How to Design an Academic Poster

Dr James Anderson

BSc(Hons) BVetMed PGCertHE MRes PhD FHEA MRCVS

Lecturer in Veterinary Anatomy

janders@liverpool.ac.uk

INSPIRE Research Summer School 2024

Overview

1. Why make a poster?

2. What information do you put on a poster?

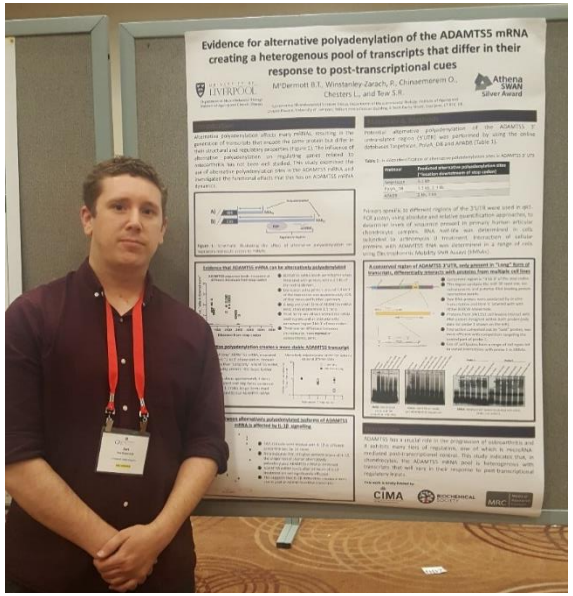
3. How to lay out a poster.

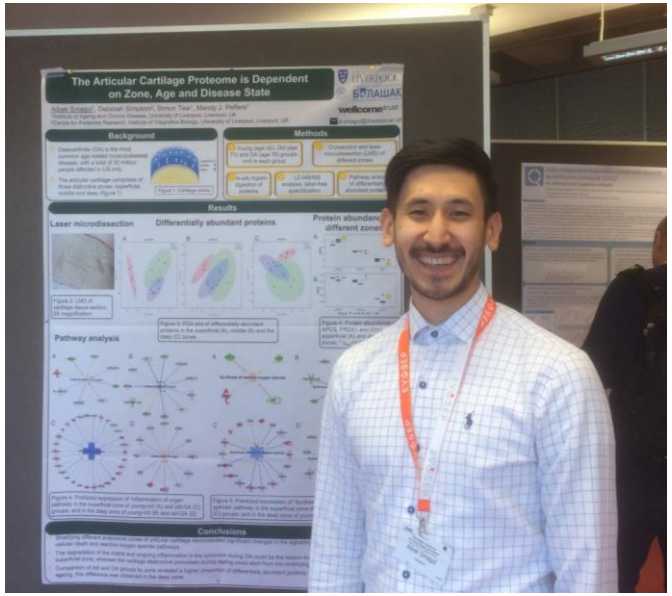
4. Presenting a poster.

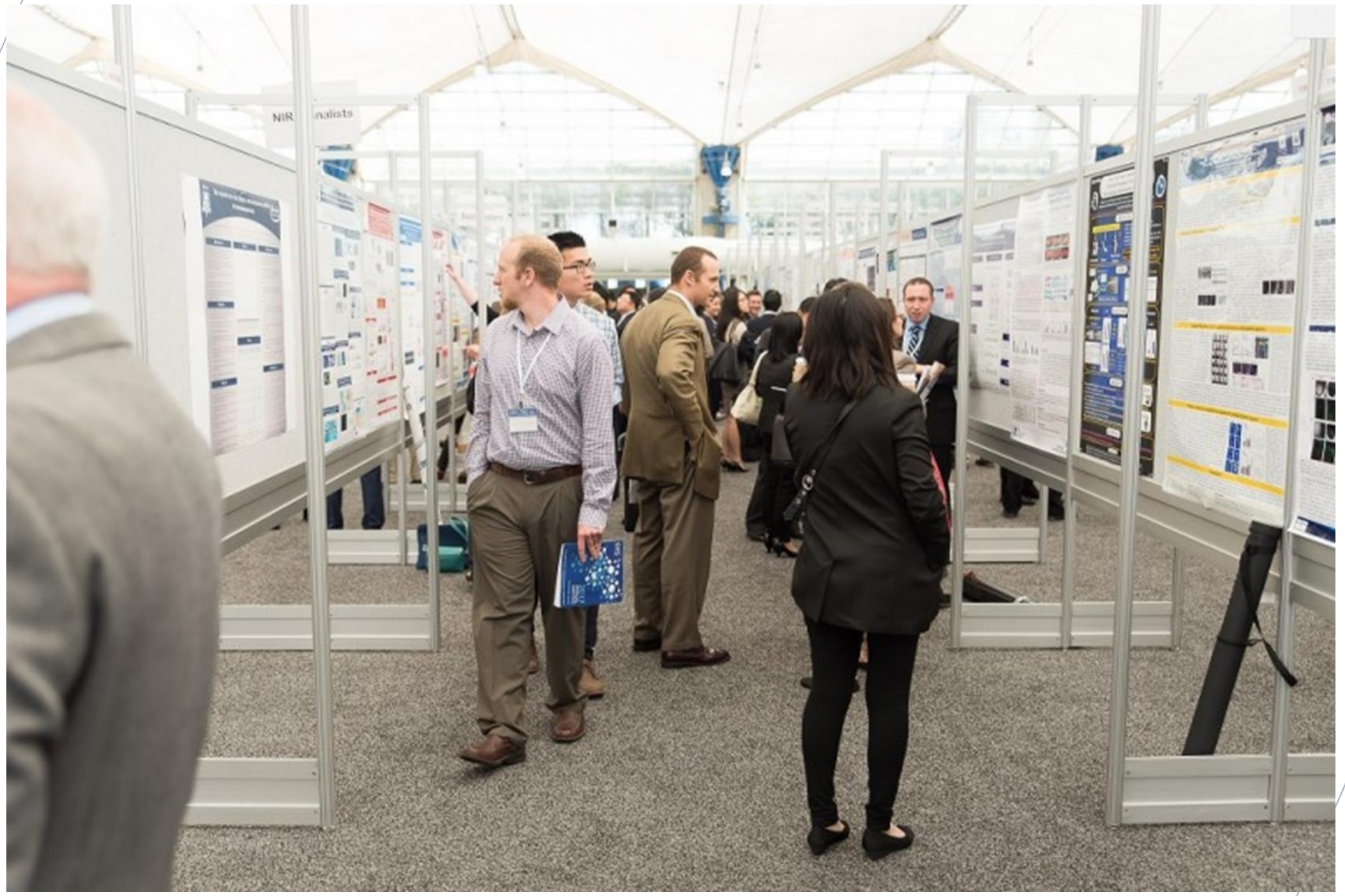
Why make a poster?

Why make a poster?

- Posters are a great way to summarise your projects.
- Posters are one of the main ways that you will present your work when attending conferences.
- For these reasons, you should make sure your poster is of good quality.







What information goes on a poster?

What information goes on a poster?

- Title

- Introduction / Background (Include Hypothesis / Aim)
- Methods
- Results
- Conclusion / Discussion
- References

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- Introduction / Background (Include Hypothesis / Aim)
- Methods
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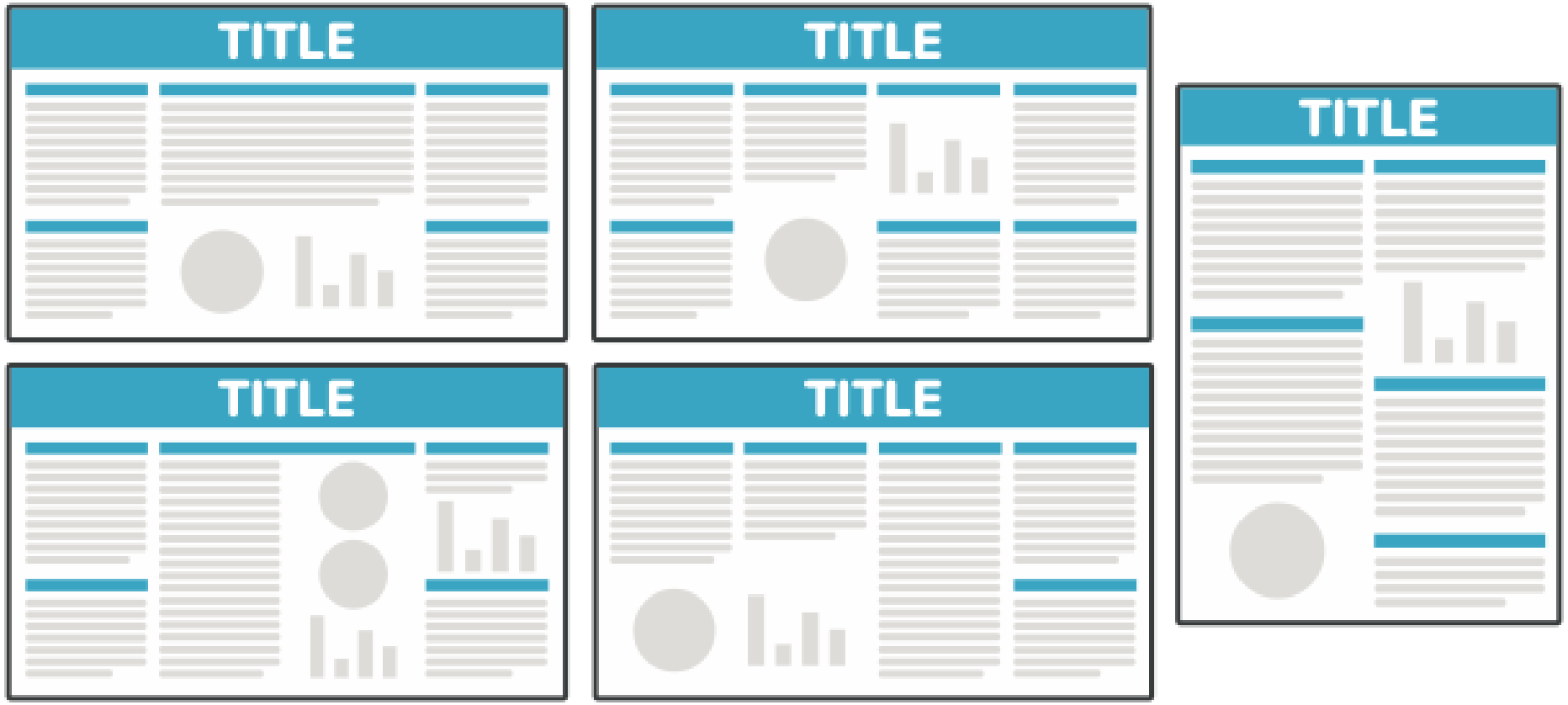
- References

Other important information:

- Authors (and institutions)
- Logos (university, funders etc)
- Email address, social media, (QR code?)
- Acknowledgements, conflicts of interest
- Sources of funding

What is the best way to lay out a poster?

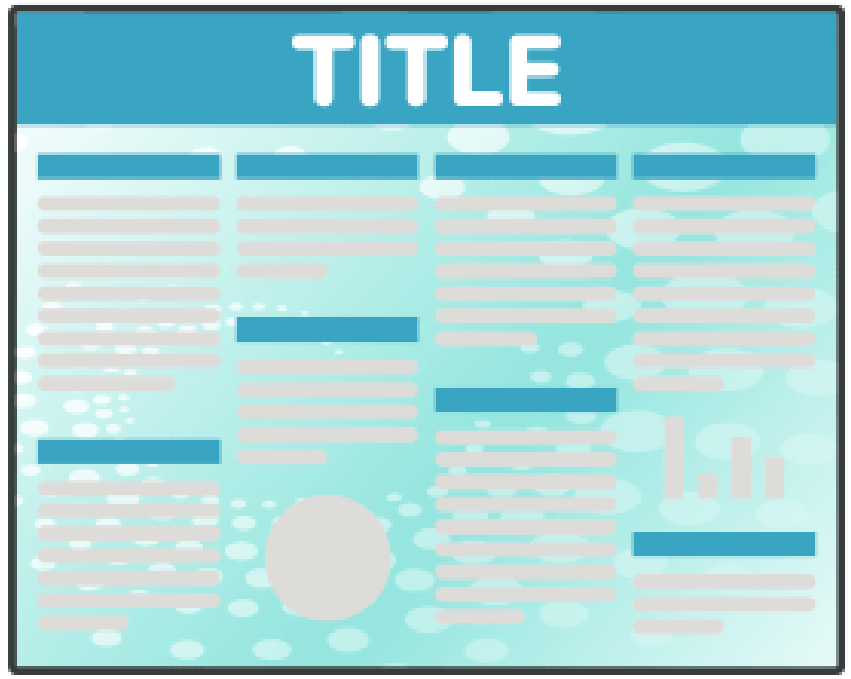


Check the conference/course website!

What is the best way to lay out a poster?

Good Background

Bad Background



What is the best way to lay out a poster?

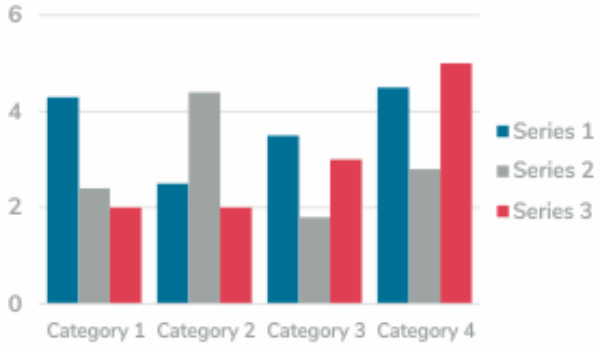
Good Alignment

Bad Alignment

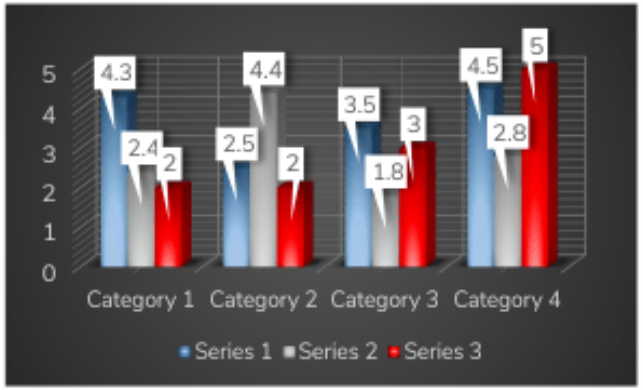
Good Headings

Bad Headings

Good Chart



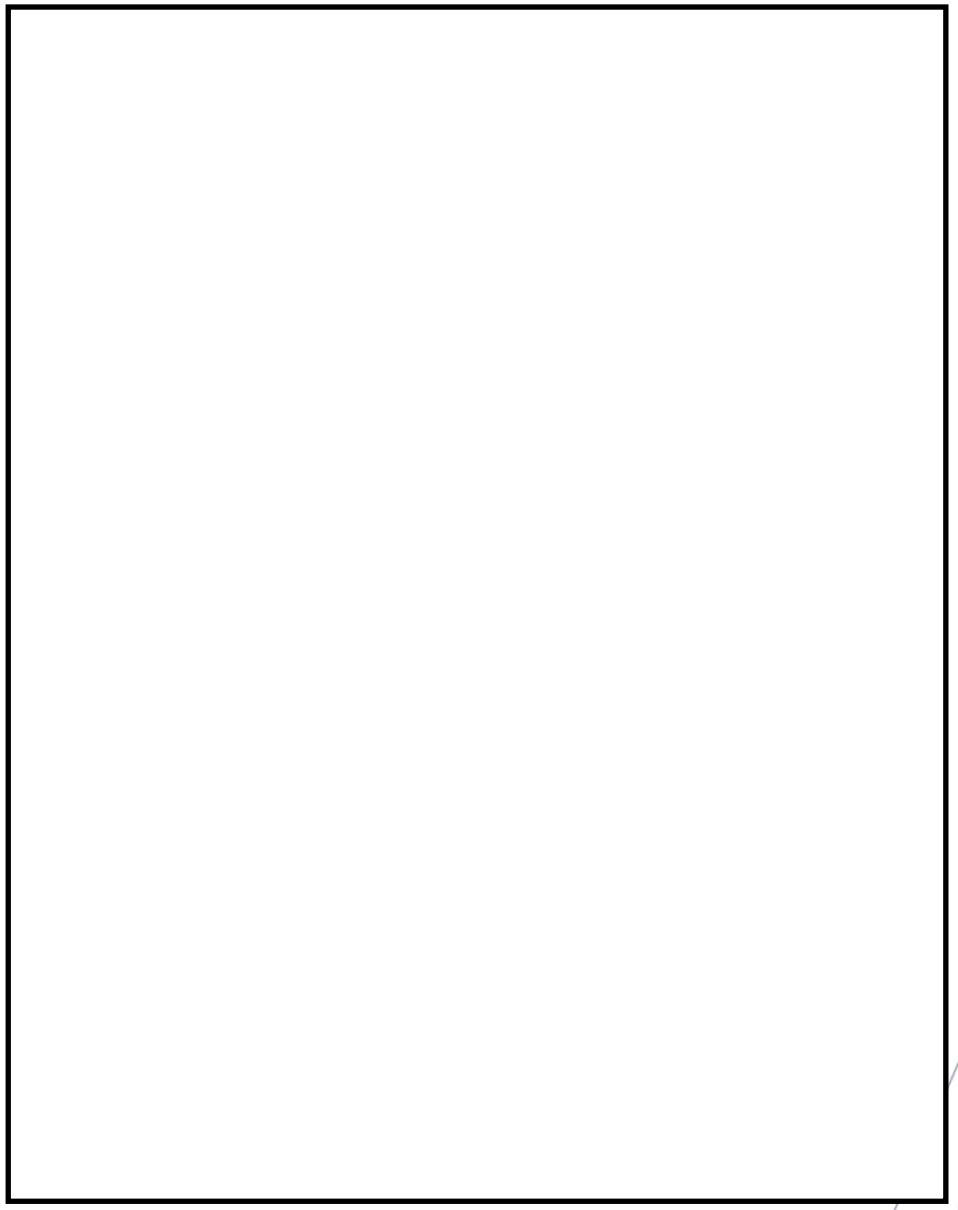
Bad Chart



Where to start?

