subjects (61%) were eligible for this study, based on the inclusion and exclusion criteria. Participants were excluded due to dental decay, suboptimal oral hygiene and the need for dental treatment necessary before tooth whitening procedures. The eligible participants in the study (44 females and 33 males) varied in age from 18 to 60+, however, 65% were between the ages of 18 and 25 years (table 3). 50% of participants had a graduate or higher level of education.

### 3.1 In-Office Kits

Table 4 shows the average change in tooth shade for the three teeth, each measured by the three shade readings off the VITA Easyshade® device for the three In-Office whitening products over four measurement episodes. Kruskall Wallis tests revealed statistically significant differences in shade changes across the three whiten-

| Age and Gender of Participants | | | | |
|---|---|---|---|---|
| | | Female | Male | Total |
| Age | 18 – 25 | 30 | 20 | 50 |
| | 26 – 35 | 10 | 10 | 20 |
| | 36 – 45 | 2 | 2 | 4 |
| | 46 – 60 | 1 | 1 | 2 |
| | 61 and over | 1 | 0 | 1 |
| | Total | 44 | 33 | 77 |

**Table 3:** Demographics of Participants

ing products, for the three teeth over time. The results showed that the Philips Zoom Speed system exhibited the best results over the period T0 to T3 on all the 3 teeth examined.

#### 3.1.1 Tooth 1

The best overall result for this system was a 5.1 shade advancement when measured by Vita 3D Master and Bleaching Score output on tooth 1 (T0–T3). A statistically significant difference was observed between Philips Zoom Speed System shade changes as compared to those achieved by the Pearl Smile Standard treatment at T2 (KW $p = 0.012$). Similar significant results were also recorded between Philips Zoom Speed system and Pearl Smile Standard treatment on tooth 1 at time T2 (KW, $p = 0.009$) and time T3 (KW, $p = 0.05$) when measured by the Classic Vita Shade.

#### 3.1.2 Tooth 2

Shade changes observed for tooth 2 by the three In-Office whitening systems at T1, T2 and T3 were not significantly different to each other when measured by the three shade guide systems.

#### 3.1.3 Tooth 3

Significantly different shade changes were however observed for Tooth 3 again between Philips Zoom Speed System and Pearl Smile Standard at T2 (KW, $p = 0.023$) and T3 (KW, $p = 0.050$) when measured using the Classic Vita shade system and also when using the Vita 3D Master Shade system at T2 (KW, $p = 0.009$) and T3 (KW, $p = 0.010$).

### 3.2 Home Kits

Table 5 shows the average change in tooth shade for the three teeth, each measured by the three shade readings off the VITA Easyshade® device for the three home-use whitening products over four measurement episodes. For the period T0 to T3, results varied from a 9.37 shade improvement score for Philips Zoom Home kit on tooth 1 when measured by the Vita 3D Master shade guide to the reading of −1.89 shade deterioration recorded for Pearl Light Home kit on tooth 1 when measured by Classic Vita shade guide. The greatest shade improvements occurred mostly in period T0 to T1, whereas the shades then deteriorated or remained the same in periods T1 to T2 and T2 to T3.

#### 3.2.1 Tooth 1

When measured using the Classic Vita shade, both Philips Zoom Home kit and the UltraDent Opalescence PF Home kit showed statistically significant differences in shade changes as compared to the Pearl Light Home kit both at T1 (KW, $p = 0.005$, $p = 0.003$, respectively) and at T2 (KW $p = 0.001$, $p = 0.001$, respectively) and at T3 (KW $p < 0.001$, KW $p < 0.001$, respectively). Beyond Corewhite Home Kit also reported significant shade differences to the Pearl Light Home kit at T3 (KW, $p = 0.034$).

Statistically significant differences in shade change were also reported between Philips Zoom Home kit and Pearl Light Home kit when measured using the 3D Master shade guide at T1 (KW, $p < 0.001$), T2 (KW, $p < 0.001$) and T3 (KW, $p < 0.001$). Similar results were observed between the Beyond Corewhite Home Kit and the Pearl Light Home kit at T1 (KW, $p < 0.001$), T2 (KW, $p < 0.001$) and T3 (KW, $p < 0.001$) and between the UltraDent Opalescence PF Home kit and the Pearl Light Home kit at T1 (KW, $p < 0.001$), T2 (KW, $p < 0.001$) and T3 (KW, $p < 0.001$).

Similar results were reported (KW, $p < 0.001$) for the products Philips Zoom Home kit, Beyond Corewhite Home Kit and UltraDent Opalescence PF Home kit when compared independently to the Pearl Light Home kit at T1 (KW, $p < 0.001$), T2 (KW, $p < 0.001$) and T3 (KW, $p < 0.001$) using the Bleaching Score output.

#### 3.2.2 Tooth 2

The upper central incisor (Tooth 2) showed statistically significant differences in shade changes when whitened

| In-Office Kits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tooth Type | 1 | | | 2 | | | 3 | | |
| **Shade measurement system** | **Classic Vita shade guide** | **Vita 3D Master shade guide** | **Bleaching score** | **Classic Vita shade guide** | **Vita 3D Master shade guide** | **Bleaching score** | **Classic Vita shade guide** | **Vita 3D Master shade guide** | **Bleaching score** |
| Whitening Product | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed | Philips Zoom Speed |
| T0 – T3 average shade improvement | 4.29 | 5.1 | 5.1 | 2.8 | 3.84 | 3.84 | 3.9 | 4.3 | 4.3 |
| Whitening Product | Beyond Osmo | Beyond Osmo | Beyond Osmo | Beyond Osmo | Beyond Osmo | Beyond Osmo | Beyond Osmo | Beyond Osmo | Beyond Osmo |
| T0 – T3 average shade improvement | 3.78 | 3.9 | 3.9 | 1.22 | 0.67 | 0.67 | 0.78 | 1.78 | 1.78 |
| Whitening Product | Pearl Smile | Pearl Smile | Pearl Smile | Pearl Smile | Pearl Smile | Pearl Smile | Pearl Smile | Pearl Smile | Pearl Smile |
| T0 – T3 average shade improvement | −0.7 | −0.45 | −0.45 | −0.11 | −0.22 | −0.22 | −0.22 | −0.89 | −0.89 |

**Table 4:** Difference in shade measurements obtained by the VITA Easyshade® device for the In-Office Kits

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| Home Kits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tooth Type | 1 | | | 2 | | | 3 | | |
| **Shade measurement system** | **Classic Vita shade guide** | **Vita 3D Master shade guide** | **Bleaching score** | **Classic Vita shade guide** | **Vita 3D Master shade guide** | **Bleaching score** | **Classic Vita shade guide** | **Vita 3D Master shade guide** | **Bleaching score** |
| Whitening Product | Philips Zoom | Philips Zoom | Philips Zoom | Philips Zoom | Philips Zoom | Philips Zoom | Philips Zoom | Philips Zoom | Philips Zoom |
| T0 – T3 average shade improvement | 7.63 | 9.37 | 9.37 | 4.62 | 3.63 | 3.63 | 2.25 | 2.51 | 2.51 |
| Whitening Product | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF | Ultra Dent Opalescence PF |
| T0 – T3 average shade improvement | 7.57 | 8 | 8 | 7.57 | 2.87 | 2.87 | 4.29 | 3.5 | 3.5 |
| Whitening Product | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO | Ultra Opalescence GO |
| T0 – T3 average shade improvement | 6 | 6.8 | 6.8 | 4.57 | 2.2 | 2.2 | 1.01 | 1.4 | 1.4 |
| Whitening Product | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit | Beyond Corewhite Home kit |
| T0 – T3 average shade improvement | 5.78 | 7.44 | 7.44 | 4.11 | 5.44 | 5.44 | 2.78 | 5.11 | 5.11 |
| Whitening Product | Pearl Light | Pearl Light | Pearl Light | Pearl Light | Pearl Light | Pearl Light | Pearl Light | Pearl Light | Pearl Light |
| T0 – T3 average shade improvement | −1.89 | −0.55 | −0.55 | 0.67 | −0.55 | −0.55 | 0.67 | 0.22 | 0.22 |

**Table 5:** Difference in shade measurements obtained by the VITA Easyshade® device for the At-Home Kits

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using the Philips Zoom Home kit versus the Pearl Light Home kit at T1 (classic vita shade guide KW, $p = 0.011$, Vita 3D Master KW, $p = 0.001$; Bleaching score KW, $p = 0.001$) at T2 (classic vita shade guide KW, $p = 0.015$, Vita 3D Master KW, $p = 0.002$; Bleaching score KW, $p = 0.002$) and at T3 (classic vita shade guide KW, $p = 0.011$, Vita 3D Master KW, $p < 0.001$; Bleaching score KW, $p < 0.001$).