- Portrait or landscape?
- **PowerPoint**, Apple Keynote, CorelDRAW, Corel PaintShop Pro, Inkscape
- Dimensions

A0 = Width: 84.111 cm
Height: 118.913 cm



Where to start?

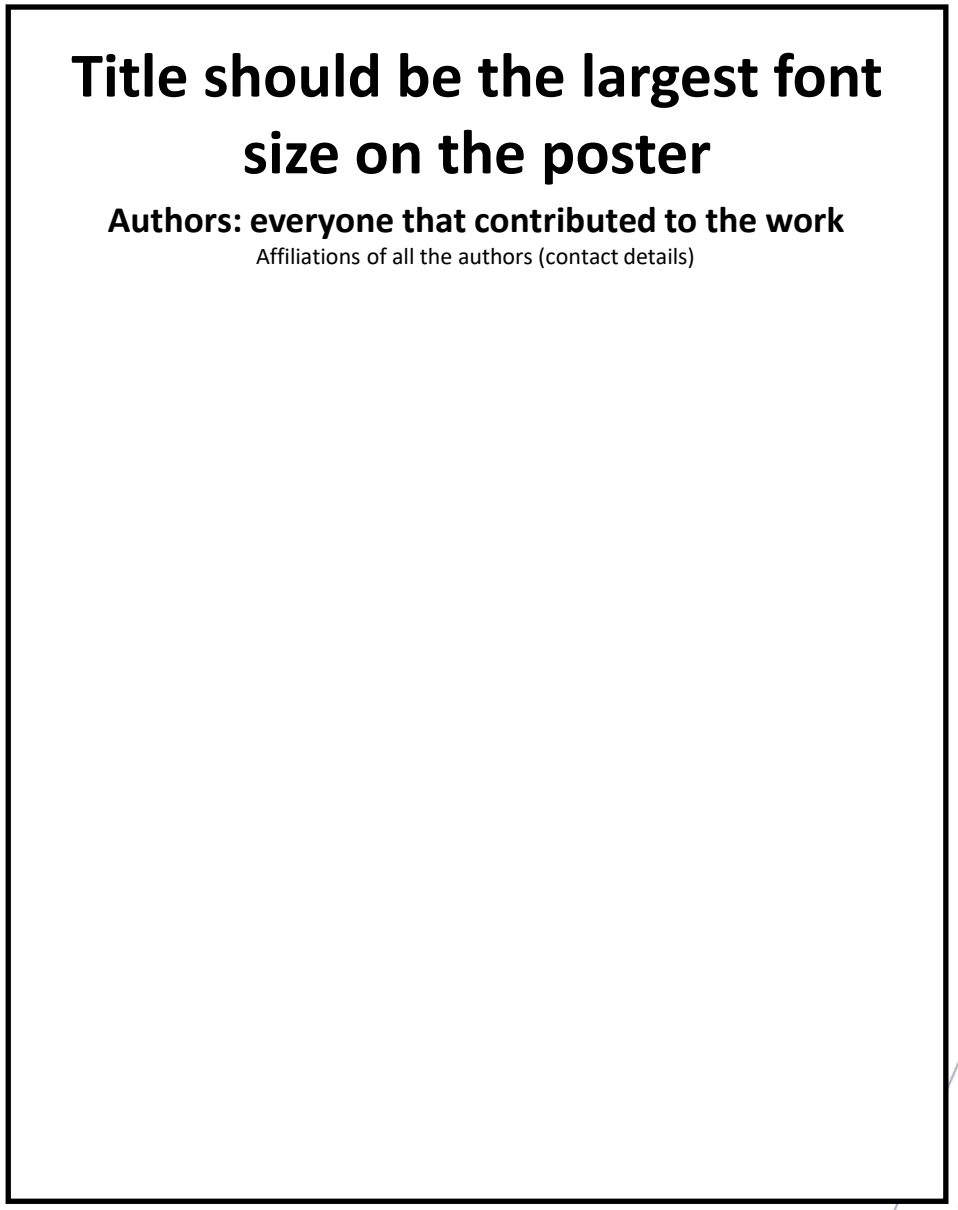
- Title

  Statements rather than questions

- Authors

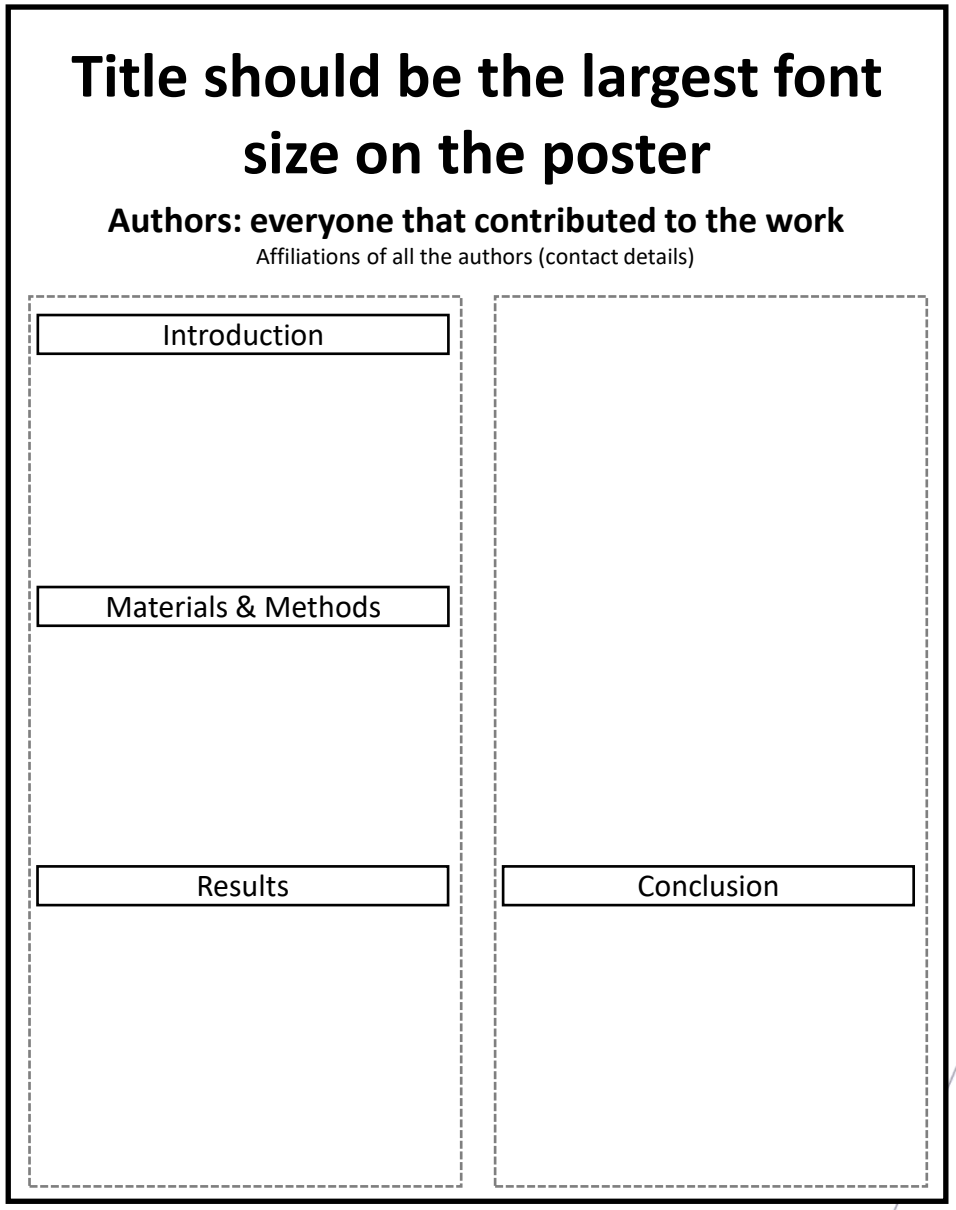
- Affiliations

  Organisations of authors, where the work took place, contact details



The main body

- Logical structure
- Concise - too much text is off-putting!
- Sub-headings



The main body

- Logical structure
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- Sub-headings

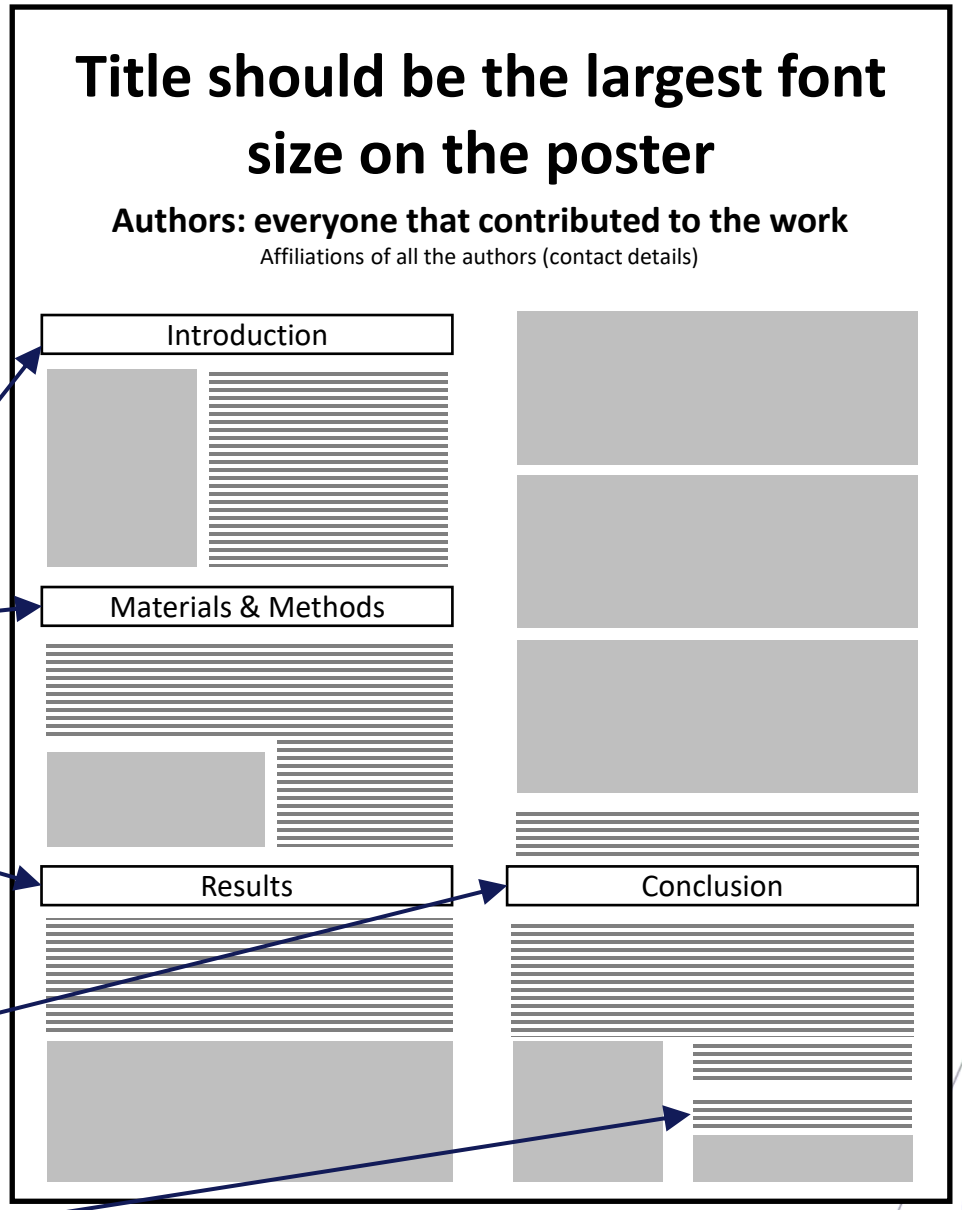
Short background on your topic to add context to the work. State your objectives and why your work is novel in its field.

Basic parameters, settings, inclusion/exclusion criteria, statistical techniques etc.

Data analysis. Only results that answer the stated hypothesis. Large figures.

Conclusions must arise from the results and answer hypothesis posed in the introduction. List any improvements, limitations and possible future work resulting from this project.