Similar results were observed between the products UltraDent Opalescence PF Home kit and the Pearl Light Home kit at T1 (classic vita shade guide KW, $p = 0.038$, Vita 3D Master KW, $p = 0.006$; Bleaching score KW, $p = 0.006$), T2 (classic vita shade guide KW, $p = 0.024$, Vita 3D Master KW, $p = 0.016$; Bleaching score KW, $p = 0.016$) and also at T3 (classic vita shade guide KW, $p = 0.018$, Vita 3D Master KW, $p = 0.004$; Bleaching score KW, $p = 0.004$).

There were no significant differences in shade changes between all the other home kit products for Tooth 2 when measured using the Classic Vita Shade guide at T1, T2 and T3.

The Vita 3D Master Shade Guide detected significant differences in shade changes for Tooth 2 between results observed for the Beyond Corewhite Home Kit and the Pearl Light Home kit at T1 (KW 0.018), T2 (KW $p = 0.017$) and T3 (KW $p = 0.006$) and between the Ultradent Opalescence GO Home-kit the Pearl Light Home kit only at point T3 (KW $p = 0.05$).

The Bleaching Score Guide results gave significant differences in shade changes for tooth 2 when whitened either using the Beyond Core white home kit or the Pearl Light Home kit at T1 (KW $p = 0.018$), T2 (KW $p = 0.017$) and T3 (KW $p = 0.006$) and between the Ultradent Opalescence GO Home-kit the Pearl Light Home kit only at point T3 (KW $p = 0.05$).

#### 3.2.3 Tooth 3

Tooth 3 showed significant changes in shade when measured using the Classic Vita shade for both the Philips Zoom Home kit and the UltraDent Opalescence PF Home kit as compared to the Pearl Light Home kit both at T1 (KW, $p = 0.036$, $p = 0.020$, respectively), at T2 (KW, $p = 0.025$, $p = 0.009$, respectively) and at T3 (KW, $p = 0.015$, $p = 0.05$, respectively). The Beyond Core white home kit and the Pearl Light Home kit only gave statistically significant different results at T2 (KW, $p = 0.034$) and T3 (KW, $p = 0.033$).

The Vita 3D Master Shade guide and the Bleaching Score guide results produced more statistically significant differences between product results for Tooth 3.

The Vita 3D Master Shade Guide Readings detected significant shade change readings between Philips Zoom Home kit and Pearl Light Home kit at T1 (KW, $p < 0.001$), T2 (KW, $p = 0.001$) and T3 (KW, $p < 0.002$). Significant changes were reported for differences between the Beyond Core white home kit or the Pearl Light Home kit at T1 (KW, $p = 0.001$), T2 (KW, $p < 0.001$) and T3 (KW, $p = 0.004$) and between the Ultradent Opalescence GO Home-kit the Pearl Light Home kit at T1 (KW, $p < 0.001$), T2 (KW, $p < 0.002$) and T3 (KW, $p = 0.002$). The Opalescence GO home kit only showed significant shade differences with those achieved by the Pearl Light Home kit at T1 (KW, $p = 0.032$) and at T2 (KW, $p < 0.0026$).

The Bleaching score guide detected the greatest number of significant colour changes between the products. The Philips Zoom Home kit, the Beyond Core white home kit, UltraDent Opalescence PF Home kit and the Ultradent Opalescence GO Home-kit all showed statistically better shade improvements than the Pearl Light Home kit at T1 (KW, $p < 0.001$, KW, $p < 0.001$, KW, KW $p < 0.001$, KW, $p = 0.032$ respectively) and at T2 (KW, $p = 0.001$, KW, $p < 0.001$, KW, $p = 0.002$, KW, $p = 0.026$ respectively). Only The Philips Zoom Home kit, the Beyond Core white home kit and the UltraDent Opalescence PF Home kit recorded significant improvements in shade over the Pearl Light Home kit at T3 (KW, $p = 0.002$, KW, $p = 0.004$, KW, KW, $p = 0.002$, respectively).