References / Acknowledgements / Funding sources



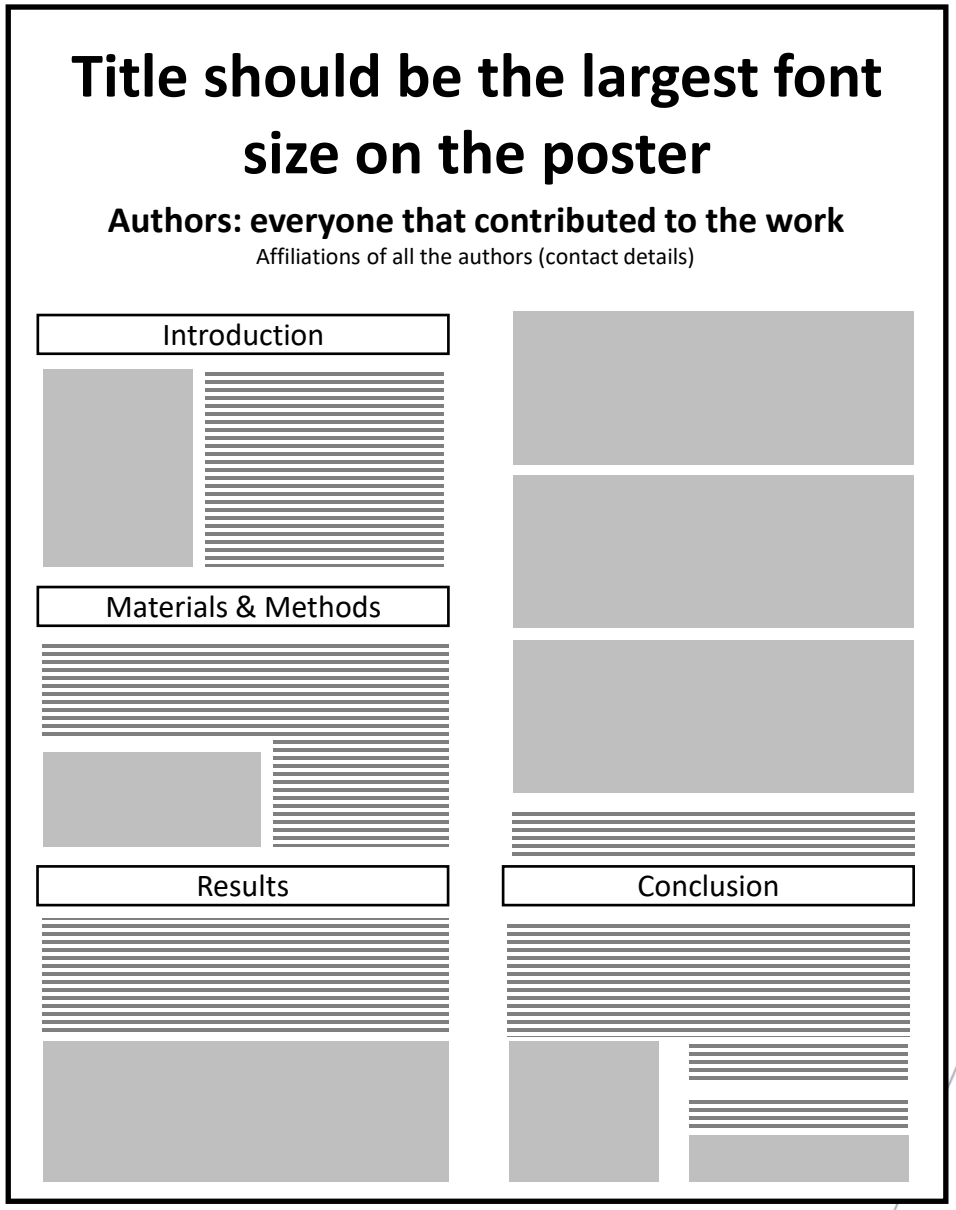
Shortened References

References

1. Summerhays GE, *Vet Rec*, 2000, 147(7), pp. 184-188
2. Blewis ME *et al.*, *Eur Cell Mater*, 2007, 13, pp. 26-39
3. Ruiz-Romero C & Blanco FJ, *Osteoarthritis Cartilage*, 2010, 18(4), pp. 500-509
4. Mateos J *et al.*, *J. Proteomics*, 2012, 75(10), pp. 2869-2878
5. Wluka AE *et al.*, *Nat. Rev. Rheumatol.*, 2013, 9(4), pp. 225-235
6. Enpevet, http://www.enpevet.de/Lexicon
7. Kherani RB & Shojania K, *CMAJ*, 2007, 176(11), pp. 1605-1608
8. Keun HC & Athersuch TJ, *Methods Mol. Biol.*, 2011, 708, pp. 321-334

The main body

- Templates / colour scheme



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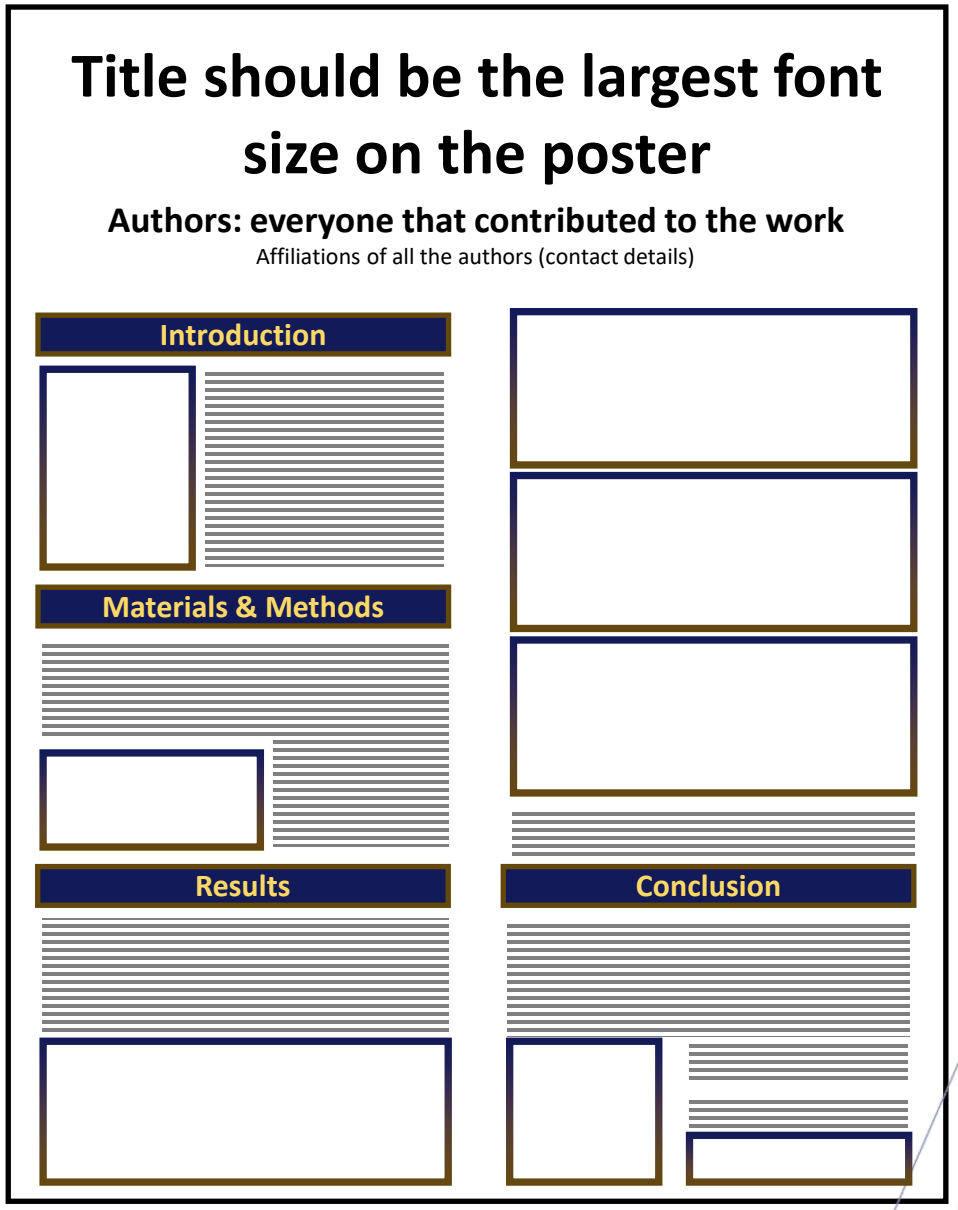

The main body

- Templates / colour scheme
- Font size
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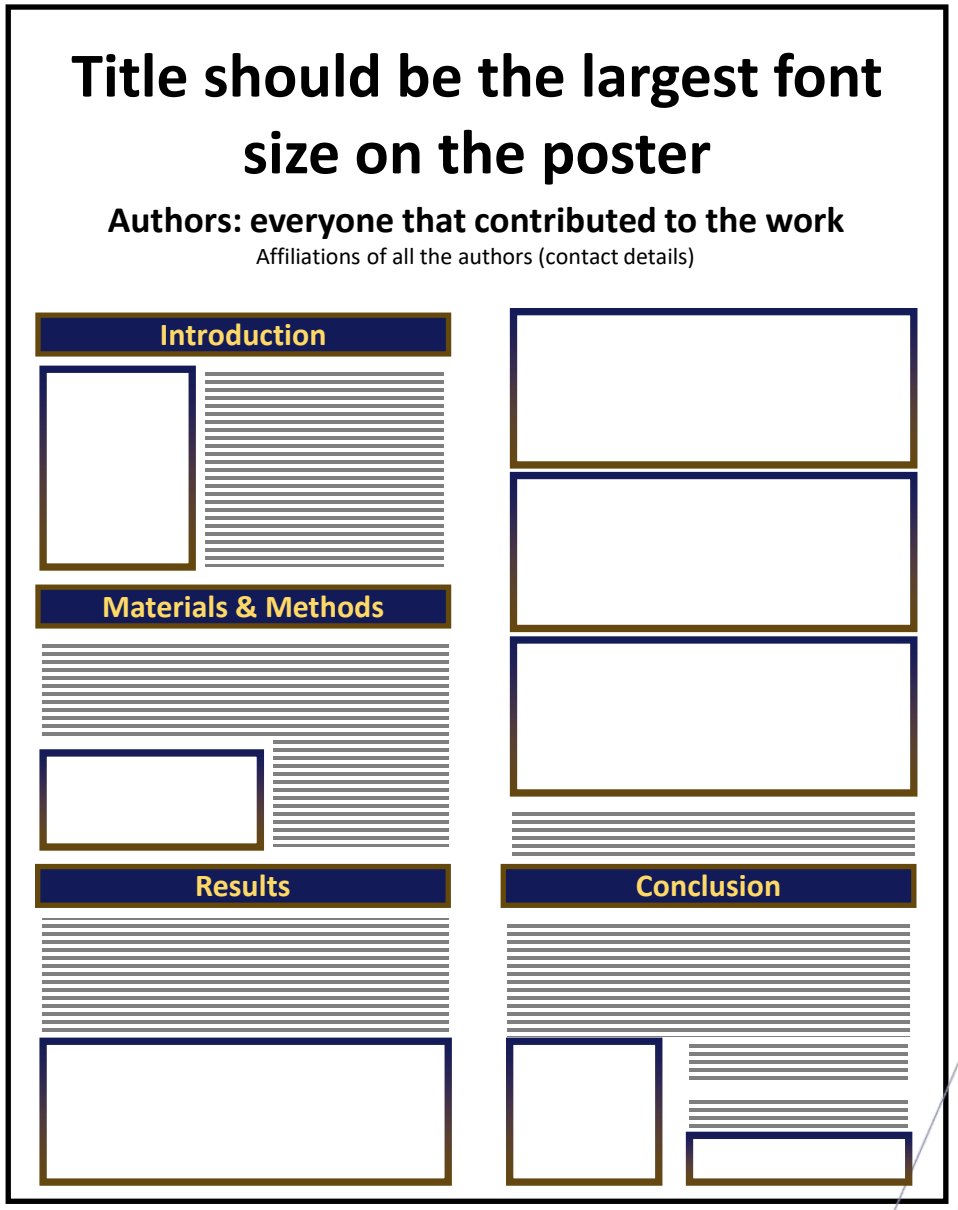
    - Authors 54
    - Affiliations 28
    - Subheadings 54
    - Main body text 40
    - Figure legends etc. 28



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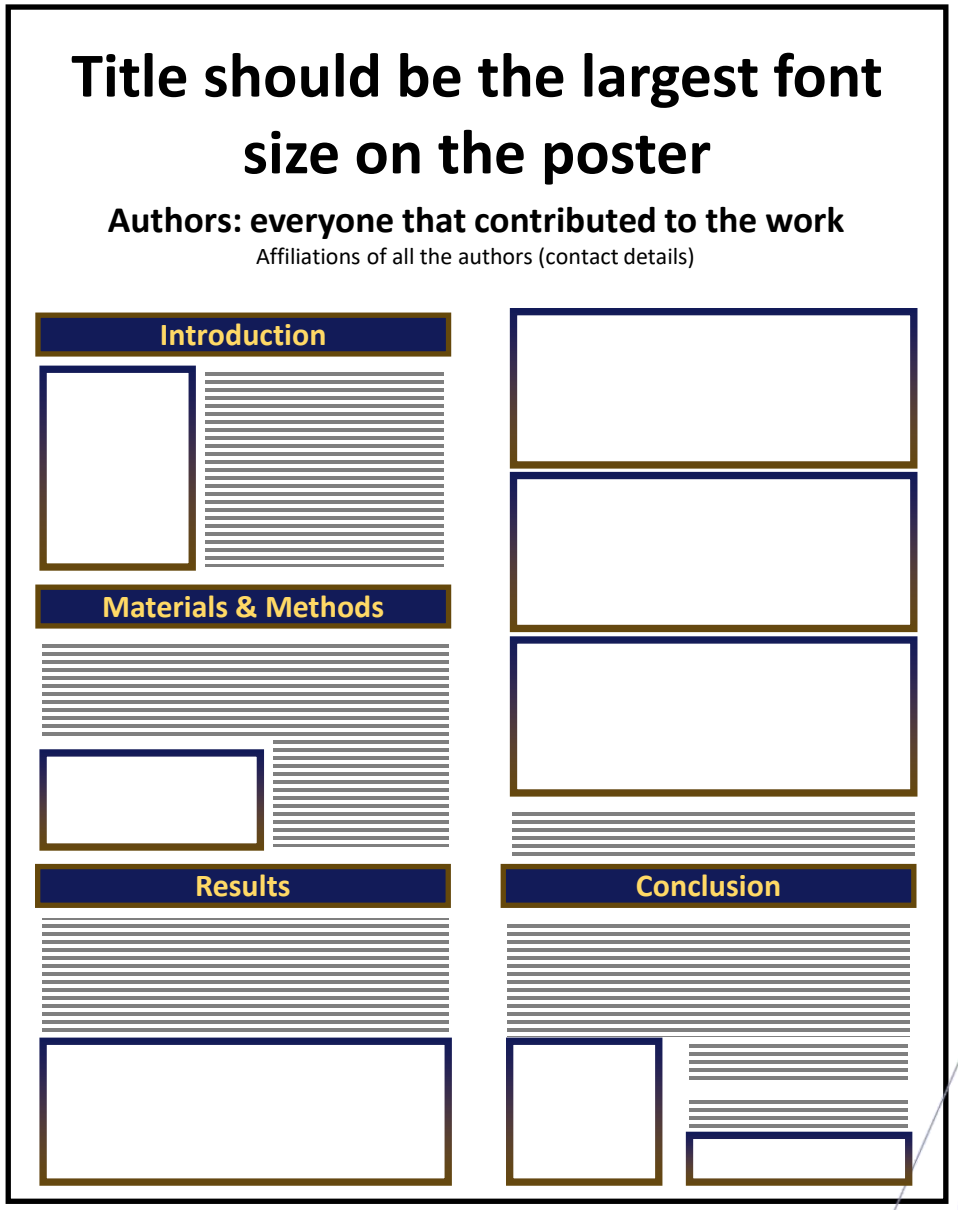
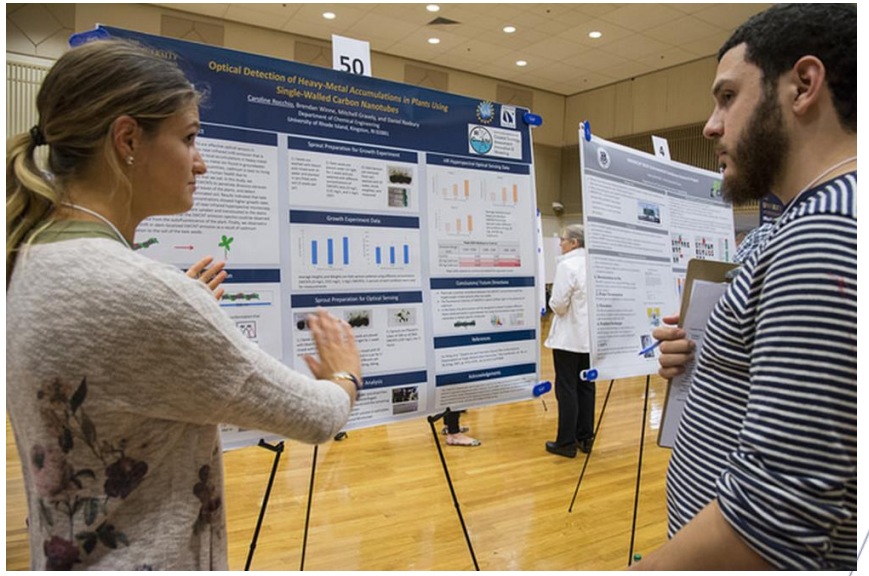
- Figures
- Printing



Presenting the poster

- Check you have a means to put the poster on the poster boards.
- Check which session you are presenting in.
- Think about what you are going to say about your poster!!!
  - Be friendly and approachable
  - 5-7 min presentation
  - Guide the reader through sections
  - Be concise
  - Highlight the main points
  - Practice!!!
  - Don’t rush!
  - Think about potential questions
  - Don’t wing it!