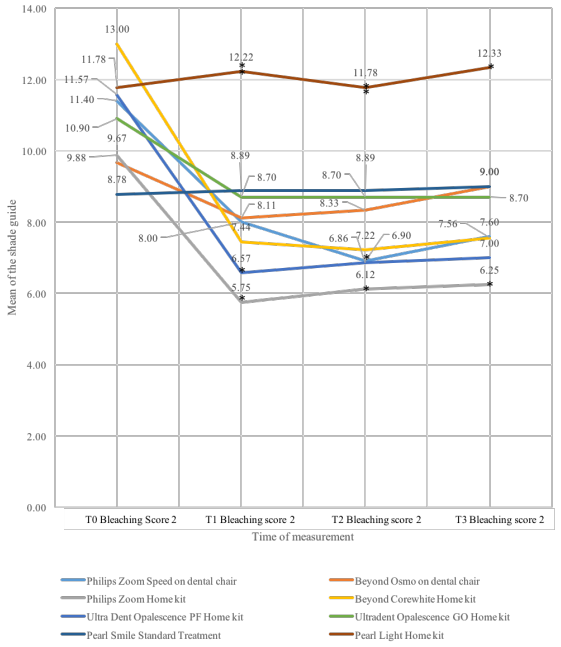
### 3.3 In-Office Kits versus Home Kits

Figure 1 depicts the results similarly obtained for Tooth 2 as recorded by the Bleaching Score Guide Output. Shade changes for this tooth were not as large as for Tooth 1. As illustrated the greatest colour change was that produced by Ultra Dent Opalescence PF Home kit and the Philips Zoom Home kit. These were however not found to be significantly different to that produced by the rest of the kits except for that produced by the Pearl Light Home Kit at T1 (KW, $p = 0.0021$, KW, $p = 0.002$, respectively). The Philips Zoom Home Kit persisted to be significantly better than the Pearl light Home kit both at T2 (KW, $p = 0.011$) and at T3 (KW, $p = 0.013$). It was however not found to be significantly better than the rest of the products.

The best result for the upper central incisor (T0 – T3 = 7.57) was that achieved by the Ultra Dent Opalescence PF Home kit as measured by both the Classic Vita Shade guide. A deterioration in shade of the upper central incisor was observed when using the Pearl Light Home Kit (T0 – T3 = −0.55), as measured by the Vita 3D Master shade guide and the Bleaching Score readings.

### 3.4 Debris and Gingival Indices

A significant improvement in patients' oral hygiene status was observed relative to outcome of the tooth whitening procedures. Patients who experienced improvement with tooth whitening also exhibited better oral hygiene as evidenced by a decrease in the debris




T1 Bleaching score 2: * Kruskal–Wallis Test: $p = 0.002$.
(Philips Zoom Home kit/Pearl light home kit)
T1 Bleaching score 2: * Kruskal–Wallis Test: $p = 0.021$.
(Ultra Dent Opalescence PF Home kit/ Pearl light home kit)
T2 Bleaching score 2: * Kruskal–Wallis Test: $p = 0.011$.
(Philips Zoom Home kit/Pearl light home kit)
T2 Bleaching score 2: * Kruskal–Wallis Test: $p = 0.031$.
(Philips Zoom speed on dental chair/Pearl light home kit)
T3 Bleaching score 2: * Kruskal–Wallis Test: $p = 0.013$.
(Philips Zoom Home kit/Pearl light home kit)

**Figure 1:** Plots of shade changes for all tooth-whitening systems on tooth 2 as measured by the Bleaching Score

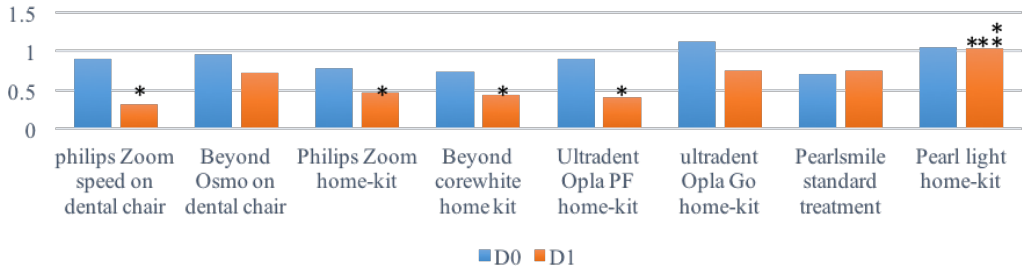
and gingival indices. Figures 2 and 3 illustrate the change in debris and gingival indices before and after treatment. None of the patients reported any major adverse soft tissue changes following whitening procedures. However, three patients, using Non-OTC home-kit products, reported minimal soft tissue changes. Clinical examination of these cases revealed minor gingival burns that healed uneventfully within 3 days.