Good or Bad?

PIGS IN SPACE: EFFECT OF ZERO GRAVITY AND AD LIBITUM FEEDING ON WEIGHT GAIN IN CAVIA PORCELLUS

Colin B. Purrington
6673 College Avenue, Swarthmore, PA 19081 USA

NRF
BLACK HOLE DIET PLANS
THE HIGH FRUCTOSE SUGAR ASSOCIATION
SPACE-EXES

ABSTRACT:

One ignored benefit of space travel is a potential elimination of obesity, a chronic problem for a growing majority in many parts of the world. In theory, when an individual is in a condition of zero gravity, weight is eliminated. Indeed, in space one could conceivably follow ad libitum feeding and never even gain an gram, and the only side effect would be the need to upgrade one's stretchy pants("exercise pants"). But because many diet schemes start as very good theories only to be found to be rather harmful, we tested our predictions with a long-term experiment in a colony of Guinea pigs (Cavia porcellus) maintained on the International Space Station. Individuals were housed separately and given unlimited amounts of high-calorie food pellets. Fresh fruits and vegetables were not available in space so were not offered. Every 30 days, each Guinea pig was weighed. After 5 years, we found that individuals, on average, weighed nothing. In addition to weighing nothing, no weight appeared to be gained over the duration of the protocol. If space continues to be gravity-free, and we believe that assumption is sound, we believe that sending the overweight — and those at risk for overweight — to space would be a lasting cure.

INTRODUCTION:

The current obesity epidemic started in the early 1960s with the invention and proliferation of elastane and related stretchy fibers, which released wearers from the rigid constraints of clothes and permitted monthly weight gain without the need to buy new outfits. Indeed, exercise today for hundreds of million people involve only the act of wearing stretchy pants in public, presumably because the constrictive pressure forces fat molecules to adopt a more compact tertiary structure (Xavier 1965).

Luckily, at the same time that fabrics became stretchy, the race to the moon between the United States and Russia yielded a useful fact: gravity in outer space is minimal to nonexistent. When gravity is zero, objects cease to have weight. Indeed, early astronauts and cosmonauts had to secure themselves to their ships with seat belts and sticky boots. The potential application to weight loss was noted immediately, but at the time travel to space was prohibitively expensive and thus the issue was not seriously pursued. Now, however, multiple companies are developing cheap extra-orbital travel options for normal consumers, and potential travelers are also creating news ways to pay for products and services that they cannot actually afford. Together, these factors open the possibility that moving to space could cure overweight syndrome quickly and permanently for a large number of humans.

We studied this potential by following weight gain in Guinea pigs, known on Earth as fond of ad libitum feeding. Guinea pigs were long envisioned to be the "Guinea pigs" of space research, too, so they seemed like the obvious choice. Studies on humans are of course desirable, but we feel this current study will be critical in acquiring the attention of granting agencies.

MATERIALS AND METHODS:

One hundred male and one hundred female Guinea pigs (Cavia porcellus) were transported to the International Space Laboratory in 2010. Each pig was housed separately and deprived of exercise wheels and fresh fruits and vegetables for 48 months. Each month, pigs were individually weighed by duct-taping them to an electronic balance sensitive to 0.0001 grams. Back on Earth, an identical cohort was similarly maintained and weighed. Data was analyzed by statistics.

RESULTS:

Mean weight of pigs in space was 0.0000 +/- 0.0002 g. Some individuals weighed less than zero, some more, but these variations were due to reaction to the duct tape, we believe, which caused them to be alarmed push briefly against the force plate in the balance. Individuals on the Earth, the control cohort, gained about 240 g/month ($p = 0.0002$). Males and females gained a similar amount of weight on Earth (no main of effect of sex), and size at any point during the study was related to starting size (which was used as a covariate in the ANCOVA). Both Earth and space pigs developed substantial dewlaps (double chins) and were lethargic at the conclusion of the study.

CONCLUSIONS:

Our view that weight and weight gain would be zero in space was confirmed. Although we have not replicated this experiment on larger animals or primates, we are confident that our result would be mirrored in other model organisms. We are currently in the process of obtaining necessary human trial permissions, and should have our planned experiment initiated within 80 years, pending expedited review by local and Federal IRBs.

ACKNOWLEDGEMENTS:

I am grateful for generous support from the National Research Foundation, Black Hole Diet Plans, and the High Fructose Sugar Association. Transport flights were funded by SPACE-EXES, the consortium of wives divorced from insanely wealthy space-flight startups. I am also grateful for comments on early drafts by Mañana Athletic Club, Corpus Christi, USA. Finally, sincere thanks to the Cuy Foundation for generously donating animal care after the conclusion of the study.

LITERATURE CITED:

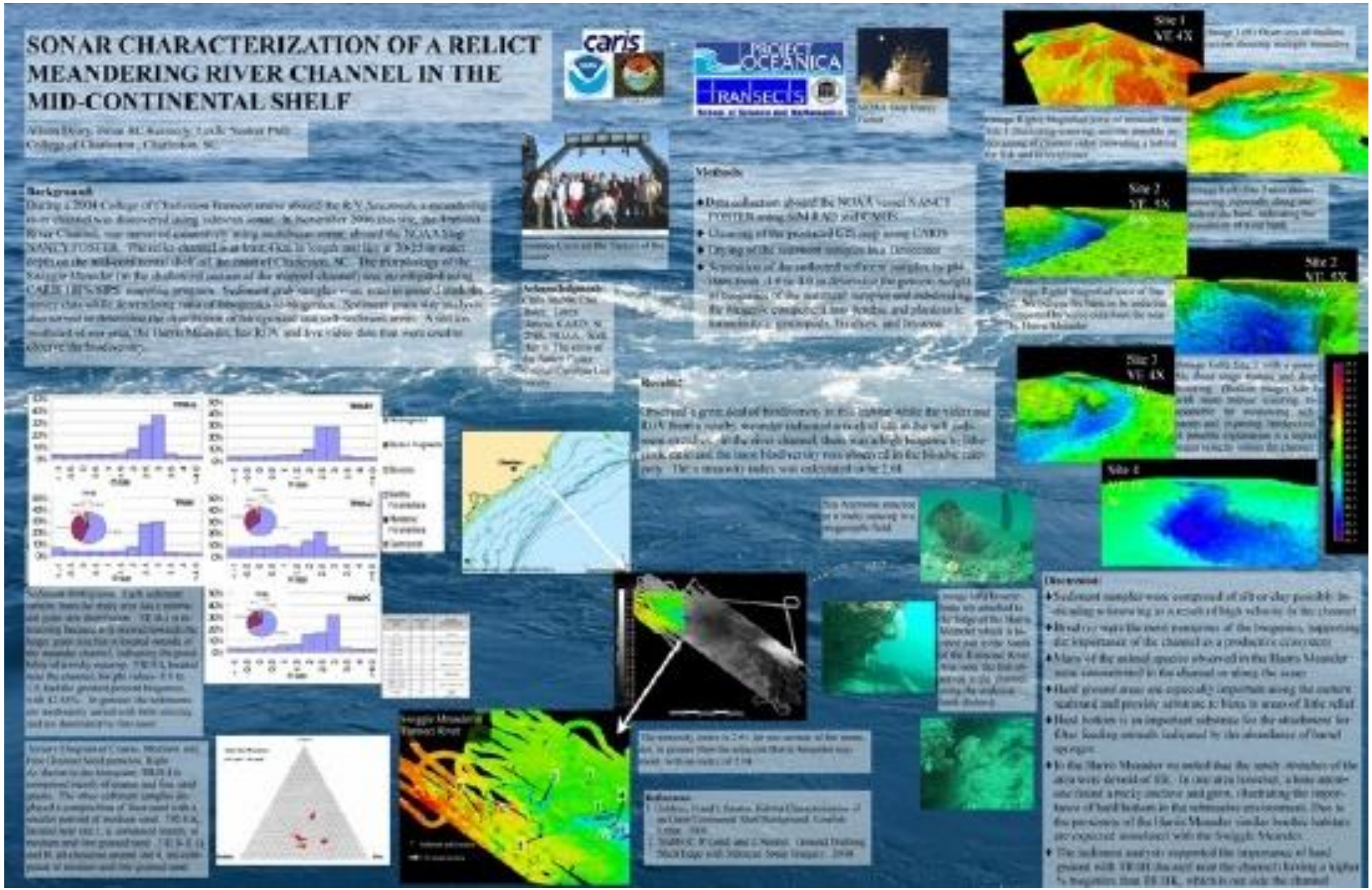
NASA. 1982. Project STS-XX: Guinea Pigs. Leaked internal memo.
Sekulić, S.R., D. D. Lukač, and N. M. Naumović. 2005. The Fetus Cannot Exercise Like An Astronaut: Gravity Loading Is Necessary For The Physiological Development During Second Half Of Pregnancy. Medical Hypotheses. 64:221-228
Xavier, M. 1965. Elastane Purchases Accelerate Weight Gain In Case-control Study. Journal of Obesity. 2:23-40.

Good or Bad?

https://colinpurrington.com/2012/02/example-of-bad-scientific-poster/

1. Too much text (Mission to push for 800 words).
2. Background image is distracting (distracts from illustrations).
3. Text box backgrounds - dark, text hard to read.
4. Text box backgrounds - different colours, distracting.
5. Text boxes - different widths, distracting, hard to follow flow of poster.
6. Some text boxes too wide (aim for 45-65 characters per line).
7. Text boxes not separated from each other by pleasing “white” space.
8. Text box edges not aligned - distracting.
9. Text justified, which causes bad inter-word spacing. Also makes reading harder (brain uses jaggedness of left-justified text).
10. Logos are distracting, useless, crowd title.
11. Title word art distracting - hard to read, juvenile!
12. Title is in all caps, which is harder to read *and* obscures Latin name.
13. Title is italicized - *also* obscures Latin name style conventions.
14. Author font and colour is annoying (comic sans should be reserved for comic books).
15. Author font colour is too loud relative to other text.
16. Results are presented in sentences instead of visually with charts.
17. Section headers have too much formatting (big font, bolded, italicized, underlined, *and* coloured).
18. Terrible graphic of guinea pig on scales. Need one of the actual set up (pigs eating while weightless, for example).
19. Inclusion of an abstract consumes space needlessly. Abstract section should be banned from posters. Posters ARE an abstract.
20. Plus the science is terrible!

Good or Bad?


SONAR CHARACTERIZATION OF A RELICT MEANDERING RIVER CHANNEL IN THE MID-CONTINENTAL SHELF
caris
PROJECT OCEANICA
TRANSECTS
Background
Methods
Results
Discussion
Site 1
Site 2
Site 3
Site 4

The Effect of Dose and Duration of Retinoic Acid upon Retinal Pigment Epithelial Cells in Proliferative Vitreoretinopathy

J.R. Anderson, S.M. Kennedy and V.R. Kearns

Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

janders@liverpool.ac.uk

1. Introduction

Rhegmatogenous retinal detachment (RRD) is the most common type of retinal detachment, involving a rupture in the neurosensory retina and subsequent movement of fluid to within the subretinal space [1, 2 & 3]. Numerous surgical options are available for RRD treatment, including pars plana vitrectomy using silicone oil as an intraocular tamponade agent [3, 5, 6 & 4]. Proliferative vitreoretinopathy (PVR) is the most common cause of surgical failure and can be equated to an excessive wound healing process [1, 4, 8 & 9]. Retinal pigment epithelial cells (RPE) are the most significant contributor throughout this process, undergoing an epithelial-mesenchymal transition due to exposure to growth factors and cytokines following blood-retina barrier disruption [10 & 11]. RPE cells migrate, produce extracellular matrix including fibronectin and collagen types I to IV, forming membranes which then exert a contractile force upon the retina leading to retinal wrinkling/alteration, formation of new retinal tears or the reopening of previous retinal breaches and subsequent complicated/repeated retinal detachment [10, 11, 12, 13, 14, 15 & 16].

all-trans Retinoic acid (atRA) has shown to be a powerful regulator of cell growth and differentiation as well as ECM formation within various cell types, with the potential to be incorporated within silicone oil [17 & 4]. Studies have shown suppression of adhesion and migration of RPE cells following atRA treatment *in vitro* 21. However, the majority of studies have not explored the effects of atRA beyond 48-72 hours, although evidence gathered to date suggests treatments of up to 3 months are required in order to prevent PVR [18].

2. Aims

The main aims of this study were to investigate the effects of numerous doses of atRA on RPE cells for up to one week post treatment, focusing on toxicity, migration, and ECM expression.

3. Methods

ARPE-19 cells were either grown for 24 hours (representing proliferating RPE cells identified during PVR) or 7 days forming a confluent monolayer (representing the normal RPE monolayer). Following these growth periods, cells were treated for either 48 hours or 7 days, producing four main study groups, 24 hour growth/48 hour treatment (24G48T), 24 hour growth/7 day treatment (24G7T), 7 day growth/48 hour treatment (7G48T) and 7 day growth/7 day treatment (7G7T). atRA powder was dissolved in dimethyl sulfoxide (DMSO) and treatments carried out in duplicate at 0 (untreated control), 1×10^{-4}, 5×10^{-5}, 1×10^{-5}, 5×10^{-6}, 1×10^{-6}, 5×10^{-7} and 1×10^{-7}M as well as DMSO 1% and 0.1% (v/v) (solvent control groups) for all four main study groups unless stated otherwise.

Cell Toxicity: Resazurin assays were carried with no treatment and DMSO 20% wells used as negative and positive controls respectively. Plates were incubated with 10% resazurin solution, covered and incubated at 37°C, 5% CO_2 for 2 hours. Four aliquots from each well were pipetted into wells on a black 96 well plate and Plates were read by a plate reader at excitation 570nm, emission 590nm. Cell morphology was observed through phase-contrast microscopy at x10, x20 and x40 magnifications. All experiments were repeated once.

Wound Healing Assays: A scratch was made through each well by a P200 yellow pipette tip and the mean distance travelled by the wound edges calculated at 4 and 8 hours following the scratch via digital images taken using phase-contrast microscopy. All experiments were repeated once.

Immunocytochemistry: Cells were fixed in NBF, permeabilised with 1% Triton X-100, blocked with 10% goat serum, incubated with primary antibody (anti-cytokeratin, anti-collagen IV or anti-fibronectin), incubated with appropriate secondary antibody, DAPI stained and fluorescence images taken at x20 magnification.

ECM Protein Expression: Along with the 7G7T group, another group were grown to confluence and treated for 24 hours or 7 days. Following SDS-PAGE, Western blotting was carried out incubating gels with anti-fibronectin or anti- collagen IV antibodies with anti-α-tubulin used as a loading control. Protein expression was quantified using densitometric analysis showing the relative differences between each sample to the control following normalisation to α-tubulin.

4. Results

Cell Toxicity: All four groups showed a significant reduction in cell viability at 1×10^{-4} and 5×10^{-5}M atRA concentrations (**Fig. 1E-H**). Cells grown for 24 hours also showed reduced cell viability following 1×10^{-5}M and 1×10^{-6}M 48 hour treatments and in addition to these also 5×10^{-6}M, DMSO 1% and DMSO 0.1% following 7 day treatments.