## 4 Discussion

In this prospective clinical study, the effectiveness of different tooth whitening products, and their impact on soft oral tissues was investigated. The products included 3 In-Office treatments and 5 Home-Kits, one of which was an over the counter product (OTC). Moreover, the authors assessed objectively whether achieving tooth whitening can serve as a motivation for patients to improve their oral hygiene.

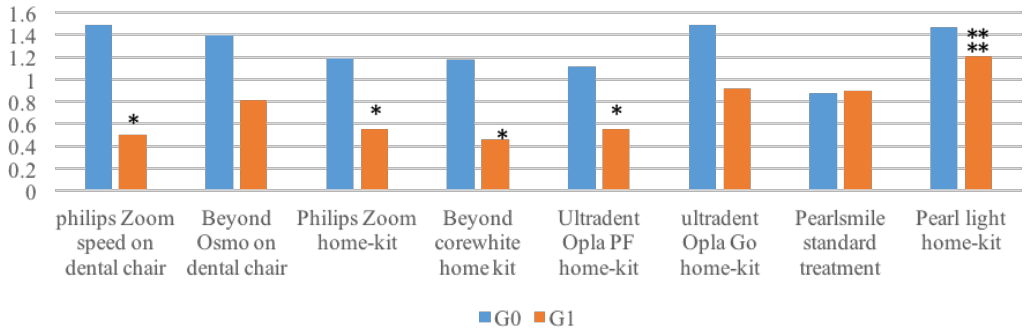
Sixty-five percent of the participants who approached this study for tooth whitening treatment were between the ages of 18–25 years. A further 26% fell within the 26-35 year old bracket. This was a slightly younger age cohort to a systematic review involving 649 patients with mean age of 36 years (Pontes et al., 2020). This could be due to this study being notified and held on a university campus and therefore attracted a younger cohort of people. Such treatment is however also becoming increasingly appealing to older age groups. Half of online respondents aged 55-65 interested in having their teeth bleached seek not only a functional but also a pleasing dentition (Wulfman et al., 2010). These could be potentially heavily restored dentitions in patients with multiple co-morbidities (Lewis, 2011) that need to be handled carefully by the trained dental professional.

One of the most important findings of this study was that roughly only half of the participants who volunteered were eligible for testing. Forty-nine percent (49%) of interested participants required dental treatment to optimise their oral health before undergoing whitening procedures. These participants were excluded from the study. This underscores a very important issue that tooth-whitening procedures should be carried out by properly qualified professionals who can diagnose oral health issues. Regrettably, OTC products are readily available online, in supermarkets, shopping mall kiosks and pharmacies away from professional supervision, beyond registered dental clinics. Such products come in the form of mouth rinses, toothpastes, strips, paint on gels, floss and tray-based tooth whiteners with many such products having no scientific studies to support them, are very often ineffective and fall into a grey zone within no regulation. Patients might not be aware of ongoing oral health issues and failure to interact with a dental professional may allow oral patho-




* Kruskal-Wallis Test: $p = 0.000$. (Philips Zoom speed on dental chair/Pearl light home kit)
* Kruskal-Wallis Test: $p = 0.014$. (Philips Zoom home kit/Pearl light home kit)
* Kruskal-Wallis Test: $p = 0.006$. (Ultra Dent Opalescence PF Home kit/ Pearl light home kit)
* Kruskal-Wallis Test: $p = 0.006$. (Beyond core white home kit/Pearl light home kit)

**Figure 2:** Debris Index Changes before and after treatment per product




* Kruskal-Wallis Test: $p = 0.000$. (Philips Zoom speed on dental chair/Pearl light home kit)
* Kruskal-Wallis Test: $p = 0.002$. (Philips Zoom home kit/Pearl light home kit)
* Kruskal-Wallis Test: $p = 0.004$. (Ultra Dent Opalescence PF Home kit/ Pearl light home kit)
* Kruskal-Wallis Test: $p = 0.000$. (Beyond core white home kit/Pearl light home kit)

**Figure 3:** Gingival Index Changes before and after treatment per product

logy to go unnoticed and preclude timely dental treatment. Furthermore what has been termed as 'bleachorexia' has been documented where in conditions similar to body dismorphic disorder, patients misuse and abuse such products away from professional care, causing tooth erosion, extreme sensitivity and gingival irritations (Demarco et al., 2009).