Despite showing a significant decrease in cell viability within the 24G7T group for 1×10^{-5}M, 5×10^{-6}, 1×10^{-6}M and DMSO 1% and 0.1%, cell viability was recorded at 65-80% of the control group with no statistical difference between these groups. However 1×10^{-4} and 5×10^{-5}M showed relative cell viability of 15% and 12% respectively which was found to be statistically lower than all other groups. These results should however be interpreted with caution due to decreased cell viability within the solvent control groups.

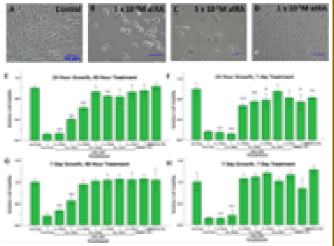


Figure 1. Cell Morphology and cell Viability Assays of ARPE-19 cells following all-trans retinoic acid treatment. Shows representative phase-contrast images of all four ARPE-19 cell treatment groups showing unchanged morphology at 1×10^{-5}M all-trans retinoic acid (atRA) (highest non-toxic dose) compared to the control, with marked morphological changes at 1×10^{-4}M and 5×10^{-5}M associated with toxicity (A-D) Scale bar represents 50μm. Cell viability assay results are shown following ARPE-19 cell growth and all-trans retinoic acid treatment periods at various concentrations shown with DMSO solvent control groups (E-H). Untreated and DMSO 20% wells used as -ve and +ve controls respectively. * indicates P<0.05, ** indicates P<0.01.

5. Results (Cont.)

For all study groups, control and 1×10^{-5}M down to 1×10^{-7}M atRA treatment groups showed RPE cells maintained their cobblestone, cuboidal appearance within the monolayer. However 1×10^{-4}M and 5×10^{-5}M concentrations showed marked signs of toxicity including a reduction in nucleus size, cell shrinkage, rounding of cells and denuded areas (**Fig 1.A-D**).

Wound Healing Assays: In all 4 group study groups 1×10^{-4} and 5×10^{-5}M treatments contained dead cells with no migratory movements. These have not been included in **Fig. 2**. The 24G48T group showed an increase in rate for all treatments at all time points except DMSO 0.1% and 1% at 4-8 hours (**Fig. 2A**). No significant differences were found at any time points for the 24G7T group (**Fig. 2B**). In the 7G48T group all treatments showed a significant increase in rate except 1×10^{-6}M at 0-4 hours (**Fig. 2C**). For the 7G7T group all treatments showed a significant increase in rate except 5×10^{-6}M, 5×10^{-7}M and DMSO 1% at 0-4 hours and DMSO 1% at 0-8 hours (**Fig. 2D**). Results collected were largely unexpected. With the exception of the 24G7T group, all study groups showed an increase in cell migration compared to untreated cells for most atRA treatments as well as DMSO 1% and 0.1%.

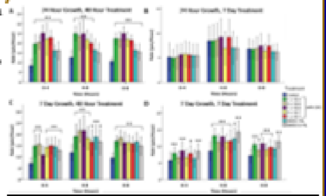


Figure 2. Wound Healing Assays on ARPE-19 cells following all-trans retinoic acid treatment. Shows wound healing assay results following ARPE-19 cell growth and all-trans retinoic acid (atRA) treatment periods at various concentrations shown as well as DMSO solvent control groups. A scratch was made through the monolayer of each well and phase-contrast images taken at 0, 4 and 8 hours at x10 magnification and the mean distance travelled by the cells into the denuded area calculated at 4 and 8 hours (A-D). * indicates P<0.05, ** indicates P<0.01.

Immunocytochemistry: Cytokeratin, fibronectin and collagen IV staining was consistent between the control groups and all non-toxic treatment concentrations (1×10^{-5}M down to 1×10^{-7}M atRA) within each of the four study groups, with no observed changes in localisation or distribution, with 7 day treatments shown in **Fig. 3**.

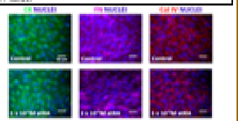


Figure 3. Immunostaining of ARPE-19 cells following atRA Treatment. Fluorescence images showing no change in localisation or distribution of cytokeratin (CK), fibronectin (FN) or collagen IV (Col IV) following NBF fixation and incubation with appropriate primary and secondary antibodies. Control and 5×10^{-5}M (highest non-toxic dose) shown, representative of all four study groups. Scale bar represents 100 μm. G = Growth period and T = Treatment period. CK pseudocoloured green, FN magenta and Col IV red.

ECM Protein Expression: The Confluent culture with 24 hour treatment showed a decrease in fibronectin production at 1×10^{-5}, 1×10^{-6} and 1×10^{-7}M concentrations of atRA. (**Fig. 4A & B**). The same culture treated for 7 days showed similar results, with decreased fibronectin expression at 1×10^{-5}, 1×10^{-6}, 1×10^{-7} as well as 1×10^{-8}M doses (**Fig. 4C & D**). For both treatment periods, expression was reduced in a largely dose dependent manner, although both groups did show a higher relative expression at 1×10^{-7}M than 1×10^{-6}M. For the 7G7T group, no α-tubulin was identified at 1×10^{-5}M due to cell toxicity. Collagen IV production was shown to increase compared to the controls for 5×10^{-6}, 1×10^{-6} & 5×10^{-6}M, with collagen production increasing with decreasing atRA doses (**Fig. 4E & F**).

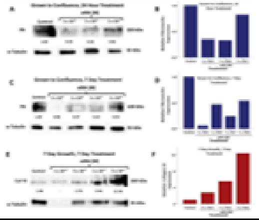


Figure 4. ARPE-19 Fibronectin and Collagen IV expression following all-trans retinoic acid treatment. Following growth and atRA treatment periods indicated, SDS-PAGE and Western blotting was carried out incubating gels with anti-fibronectin (FN) or anti-collagen IV (Col IV) antibodies with anti- α-tubulin used as a loading control (A, C & E). Protein quantification shown below sample (A, C & E) and in graphs (B, D & F).

6. Conclusions

1. 1×10^{-4}M and 5×10^{-5}M atRA concentrations were shown to be toxic to RPE cells grown for 24 hours or 7 days following 48 hour or 7 day treatment.
2. Following atRA treatments no changes in the localisation or distribution of epithelial marker cytokeratin or ECM proteins fibronectin or collagen IV were identified. However atRA did cause a largely dose dependent decrease in fibronectin production on confluent RPE cultures following 24 hour and 7 day treatments and a dose dependent increase of collagen IV on a confluent culture after 5×10^{-6}M, 1×10^{-6}M and 1×10^{-6}M 7 day treatments.
3. Wound healing assay results were unexpected with atRA treatments in the majority of cases showing an increase in migration, however this increase may be due, at least in part, to the solvent DMSO used to solubilise atRA in this study and thus these assay results are currently inconclusive.

References

1. Papaioannou, [illegible] et al (2013), J. Comp. Path., 148, pp. 230-239
2. Ma, X, et al (2014), Plosone, 9(10), pp. 1-8
3. [illegible] & [illegible] (2000), Eye, 14, pp. 411-421
4. [illegible] et al (2014), Retinal Pharmacotherapy, pp. 147-151
5. [illegible] et al (2009), Arq. Bras. de Oftalm., 72(1), pp. 28-32
6. [illegible] & Miller, [illegible] (1989), Sem. in Ophth., 24, 42-48
7. Schwartz, [illegible] & [illegible] (1991), Ophth., 100(1), pp. 785-788
8. [illegible] et al (1993), Invest. Ophth. and Vis. Sci., 34(3), pp. 820-830
9. [illegible] et al (1998), Prog. in Retinal & Eye Res., 17(1), pp. 77-98
10. Li, [illegible] et al (2011), Plosone, 6(8), e23833
11. Chen, [illegible] et al (2014), Plosone, 9(9), e[illegible]
12. [illegible] et al (2010), Invest Ophth & Vis Sci, 51(4), pp. 2099-2063
13. [illegible] & [illegible] (1991), Br J Ophth., (75), p. 53
14. [illegible] et al (2013), Exp. Eye Res., 109, pp. 33-39
15. [illegible] et al (2008), Br J Ophth., 92(11), pp. 1506-1511
16. [illegible] et al (1992), Ophth., 94(4), pp. 327-332.
17. Chang, Y, et al (2008), Exp. Eye Res., 86(5), pp. 500-509
18. [illegible] et al (2006), J Ocular Pharm. & Therap., 22(1), pp. 44-52

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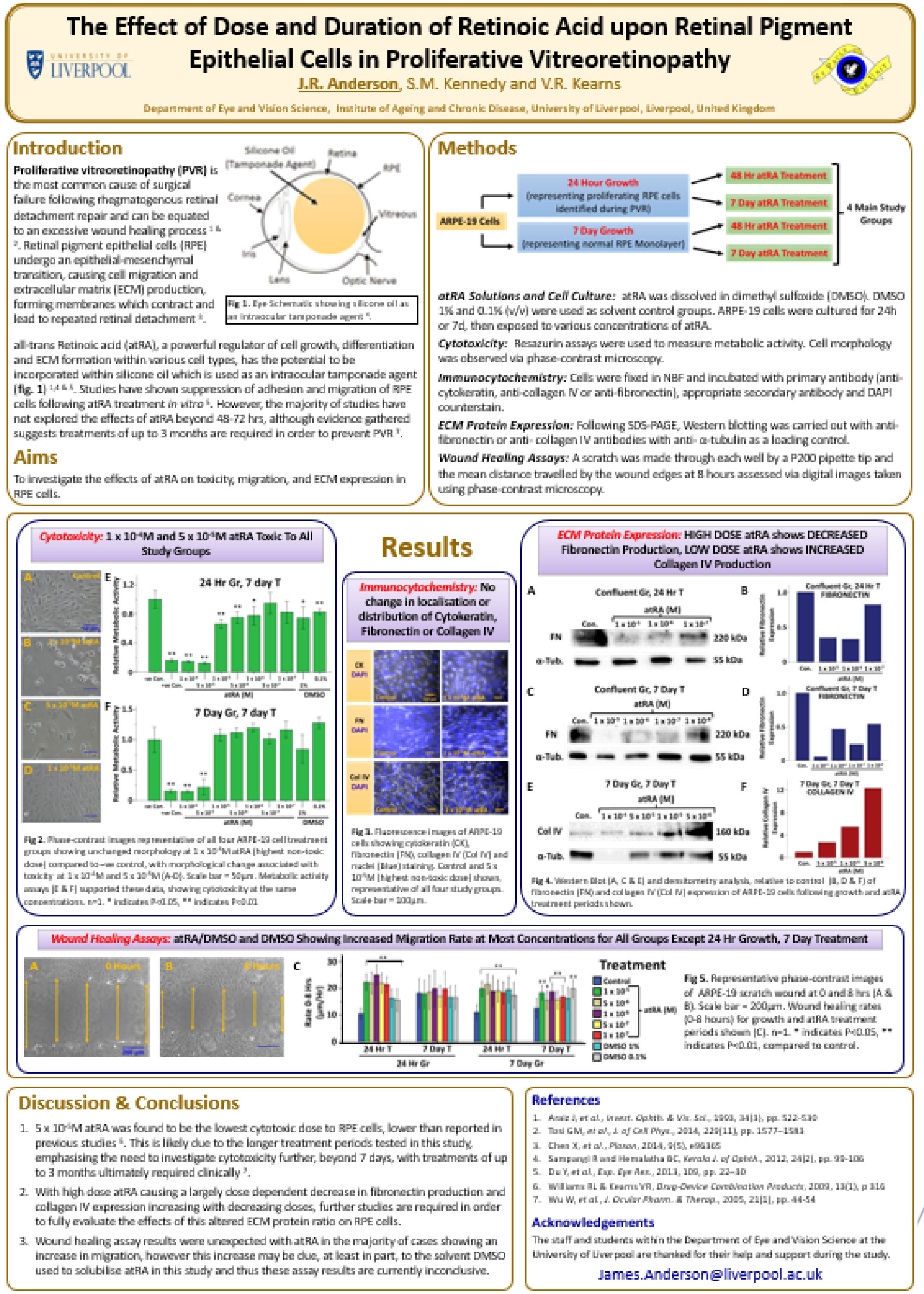
The staff and students within the Eye and Vision Department at the University of Liverpool are thanked for their help and support during the project.


The Effect of Dose and Duration of Retinoic Acid upon Retinal Pigment Epithelial Cells in Proliferative Vitreoretinopathy
J.R. Anderson, S.M. Kennedy and V.R. Kearns
Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom
janders@liverpool.ac.uk
1. Introduction
2. Aims
3. Methods
4. Results
5. Results (Cont.)
6. Conclusions
References
Acknowledgements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J.R. Anderson, S.M. Kennedy and V.R. Kearns
Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom
Introduction
Proliferative vitreoretinopathy (PVR) is the most common cause of surgical failure following rhegmatogenous retinal detachment repair and can be equated to an excessive wound healing process [1 & 2]. Retinal pigment epithelial cells (RPE) undergo an epithelial-mesenchymal transition, causing cell migration and extracellular matrix (ECM) production, forming membranes which contract and lead to repeated retinal detachment [3].
Silicone Oil (Tamponade Agent)
Retina
RPE
Cornea
Vitreous
Iris
Lens
Optic Nerve
Fig 1. Eye Schematic showing silicone oil as an intraocular tamponade agent [4].
all-trans Retinoic acid (atRA), a powerful regulator of cell growth, differentiation and ECM formation within various cell types, has the potential to be incorporated within silicone oil which is used as an intraocular tamponade agent (fig. 1) [1,4 & 5]. Studies have shown suppression of adhesion and migration of RPE cells following atRA treatment in vitro [6]. However, the majority of studies have not explored the effects of atRA beyond 48-72 hrs, although evidence gathered suggests treatments of up to 3 months are required in order to prevent PVR [7].
Aims
To investigate the effects of atRA on toxicity, migration, and ECM expression in RPE cells.
Methods
ARPE-19 Cells
24 Hour Growth (representing proliferating RPE cells identified during PVR)
7 Day Growth (representing normal RPE Monolayer)
48 Hr atRA Treatment
7 Day atRA Treatment
48 Hr atRA Treatment
7 Day atRA Treatment
4 Main Study Groups
atRA Solutions and Cell Culture: atRA was dissolved in dimethyl sulfoxide (DMSO). DMSO 1% and 0.1% (v/v) were used as solvent control groups. ARPE-19 cells were cultured for 24h or 7d, then exposed to various concentrations of atRA.
Cytotoxicity: Resazurin assays were used to measure metabolic activity. Cell morphology was observed via phase-contrast microscopy.
Immunocytochemistry: Cells were fixed in NBF and incubated with primary antibody (anti-cytokeratin, anti-collagen IV or anti-fibronectin), appropriate secondary antibody and DAPI counterstain.
ECM Protein Expression: Following SDS-PAGE, Western blotting was carried out with anti-fibronectin or anti- collagen IV antibodies with anti- α-tubulin as a loading control.
Wound Healing Assays: A scratch was made through each well by a P200 pipette tip and the mean distance travelled by the wound edges at 8 hours assessed via digital images taken using phase-contrast microscopy.
Results
Cytotoxicity: 1 x 10⁻⁴M and 5 x 10⁻⁵M atRA Toxic To All Study Groups
24 Hr Gr, 7 day T
7 Day Gr, 7 day T
Immunocytochemistry: No change in localisation or distribution of Cytokeratin, Fibronectin or Collagen IV
ECM Protein Expression: HIGH DOSE atRA shows DECREASED Fibronectin Production, LOW DOSE atRA shows INCREASED Collagen IV Production
Confluent Gr, 24 Hr T
Confluent Gr, 7 Day T
7 Day Gr, 7 Day T
Wound Healing Assays: atRA/DMSO and DMSO Showing Increased Migration Rate at Most Concentrations for All Groups Except 24 Hr Growth, 7 Day Treatment
Treatment
Control
DMSO 1%
DMSO 0.1%
24 Hr T
7 Day T
24 Hr Gr
7 Day Gr
Fig 5. Representative phase-contrast images of ARPE-19 scratch wound at 0 and 8 hrs (A & B). Scale bar = 200µm. Wound healing rates (0-8 hours) for growth and atRA treatment periods shown (C). n=1. * indicates P<0.05, ** indicates P<0.01, compared to control.
Discussion & Conclusions
1. 5 x 10⁻⁵M atRA was found to be the lowest cytotoxic dose to RPE cells, lower than reported in previous studies [5]. This is likely due to the longer treatment periods tested in this study, emphasising the need to investigate cytotoxicity further, beyond 7 days, with treatments of up to 3 months ultimately required clinically [7].
2. With high dose atRA causing a largely dose dependent decrease in fibronectin production and collagen IV expression increasing with decreasing doses, further studies are required in order to fully evaluate the effects of this altered ECM protein ratio on RPE cells.
3. Wound healing assay results were unexpected with atRA in the majority of cases showing an increase in migration, however this increase may be due, at least in part, to the solvent DMSO used to solubilise atRA in this study and thus these assay results are currently inconclusive.
References
1. Araiz J, et al., Invest. Ophth. & Vis. Sci., 1993, 34(3), pp. 522-530
2. Tosi GM, et al., J. of Cell Phys., 2014, 229(11), pp. 1577–1583
3. Chen X, et al., PLoS One, 2014, 9(5), e96365
4. Sampangi R and Hemalatha BC, Kerala J. of Ophth., 2012, 24(2), pp. 99-106
5. Du Y, et al., Exp. Eye Res., 2013, 109, pp. 22–30
6. Williams RL & Kearns VR, Drug-Device Combination Products, 2009, 13(3), p 316
7. Wu W, et al., J. Ocular Pharm. & Therap., 2005, 21(1), pp. 44-54
Acknowledgements
The staff and students within the Department of Eye and Vision Science at the University of Liverpool are thanked for their help and support during the study.
James.Anderson@liverpool.ac.uk

Does Blood Spot Quality Make a Difference?

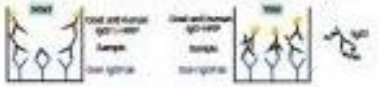
Dabbs, R. A.[1], Hall, T.[1], Drakeley, C. J.[1]
[1] Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK

Introduction

- Dried blood spots (DBS) provide a robust, inexpensive, convenient method of collecting and storing blood samples in the field[1,2].
- DBS can be used as a source of antibodies for serological assays such as ELISA and as a source of DNA for PCR[1,2].
- The quality of the DBS depends on both collection and storage conditions.
- Variation in DBS quality affects the amount and quality of recoverable antibody, which can have adverse effects on subsequent serological assays.

Methods

- **Sample Selection**
  - Samples from Tanzania where collection quality varied.
  - Samples contaminated with a variety of environmental moulds from Vanuatu.
  - Samples collected in Mozambique and stored under different conditions.
- **ELISA to Detect Intact vs Total IgG in a Sample**



- **ELISA to Detect Malaria Specific Antibodies**
  - Malaria antigen (AMA1 or MSP1-19) bound to plate (4°C, overnight); plates blocked with 1% skim milk in PBS + 0.05% Tween20 (3 h room temperature); samples added (4°C, overnight); Rabbit anti human IgG-HRP added (3 h room temperature); developed using TMB.

Results

Figure 1 – Examples of both "good" and "bad" DBS. Spots which have been involved are indicated by an arrow.

- There is significant difference ($p < 0.0001$) between the amount of intact IgG eluted from samples that were either collected properly (bled through) or not (Figure 2)

Figure 2 – The quality of the DBS (bled through or not) impacts on the recoverable amount of intact IgG from the sample.

- If the quality of the DBS is poor there seems to be a slight bias toward lower OD values (Figure 3).

Figure 3 – The quality of the DBS (bled through or not) may bias the obtained malaria specific OD towards lower values.

- Mould and incorrect storage of DBS can dramatically impact on the integrity of the eluted sample. In this case DBS contaminated with mould show reduced amounts of intact IgG (Figure 4).

Figure 4 – Mould and incorrect storage conditions can result in IgG degradation.

- Storage under elevated temperatures can result in a "baking" effect where the IgG is no longer able to elute from the DBS (Figure 5)

Figure 5 – Storage at elevated temperatures results in a "baking" effect, whereby the IgG contained on the DBS is no longer able to elute. This can be judged visually (see inset images) or more accurately by measuring the amount of eluted intact IgG by ELISA.

Conclusions

- It is important to ensure the DBS are correctly collected, as poor quality DBS result in significantly lower levels of eluted IgG and may bias your results towards a lower malaria antigen specific ELISA result.
- Ensuring storage and shipping conditions is vital as elevated temperatures and humidity may result in a "baking" effect or mould contamination, respectively. These both result in unusable samples, which need to be discarded.

References: [1] Garcia, D. H., Cook, J., et al. (2006) Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. Malaria Journal 5: 106.
[2] Hall, T. W., Filed, I., et al. (2010) An overview of the clinical use of filter paper in the diagnosis of tropical diseases. Am J Trop Med Hyg 83(1): 163-169.

Does blood spot quality make a difference?

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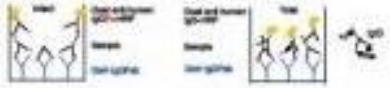

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Results

Fig. 1 Examples of both "good" and "bad" DBS. Spots which have been involved are indicated by an arrow.

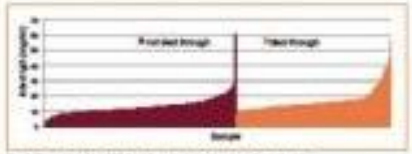
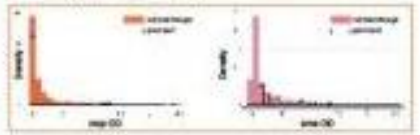


Fig. 2 The quality of the DBS (bled through or not) impacts on the recoverable amount of intact IgG from the sample.

- There is significant difference ($p < 0.0001$) between the amount of intact IgG eluted from samples that were either collected properly (bled through) or not (Fig. 2)
- If the quality of the DBS is poor there seems to be a slight bias toward lower OD values (Fig. 3).



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- Mould and incorrect storage of DBS can dramatically impact on the integrity of the eluted sample. In this case DBS contaminated with mould show reduced amounts of intact IgG (Fig. 4).

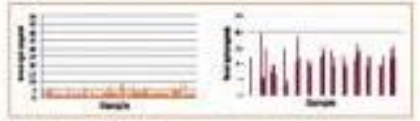

Fig. 4 Mould and incorrect storage conditions can result in IgG degradation.

- Storage under elevated temperatures can result in a "baking" effect where the IgG is no longer able to elute from the DBS (Fig.5).

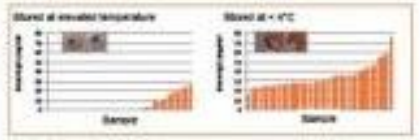

Fig. 5 Storage at elevated temperatures results in a "baking" effect, whereby the IgG contained on the DBS is no longer able to elute. This can be judged visually (see inset images) or more accurately by measuring the amount of eluted intact IgG by ELISA.

Conclusions

- It is important to ensure the DBS are correctly collected, as poor quality DBS result in significantly lower levels of eluted IgG and may bias results towards a lower malaria antigen specific ELISA.
- Ensuring storage and shipping conditions is vital as elevated temperatures and humidity may result in a "baking" effect or mould contamination, respectively. These both result in unusable samples, which need to be discarded.

References

Examining the Effects of Hyperosmolarity on Circadian Rhythms in Chondrocytes

J.R. Anderson, V. Pekovic-Vaughan and S.R. Tew

Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Leahurst Campus, Neston, CH64 7TE, United Kingdom

Introduction

Circadian rhythms are intrinsic, near 24 hour biological cycles that regulate physiology and behaviour [1&2]. Chronic disturbances of normal circadian rhythms have been associated with an elevated risk/acceleration of various conditions including rheumatoid arthritis and osteoarthritis [3&4]. Although circadian rhythms of chondrocytes have been studied in various species, equine chondrocyte circadian rhythms are yet to be characterised [5].

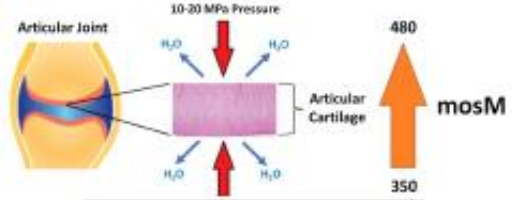

Fig 1. Increased osmolarity of articular cartilage under sustained loading force [4].

How circadian rhythms of chondrocytes in articular cartilage are regulated/synchronised within an isolated, avascular, anaural environment is not understood [5,6&7]. Mechanical loading during activity expresses water from articular cartilage leading to elevated osmotic pressure of up to 480mosM (Fig. 1) [4]. Interestingly, one of the key genes regulating circadian rhythms, *Bmal1*, is upregulated by hyperosmotic conditions in human articular chondrocytes [8].

Aims

To investigate the effect of hyperosmotic conditions on chondrocyte circadian rhythms.

Methods

Circadian Curves:

- Circadian rhythms of human SW-1353 chondrosarcoma cells and primary equine chondrocytes from fetlock joints were synchronised by culturing in 100nM dexamethasone for 1 hr.
- Cells were lysed in TRI Reagent® at 4 hour intervals between 16-44 hrs following synchronisation, RNA extracted and expression of circadian genes *Bmal1* and *Per2* quantified using qRT-PCR. Data was normalised to *GAPDH* expression and the 16 hr value using the $2^{-\Delta\Delta Ct}$ method.

Hyperosmotic Treatments:

- Circadian rhythms of human SW-1353 chondrosarcoma cells and primary equine chondrocytes from fetlock joints were again synchronised by culturing in dexamethasone.
- Cells were cultured in either untreated control media (325mosM) or media adjusted to 450mosM with either sodium chloride or D-Mannitol and lysed at two time points (20 and 28 hrs following synchronisation) corresponding with troughs/peaks in *Bmal1* and *Per2* expression.
- Gene expression levels were again calculated using the $2^{-\Delta\Delta Ct}$ method, normalising to *GAPDH* and subsequently the 20 hr control group.

Cell Morphology:

- Cell morphology was assessed via phase-contrast microscopy.

Results

Circadian Curves: **SW-1353 Cells and Primary Equine Chondrocytes**

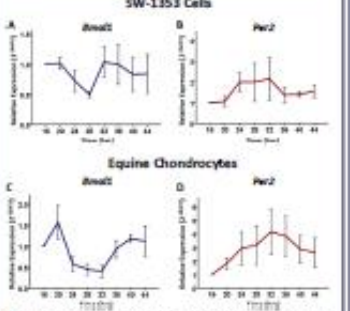

Fig 2. (A) *Bmal1* expression in SW-1353 cells peaked at 28 and 32 hrs with cyclic expression at 24 hours following synchronisation. (B) SW-1353 *Per2* expression showed an inverted pattern to *Bmal1* with troughs of expression at 16-20 hrs and 40-44 hrs with expression peaking between 28-32 hrs. (C) *Bmal1* expression in equine chondrocytes peaked at 20 hrs with a trough between 24-32 hrs (nadir at 32 hrs) then rising until 40 hrs. (D) Equine *Per2* cyclic expression was recorded at 16 hrs with expression increasing until a peak at 32 hrs and falling until the final reading at 44 hrs. Error Bars = ± 1 s.e. SW-1353, n=6. Equine Chondrocytes, n=3.

Cell Morphology: **No change to cell morphology following hyperosmotic treatments**

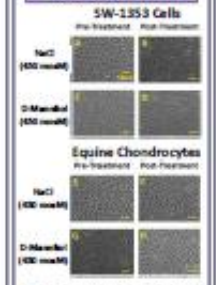

Fig 3. Phase-contrast images representative of SW-1353 cells (A-D) and primary equine chondrocytes (E-H) prior to and following 28 hr incubation with hyperosmotic 450mosM solutions containing complete media supplemented with NaCl or D-Mannitol. No changes to cell morphology were identified. Scale bar = 400μm.

Hyperosmotic Treatments: **Consistent trend shows hyperosmotic media supplemented with NaCl INCREASES expression of circadian genes *Bmal1* and *Per2***

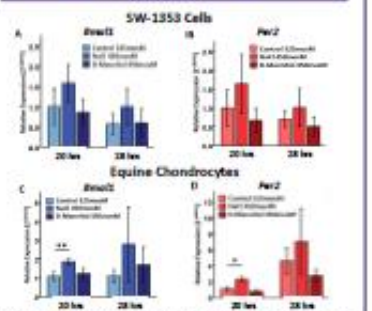

Fig 4. Both SW-1353 cells (A & B) and equine chondrocytes (C & D) showed a consistent trend with expression of *Bmal1* and *Per2* at 20 and 28 hrs highest under NaCl hyperosmotic conditions and cells cultured in D-Mannitol hyperosmotic media showing similar expression to that of the control group in the majority of cases. A statistically significant increase in gene expression was only found for *Bmal1* and *Per2* in equine chondrocytes at 20 hrs, with NaCl hyperosmotic media leading to an upregulation of both genes. Error Bars = ± 1 s.e. SW-1353, n=6. Equine Chondrocytes, n=3. * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$.

Discussion & Conclusions

- Consistent trends suggest elevated osmolarity caused by supplementation with NaCl leads to an increase in the expression of the clock genes *Bmal1* and *Per2* regardless of the stage of the circadian cycle. Thus hyperosmolarity may play a role in the synchronisation of chondrocyte circadian rhythms during locomotion.
- D-Mannitol hyperosmotic media did not maintain its elevated osmolarity during experimentation (decreasing from 450 to 376mosM). Thus the disparity in trends between hyperosmotic NaCl and D-Mannitol would appear to be due to technical error leading to differing osmolarities as opposed to an intrinsic biological difference between NaCl and D-Mannitol.
- With the disruption of circadian rhythms having been shown to predispose mice to osteoarthritis [3], synchronisation through the mechanism of altering osmolarity (i.e. targeting chondrocyte osmotic stress genes) may have a therapeutic role to play in the treatment/prevention of osteoarthritis.

References

1. Gekakis N, *et al.*, Science, 1998, 280(5369), pp. 1564-1569
2. Nohara K, *et al.*, Chronobiology International, 2015, pp.1-12
3. Xu H, *et al.*, J. of Cellular Physiology, 2015, 230(8), pp. 2174-2180
4. Hashimoto K, *et al.*, J. of Immunology, 2015, 194, pp. 1560-1568
5. Gossan N, *et al.*, Arthritis & Rheumatism, 2013, 65(8), pp. 2080-2085
6. Urban JP, *et al.*, J. of Rheumatology, 1996, 66(10), pp. 901-908
7. Technyflex, Technyflex® Canine, 2012, www.technyflex.co.uk/technyflex-canine.html
8. Tew SR, *et al.*, Osteoarthritis and Cartilage, 2013, 19, pp. 1086-1094

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James.Anderson@liverpool.ac.uk

Human Synovial Fluid Metabolite Profiles In Inflammatory And Non-inflammatory Arthritis

J. R. Anderson[1], S. Chokesuwattanaskul[2], M. M. Phelan[2,3], T. J. Welting[4], P. D. Clegg[1], L.-Y. Lian[2], H. L. Wright[2] & M. J. Peffers[1]

[1] Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, William Henry Duncan Building, L7 8TX, UK
[2] Institute of Integrative Biology, University of Liverpool, Biosciences Building, L69 7ZB, UK [3] HLS Technology Directorate, University of Liverpool, UK
[4] Laboratory for Experimental Orthopaedics, Department of Orthopaedic Surgery, Maastricht University Medical Centre, NL

Introduction

Non-inflammatory osteoarthritis (OA) and **inflammatory rheumatoid arthritis (RA)** lead to significant disability and reduction in quality of life [1]. However, despite their severity, relatively little is known about their complex pathogeneses [2]. The ability to diagnose RA and OA at an early stage is poor, due to their insidious onset and clinical signs developing after a considerable period of disease [3&4].