In this study, the bleaching effects of three In-Office kits (Philips Zoom Speed In Office, Beyond Osmo In Office, PearlSmile Standard Treatment In Office) and five Home kits (Philips Zoom Home-kit (daywear), Beyond Corewhite Home-kit (daywear); Ultradent Opalescence PF Home-kit (night wear), Ultradent Opalescence GO Home-kit (day wear), Pearl Light Home-Kit) were compared using three shade readings (VITA SYSTEM 3D-MASTER, VITA classical and Bleach Index) on three teeth using the The VITA Easyshade® device in vivo.

A number of methods are available for measuring the colour of teeth and the colour changes undergone during tooth whitening procedures. One of the most common methods is the simultaneous comparison of the tooth with a standard shade guide. This is however, a subjective method and a number of factors can influence this process. These may include lighting conditions, experience, age, fatigue of the human eye, make-up, room decor and colour blindness (Joiner, 2006b). Alternative methods include the use of instrumental measurements using spectrophotometry, chromameters and digital image analysis. This study utilised instrumental measurements by using a digital imaging device - VITA Easyshade® V digital spectrophotometer (VITA Zahnfabrik, Germany). This allowed standardised measurements to be repeatedly taken over time and changes in shade to be recorded numerically allowing for statistical analysis.

The Pearl Smile Standard Treatment (< 0.1% HP) and Pearl Light Home kit (< 0.1% HP) produced very little change in tooth shade throughout the study period. Some data displayed minimal improvement while most read a final darker shade to that present initially. These findings are in accordance with previous published literature which states that there is a tendency to reversion to a darker shade with time (Al-Tarakemah et al., 2016); the lighter the initial presenting shade, the greater the risk for a worse outcome. Al-Tarakemah et al. (2016), suggest that reversion to darker tooth shade is caused by the substantial enamel matrix breakdown by the bleaching agent allowing for an increased permeability and therefore diffusion of dietary derived colorants into the enamel. This study observed that (Figure 1) for kits such as In Office Philips Zoom Speed, Beyond Core White Home Kit and Ultradent Opalescence GO Home kit and Beyond Osmo, Ultra Dent Opalescence PF Home kit and Philips Zoom Home kit, there was some regression of tooth colour from time T1 to T3 but however still showed an improvement over the shade recorded initially at T0. Azer et al. (2011) explain how an increased surface roughness caused by the bleaching agent allows subsurface penetration of dietary derived colourants producing extrinsic stain. Such highly pigmented foodstuffs include black tea and red wine (Azer et al., 2011). However a double blind randomized clinical trial of at home tooth bleaching systems with a 6 month recall reported that high consumption of staining beverages and foods did not affect longevity of whitening effect (Meireles et al., 2008). Outcome is not affected by gender, but seems to be influenced by the nature of the intrinsic stain, initial tooth colour and age (Joiner, 2006a).

This study reported that apart from The Pearl Smile Standard treatment and the Pearl Light home kits, there were no significant differences in bleaching results achieved when comparing all In-Office and all Home kits to each other. The best overall result achieved was that for tooth 1 using the Philips Zoom Home kit.

In-Office tooth bleaching is done under direct supervision of a dentist ensuring protection of gingival tissues and requiring minimal dependence on patient compliance, allows for proper treatment planning prior to treatment, is carried out in a short period of time and gives immediate results, albeit using higher concentration of product. On the other hand, this treatment option is costlier to the patient and requires more chair time. Additionally, some products require further at – home applications and tend to produce more hypersensitivity (Rodríguez-Martínez et al., 2019). In this study, the Philips Zoom Speed system exhibited the best results over the period T0 to T3 on all the 3 teeth examined.