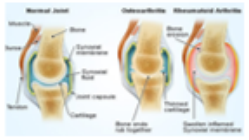


Fig 1. Joint pathology associated with osteoarthritis and rheumatoid arthritis [5].

Synovial fluid (SF) holds huge potential for earlier diagnosis of these conditions, including identification of metabolite markers, yet few studies have investigated the whole profile of metabolites within human SF [6, 7 & 8]. Previous analyses have been inhibited by the low volumes of SF able to be aspirated from human joints. Nuclear magnetic resonance (NMR) allows analysis of a small volumes of SF with minimal sample preparation using non-invasive and non-destructive methods [9].

Aim

To compare the metabolomic profiles of human synovial fluid identified in inflammatory and non-inflammatory arthritis.

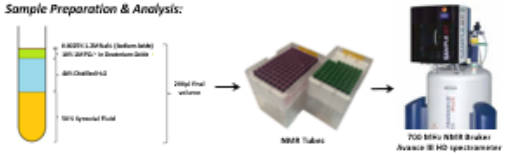
Methods

Sample Collection:

	Cohort	Number of Patients	Sex	Average Age (Yrs, S.D.)
Non-Inflamatory	Osteoarthritis (OA)	10	(5F, 5M)	67 (12)
Inflammatory	Rheumatoid Arthritis (RA)	14	(9F, 5M)	65 (9)
	Inflammatory Arthritis (IA (nonRA))	14	(5F, 9M)	47 (16)

SF was collected with ethical approval from the knee joint of patients diagnosed with OA, RA and IA (nonRA) (11 conditions including Behçet's disease, gout, reactive arthritis and calcium pyrophosphate arthritis), placed into heparinized tubes and processed within 1 hr. Native SF was centrifuged, cell-free SF removed and frozen at -20°C.

Sample Preparation & Analysis:



Samples were analysed following ^{1}H NMR spectroscopy on a 700 MHz NMR Bruker Avance III HD spectrometer with a TCI cryoprobe and chilled sample-jet autosampler. For each sample three 1D ^{1}H spectra were acquired (NOE, CPMG and LED) at 37°C. Acquisition and processing was carried out using Topsin3.1 and IconNMR 4.6.7 with MetaboAnalyst 3.0 used to carry out principle component analysis and to identify differential metabolite abundances between cohorts *via* one-way ANOVA and Fisher's LSD *post-hoc* test with $p<0.01$ considered significant.

Results

Principle Component Analysis: **Separation identified between inflammatory and non-inflammatory arthritis**

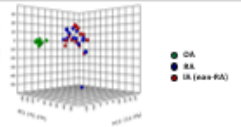


Fig 2. Separation between cohorts using PC1, PC2 and PC3 principle components.

^{1}H NMR Spectra: **Small metabolite identifications and cohort abundances analysed using CPMG spectra**

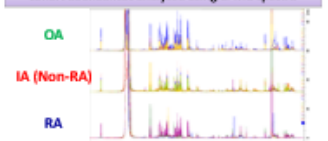


Fig 3. CPMG spectral peaks were correlated to metabolite standards to carry out metabolite identification, identify differential cohort metabolomes with the area per peak calculated to determine metabolite abundances.

Differential Metabolite Abundances: **Elevated levels of glucose, glycine, pyruvate, creatinine and glutamine and lower levels of choline and acetate were identified in OA SF compared to RA and IA (non-RA) SF**

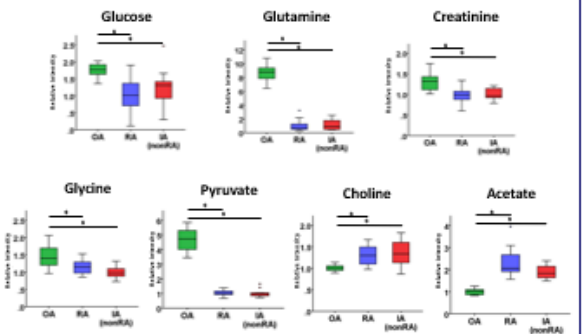


Fig 4. Metabolite Abundances shown as relative intensities corresponding to the most representative peak for each metabolite. * indicates $p<0.01$

Discussion & Conclusions

1. Global NMR metabolome identification indicated SF to be a discriminant between inflammatory and non-inflammatory rheumatological conditions.
2. Identified quantifiable differences in metabolite abundances between non-inflammatory arthritis (OA) and inflammatory arthritis (RA) may prove beneficial as a diagnostic aid as well as improving our understanding of the pathogenesis of these conditions.
3. During this study the protocols implemented using ^{1}H NMR have shown to be effective in producing high quality spectra with quantifiable differences in metabolite abundance identified using only 100μl of SF from each individual.
4. For future studies, increased sample size and improved clinical standard operating procedures would aid further (and more in-depth) analysis.

References

1. Sprangers MA *et al.*, *J. Clin. Epidemiol.*, 2000, 53, pp. 895-907
2. Wilkinson J *et al.*, *Arthritis Research & Therapy*, 2016, 18(184)
3. Gabasio R *et al.*, *Arthritis Res. Ther.*, 2017, 9, 936
4. Jansonius NM & Bykerk VP, *Best Pract. Res. Clin. Rheumatol.*, 2013, 27, pp. 651-664
5. Ola Health, http://www.olahealth.com/content/1/rheumatoid_arthritis_ra
6. Naughton D *et al.*, *NMR Lett.*, 1993, 8, 317(1-2), pp. 155-159
7. Hagle T *et al.*, *Clin. Exp. Rheumatol.*, 2013, 31(2), pp. 240-244
8. Kang KY *et al.*, *J. Rheumatol.*, 2015, 42(11)
9. Kean & Abbenseth TD, *Methods. Mol. Biol.*, 2011, 708, pp. 321-334

Acknowledgements

The authors would like to thank the study participants and staff at The Royal Liverpool Hospital and Maastricht University Medical Centre for SF samples and sample collection, NI fluid Session for NMR support and The Wellcome Trust, Pfizer, The Liverpool Technology Directorate and The Horse Trust for contributing to the funding of this research.

James.Anderson@liverpool.ac.uk

Synovial Fluid Metabolites Differentiate between Septic and Non-Septic Joint Pathologies

J. R. Anderson[1], M. M. Phelan[2,3], L.-Y. Lian[2], P. D. Clegg[1], M. J. Peffers[1] & L. M. Rubio-Martinez[4]

[1] Department of Musculoskeletal Biology , Institute of Ageing and Chronic Disease, University of Liverpool, William Henry Duncan Building, L7 8TX, UK
[2] Institute of Integrative Biology, University of Liverpool, Biosciences Building, L69 7ZB, UK [3] HLS Technology Directorate, University of Liverpool, UK
[4] Institute of Veterinary Science, University of Liverpool, Leahurst Campus, CH64 7TE, UK

Introduction

Articular pathology is common in horses causing loss of function and pain. These include **osteoarthritis (OA)**, **osteochondrosis (OC)** and **sepsis**, the latter being life-threatening [1]. However diagnosis, staging, monitoring and accurate prognostication remains a challenge for practising veterinarians and there is therefore a need to identify reliable biomarkers for accurate and rapid diagnosis as well as gaining a greater understanding of the underlying pathogenesis. Synovial fluid (SF) is an integral articular component closely associated with other articular tissues which are primarily altered during joint pathologies [2, 3 & 4].

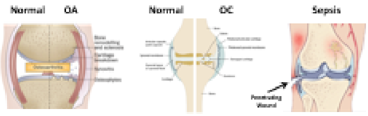


Fig 1. Joint pathology associated with osteoarthritis, osteochondrosis and sepsis [5,6 & 7].

However, to date no studies have investigated the whole profile of metabolites of equine SF in different joint conditions. Nuclear magnetic resonance (NMR) spectroscopy allows for the analysis of a small volume of native SF with a minimal level of sample pre-processing using a non-invasive and non-destructive method [8].

Aim

To define the metabolomic profile of SF samples obtained from equine joints affected by septic and non-septic articular pathologies.

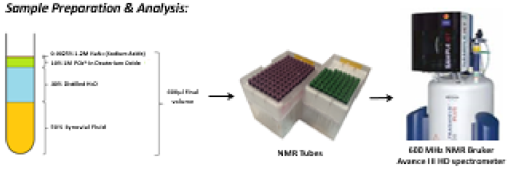
Methods

Sample Collection:

	Cohort	Number of Horses	Sex (Female; F, Male; M)	Average Age (Yrs, S.D.)
Septic	Sepsis	7	7M	7 (5)
Non-Septic	Osteoarthritis (OA)	6	2F, 4M	11 (5)
	Osteochondrosis (OC)	6	4F, 2M	5 (2)

Following institutional ethical approval and owner consent, SF was aspirated from non-septic equine joints (OA and OC) and septic equine joints, placed into plain Eppendorf tubes and stored at -80°C following centrifugation.

Sample Preparation & Analysis:



Samples were analysed following ^{1}H NMR spectroscopy on a 600 MHz NMR Bruker Avance III HD spectrometer with a TCI cryoprobe and chilled sample-jet autosampler. For each sample three 1D ^{1}H spectra were acquired (NOE, CPMG and LED) at 25°C. Acquisition and processing was carried out using Topsin3.1 and IconNMR 4.6.7. MetaboAnalyst 3.0 was used to carry out univariate analysis to identify differential metabolite abundances between cohorts *via* one-way ANOVA and Fisher's LSD *post-hoc* test with $p<0.05$ considered significant. 'R' software was used for multivariate analysis. Metabolites identified in ^{1}H NMR using in-house libraries and the 1D NMR identification software Chenomx were confirmed utilising 2D ^{1}H ^{13}C NMR and 2D NMR identification software CCPNMR Analysis.

Results

Principle Component Analysis: **Separation identified between septic and non-septic joint pathologies**

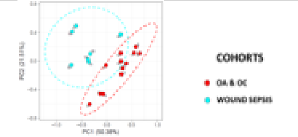


Fig 2. PC1 and PC2 principle components, containing the majority of sample variation, differentiate between the global metabolite profiles of septic and non-septic joint pathologies.

^{1}H NMR Spectra: **Small metabolite identifications and cohort abundances analysed using CPMG spectra**

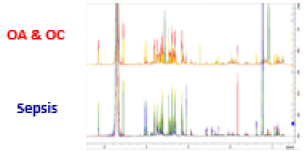


Fig 3. CPMG spectral peaks were correlated to metabolite standards to carry out metabolite identification, with peak areas used to determine relative metabolite abundances and identify differential cohort metabolite profiles.

Differential Metabolite Abundances: **Elevated levels of alanine, citrate, creatinine, glucose, glycine, phenylalanine, pyruvate, urea and valine and lower levels of acetate were identified in non-septic SF compared to septic SF**

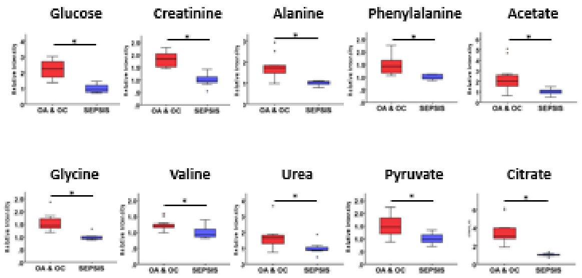


Fig 4. Metabolite Abundances shown as relative intensities corresponding to the most representative peak for each metabolite. * indicates p< 0.05

Discussion & Conclusions

1. Global NMR metabolome identification indicated SF to be a discriminant between septic and non-septic equine joint pathologies.
2. Identified quantifiable differences in metabolite abundances between non-septic (OA and OC) and septic joint pathologies may prove beneficial as a diagnostic aid as well as improving our understanding of the pathogenesis of these conditions.
3. During this study the protocols implemented using ^{1}H NMR have shown to be effective in producing high quality spectra with quantifiable differences in metabolite abundance identified using only 500µl of SF from each equine joint.
4. For future studies, increased sample size and improved clinical standard operating procedures would aid further (and more in-depth) analysis.

References

1. Summerhays GE, *Vet Rec*, 2000, 147(7), pp. 184-188
2. Blewis ME *et al.*, *Eur Cell Mater*, 2007, 13, pp. 26-39
3. Ruiz-Romero C & Blanco FJ, *Osteoarthritis Cartilage*, 2010, 18(4), pp. 500-509
4. Mateos J *et al.*, *J. Proteomics*, 2012, 75(10), pp. 2869-2878
5. Wiluka AE *et al.*, *Nat. Rev. Rheumatol.*, 2013, 9(4), pp. 225-235
6. Enpevet, http://www.enpevet.de/Lexicon
7. Kherani RB & Shojania K, *CMAJ*, 2007, 176(11), pp. 1605-1608
8. Keun HC & Athersuch TJ, *Methods Mol. Biol.*, 2011, 708, pp. 321-334

Acknowledgements

The authors would like to thank staff at the The Philip Leverhulme Equine Hospital, University of Liverpool, for SF samples and sample collection, Mr Rudi Grosman for NMR support, Dr Eva Caamaño-Gutiérrez and Dr Arturas Grauslys for statistical support and The University of Liverpool Institute of Veterinary Science, The Liverpool Technology Directorate, The Horse Trust and The Wellcome Trust for contributing to the funding of this research.

James.Anderson@liverpool.ac.uk

On Lessons Learned from Remote Sensing of Irish Grasslands, and Potential for Sentinel Data

Fiona Cawkwell*, Ingmar Nitze, Brian Barrett
School of Geography & Archaeology, University College Cork (UCC), Ireland
* email: f.cawkwell@ucc.ie

1. Introduction

- Grassland is the dominant land cover in Ireland (Fig.1), accounting for approximately 64% of the country's land area and representing over 90% of all agricultural land (~4,000,000ha).
- Thus, there is considerable potential to increase carbon sequestration in grasslands through improved land management and restoration of degraded grasslands (O'Mara, 2012).
- So far there are no operational RS-based systems in Ireland for the detection of grassland management types. This study aims to close this gap in order to achieve more reliable figures for the reporting of the national carbon budget.

Figure 1: Improved (A) and Semi-improved (B) grasslands

2. Study Area & Data

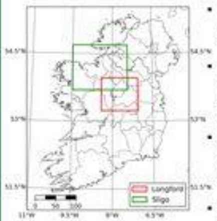


Figure 2: Location of study areas

- Two study areas in central and north-western Ireland encompassing Counties Longford and Sligo (Fig. 2)
- Climatic conditions with frequent cloud-cover and other atmospheric disturbances are the limiting factor for the use of optical RS data in Ireland
- A 13-year time-series from 2001 to 2013 of MODIS 16-day composites (MOD13Q1 – 250 m resolution) giving 23 images per year
- 2 Landsat -8 (summer 2013) and 4 DMC –UK2 (3 from 2011 and 1 from 2013) images

3. Methodology

- Four general land cover classes (Forest, Water, Settlement, Peatland) and two Grassland classes (Improved GL [GA], Semi-Improved GL [GS]) were classified. A further subdivision of the grassland classes was prevented by the spatial resolution of the MODIS data given the high fragmentation of the landscape, and of the Landsat/DMC data given their infrequent acquisition.
- Time-series pre-processing of MODIS data, taking quality measures into account and applying temporal filters to reduce data noise (Fig. 3), to reveal spectral classes.
- Random Forest (RF), Extremely Randomized Trees (ERT), Support Vector Machine (SVM) and Maximum Likelihood (ML) classifiers used.
- 5-fold cross validation performed for training and validation of the classifiers on 1051 and 2134 samples from the Longford and Sligo datasets respectively.

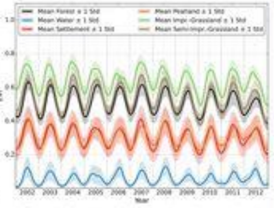


Figure 3: Smoothed EVI time-series of the examined land cover classes.