Home Kits are divided into those that are professionally supervised and the OTC products. The professionally supervised products require the fabrication of custom bleaching trays however require much reduced office chair time. These products require greater patient commitment to follow instructions carefully and longer treatment time to achieve results. Research shows that a whitening endpoint is usually reached at 6 weeks independent of concentration and type of peroxide used (Matis et al., 2000). This study followed up participants for 4 weeks. This study noted the greatest improvement in shade at initial application (T1), with very little change observed from periods T1 to T2 and T2 to T3. Purely OTC systems are widely used, are entirely patient controlled may be used indiscriminately or inappropriately without prior diagnosis of tooth discolouration. The dental professional may not be aware of the use of such products by the patient who does not disclose such information. This is critical as research shows

that bleaching agents affect shear bond strength of resin composite to acid-etched enamel when composite resin restorations are placed soon after bleaching (Khamverdi et al., 2016). OTC products do require a longer time to achieve results due to their lower hydrogen peroxide concentration however; this may be a factor patients accept, as the systems are inexpensive. This study reports the greatest shade improvement observed by Philips Zoom Home kit on tooth 1 when measured by the Vita 3D Master shade guide.

Upon comparing all In-Office to all At Home kits this study reports that apart from the Pearl Smile Standard treatment and the Pearl light Home Kit, there was no statistically significant difference reported between the results obtained by all the rest of the products. Additionally, the greatest shade improvement (T0 – T3 = 9.37) for tooth 1 was that obtained by the Philips Zoom Home Kit. This study therefore concludes that professional tooth whitening Home-Kits were more effective than In-Office systems. These findings correlate with published literature that states that ultimately the two key factors that determine whitening efficacy are the concentration of the oxidizing agent and the duration of application (Joiner, 2006b). Bleaching efficacy is found to be greater for higher concentration products however, upon extending treatment time, the differences in tooth lightness were no longer of statistical significance (Leonard et al., 1998; Matis et al., 2000). This may be of clinical relevance if one is to consider that higher concentrations of product increase the possibility of alterations in enamel surface morphology and hardness values together with soft tissue burns (Rodríguez-Martínez et al., 2019).

The debris and gingival indices results showed significant improvements in patients' oral hygiene, associated with a positive tooth whitening experience. Participants exhibiting positive tooth whitening improved their oral hygiene from fair-to-poor to good-to-fair oral hygiene levels as evident by a decrease in debris and gingival indices. However, this improvement could also be attributed to the peroxide itself which is antimicrobial and thus may have a cleansing effect on the mouth (Nuss, 2004). Furthermore, it was noted that many participants requested oral hygiene advice during the final visit. The same oral hygiene advice was given before the treatment and after the treatment. Study participants who did not achieve the desired whitening results had a minimal increase in the debris index while their gingival index decreased slightly. This can be a result of participants being made more aware of their aesthetic look upon visualising the change in shade thereby increasing their motivation towards their oral hygiene habits and maintaining the results obtained. Additionally, when all non-OTC products were compared to OTC products, statistical significance was observed in terms of debris and gingival indices reduction.

Gingival irritation is one of the most common side effects of tooth bleaching (Majeed et al., 2015). This was observed in three participants using non-OTC home kits. This can be due to excessive application of whitening gel, an ill-fitting tray or improper use of the home kit in breach of the manufacturer's guidelines. Home kits caused minimal gingival burns in five patients, one of which dropped out of the study due to the pain. This is one of the most common side effects. However, these adverse effects did not last longer than 3 days, and they did not reappear within the period of one month. Long-lasting adverse effects could be avoided or reduced by using low concentrations of hydrogen peroxide, which is the causative agent for these events. OTC products showed no undesirable side effects on soft oral tissues. HP is a caustic substance and can cause burns of the gingival or mucosal tissue. Rubber dam or light-cured resin, provided by the manufacturer, should always be used to protect the soft tissues during In-Office bleaching procedures (Majeed et al., 2015).

## 5 Conclusion

The mechanism of tooth whitening and the processes involved in the tooth sensitivity that may ensue are not yet fully understood (Rodríguez-Martínez et al., 2019). Additionally, new products are constantly emerging on the market and the provision of tooth whitening services by non-dental professionals if of concern. This study highlights that the use of bleaching agents may be effective and of value in carefully selected cases and that all products are not to be used indiscriminately. Dental practitioners are to help patients make informed decisions after considering all factors involved and providing professional advice and realistic expectations.

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