- STARFM (Gao et al., 2008) was used to fuse a pair of Landsat and MODIS images from June 9th and compared with modelled MODIS data for July 11th
- Three DMC images from March, April and November 2011 were classified with the same classes and methods as the MODIS time series.

4. Results

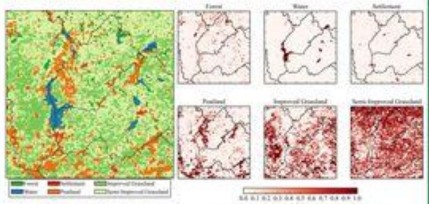


Figure 4: MODIS classification results and class specific probabilities for Longford

- High classification accuracies for MODIS data in homogeneous areas (Fig. 4), with best results from SVM and ERT (typically 97% accuracy), but low spatial resolution insufficient for heterogeneous areas.
- MODIS fusion model trained using cloud-free Landsat image, but due to changes in landscape (e.g. grass cutting and grass growth) modelled image showed greater correlation to June template than July target date, especially in the near and middle infra-red (Fig. 5)
- The multi-temporal DMC-UK2 classification accuracies were generally very high using the machine learning classifiers (> 95 %), but lacked accuracy if mono-temporal classification was conducted (Fig.6)

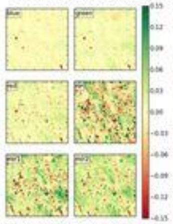


Figure 5: Differences in reflectance between modelled and observed Landsat results for July 11th 2013

Figure 6: Comparison of classification results of MODIS and DMC-UK2 in a small subset of Longford with multi-temporal data from 2011

While the spatial resolution of Landsat and DMC are comparable, the former offers a more systematic acquisition schedule and superior spectral resolution, although less frequently

5. Conclusions

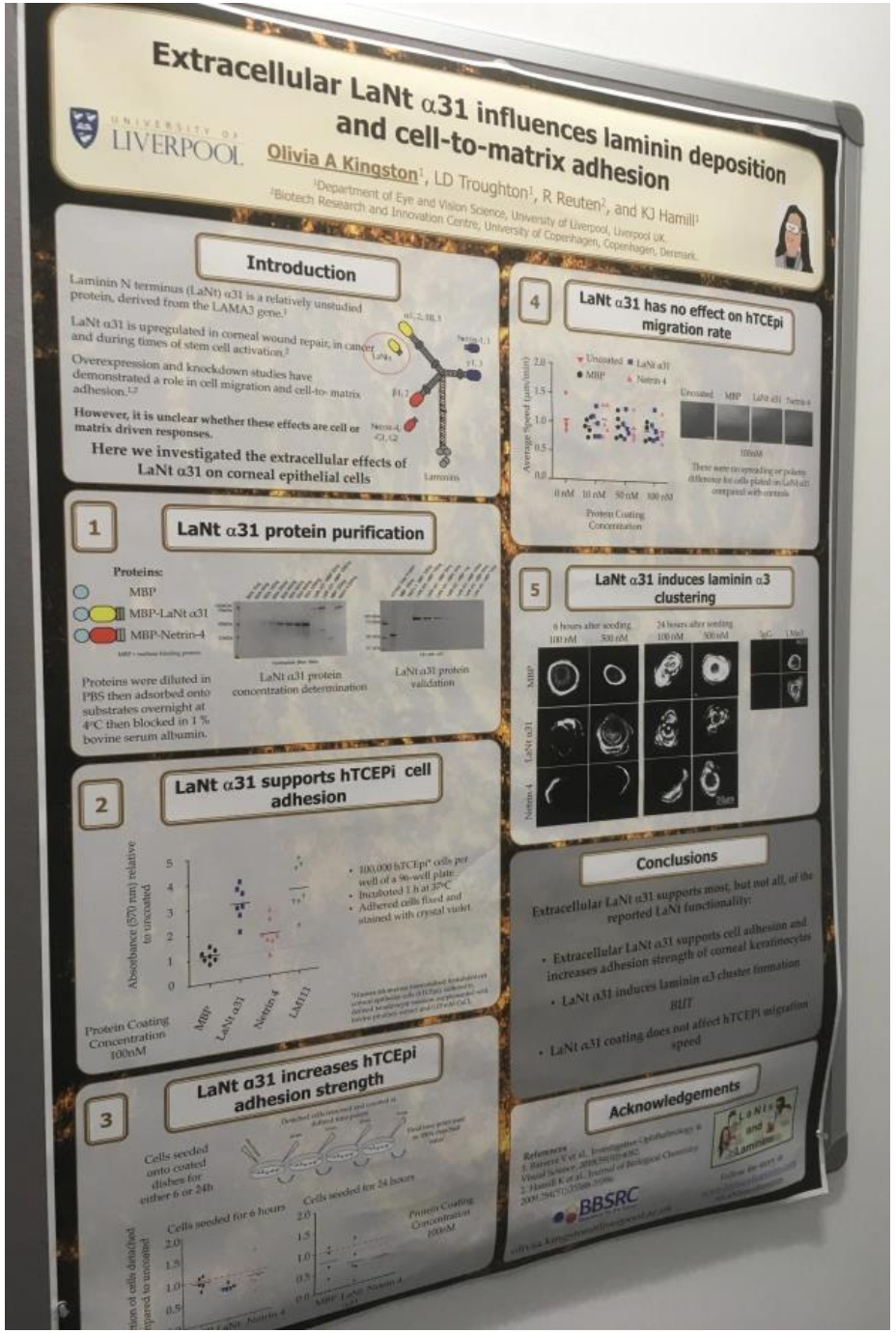
- Homogeneous areas were classified using multi-temporal MODIS data with accuracies over 97%, but Ireland has a very heterogeneous landscape
- Fusion of MODIS and Landsat data is inappropriate given the dynamic nature of the landscape which cannot be captured by monthly data
- Three UK-DMC2 images were able to distinguish grassland classes almost as accurately as a full year of MODIS data, and if images had been spaced through the growing season this would probably have improved further
- The high spatial and temporal resolution of **Sentinel-2 offers promising potential for mapping the dynamic agricultural landscape of Ireland**, for greenhouse gas inventories and agro-environmental management

References

O'Mara, F. (2012) The role of grasslands in food security and climate change, *Annals of Botany*, 1-8.
Gao, F., et al. (2008) An algorithm to produce temporally and spatially continuous MODIS-LAI time series; *Geoscience and Remote Sensing Letters*, IEEE 5.1, p. 60-64

Acknowledgements

The authors would like to acknowledge the Irish Environmental Protection Agency (EPA) for funding the Irish Land Mapping Observatory (ILMO) project, and collaborators from Teagasc, Ordnance Survey Ireland and FERS Ltd.



The Articular Cartilage Proteome is Dependent on Zone, Age and Disease State

Aibek Smagul[1], Deborah Simpson[2], Simon Tew[1], Mandy J. Peffers[1]
[1]Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK
[2]Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Liverpool, UK
a.smagul@liverpool.ac.uk

Background

- Osteoarthritis (OA) is the most common age related musculoskeletal disease, with a total of 30 million people affected in US only.
- The articular cartilage comprises of three distinctive zones: superficial, middle and deep (figure 1).

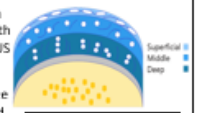


Figure 1. Cartilage zones

Methods

1. Young (age 32), Old (age 71) and OA (age 76) groups, n=5 in each group
2. Cryosection and laser microdissection (LMD) of different zones
3. *in-situ* trypsin digestion of proteins
4. LC-MS/MS analysis, label-free quantification
5. Pathway analysis of differentially abundant proteins

Results

Laser microdissection

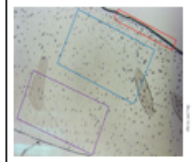


Figure 2. LMD of cartilage tissue section, 5X magnification

Differentially abundant proteins

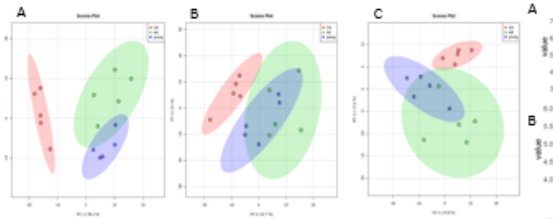


Figure 3. PCA plot of differentially abundant proteins in the superficial (A), middle (B) and the deep (C) zones

Protein abundance in different zones

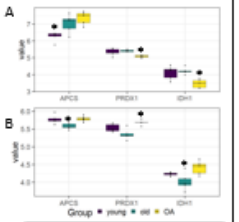


Figure 4. Protein abundance of APCS, PRDX1 and IDH1 in the superficial (A) and deep (B) zones, * $P_{adj}<0.05$

Pathway analysis

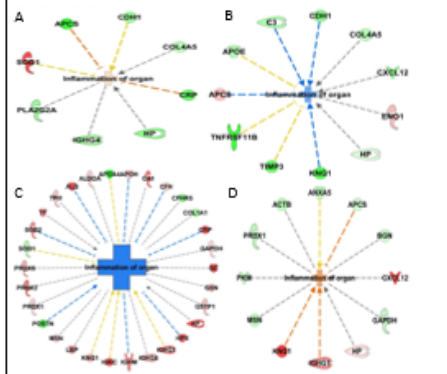


Figure 4. Predicted expression of 'Inflammation of organ' pathway in the superficial zone of young-old (A) and old-OA (C) groups; and in the deep zone of young-old (B) and old-OA (D)

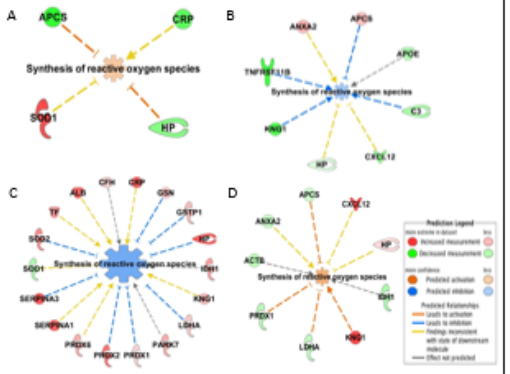


Figure 5. Predicted expression of 'Synthesis of reactive oxygen species' pathway in the superficial zone of young-old (A) and old-OA (C) groups; and in the deep zone of young-old (B) and old-OA (D)

Conclusions

- Stratifying different anatomical zones of articular cartilage demonstrated significant changes in the signalling pathways, including inflammatory, cellular death and reactive oxygen species pathways.
- The degradation of the matrix and ongoing inflammation in the synovium during OA could be the reason for substantial differences in the superficial zone, whereas the cartilage destructive processes during ageing could stem from the underlying subchondral bone.
- Comparison of old and OA groups by zone revealed a higher proportion of differentially abundant proteins in the superficial zone, however in ageing, this difference was observed in the deep zone.

Colin Purrington

nature photography / natural history / misc

https://colinpurrington.com/tips/poster-design

Designing conference posters

A one-sentence overview of the poster concept

A large-format poster is a big piece of paper or image on a wall-mounted monitor featuring a short title, an introduction to your burning question, an overview of your novel experimental approach, your amazing results in graphical form, some insightful discussion of aforementioned results, a listing of previously published articles that are important to your research, and some brief acknowledgement of the tremendous assistance and financial support conned from others — if all text is kept to a minimum (500-1000 words), a person could fully read your poster in 5-10 minutes.

Downloadable templates

Below are templates that can be used to make a meeting poster. Just download, adjust the dimensions (if you need to), and start typing. You can, of course, also change background color, text box color, font, etc. The templates are just starting points that can save you a few hours of fussing over the basics.

1. Horizontal template with results arena

DO. NOT. PUT. LOGOS. HERE.

Doing so crowds the title and visually distracts from important graphics. Put logo on your business card, not poster.

Title pitched at general audience that provides conclusion or at least hints at something interesting

Colin B. Purrington, Department of Posterology, Hudson University

DO NOT PUT LOGOS here, either.

Introduction

Three sentences max.

Persuade reader you have novel, interesting question(s) and hypothesis. Resist urge to use all the white space.

Materials and methods

Three sentences max.

If viewer truly wants to know gruesome details, they'll ask or email you. Sometimes adding a pic is good.

Results

Highlight your LARGE photographs, charts, maps, or in this central arena.

Don't include every graphic you've made that relates to project. Choose one. Or two. And separate graphics with plenty of white space.

If you have just one or two simple graphics, viewers will be drawn to explore them. If you have too many or they are too complicated, they will be repelled.

Annotate graphics with arrows and callout boxes so that viewer is **visually** led through how hypothesis is addressed. The goal is to enable viewers to understand the logic behind your conclusions *without you needing to be there.*

Keep font size of all text (even graph labels) as big or bigger than in rest of poster.

Conclusions

Explain why outcome is interesting. Don't assume it's obvious. Three sentences max.

Maybe include a sentence about what you plan to do next.

As for Introduction, don't feel like you need to fill the entire box.

I.e., if you retain a lot of white space you will attract more viewers. Seriously.

Literature cited

Author, J. 2012. Article title. *Journal of Something* 1:1-2.

Acknowledgments

Be brief.

Further information

Please see https://colinpurrington.com/tips/poster-design for more templates and tips. I'm at colinpurrington@gmail.com if you have a question or comment.

Title, formatted in sentence case (Not Title Case and NOT ALL CAPS), that hints at an interesting issue and/or methodology, doesn't spill onto a third line (ideally), and isn't hot pink

Colin Purrington
666 Teipai Street, Posterville, PA 19801, USA

Introduction

Congratulations: a reader was mildly intrigued by your title. Now you have 2-3 sentences to hook him/her into reading more by describing what your question was and why the answer might be of general interest. Gratuitous background information will cause them to walk away (if you're standing next to your poster, that can be awkward).

Typography research has shown that body text is easier to read if you use a serif font such as Times. But non-serif fonts are great for title, headings, figure legends, etc. Research also shows that fully justified text (this paragraph) is slightly harder to read even though it looks really cool.

Figure 1. A photograph in your introduction can help lure people to your otherwise non-photogenic research. If it's not your image, ask photographer for permission to use, and cite him/her.

Materials and methods

Few people, if any, really want to know the gruesome details of what you've been up to, so be brief. Use lightly-annotated photographs, drawings, or flow charts to visually convey your *general* experimental approach. To better engage viewers in your protocol or system, try attaching actual objects such as study organism (dead specimen), research gizmo, photo flip book, or a short movie (attach an old smartphone with Velcro).

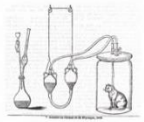


Figure 2. Hire an artist to illustrate the important step in your protocol. A photograph of you actually doing something might be nice, too. [image by John Snow 1853]

Results

The overall layout in this arena should be visually compelling, with clear cues on how a reader should travel through the components. Be creative. You might want a large map with inset graphs, or have questions on left with answers and supporting graphs on right. Be sure to separate figures from other figures by generous use of white space. When figures are too cramped, viewers get confused about which figures to read first and which legend goes with which figure.

If you can add small drawings or icons to your figures, those visual cues can be priceless aids in orienting viewers. And use colored arrows or callouts to focus attention on important parts of graphs. You can even put text annotations next to arrows to tell reader what's going on that's interesting in relation to the how the hypothesis is being evaluated. E.g., "This outlier was most likely caused by contamination when I sneezed into tube." Also, don't be afraid of using colored connector lines to show how one part of a figure relates to another figure. These tips might induce gasps for published manuscript, but posters can be more personal and thus better guide viewers.

Figures are preferred but tables are sometimes unavoidable, like death. But go to great efforts to make it look professional. Look in a respected journal and emulate the layout, line types, line thickness, text alignment, etc., exactly. Again, use colored text or arrows to draw attention to important parts of the table.

Paragraph format is fine, but so are bullet lists of results:

- 9 out of 12 brainectomized rats survived
- Brainectomized rats ate less
- Control rats completed maze faster, on average, than rats without brains

Do treatments differ in their effects?

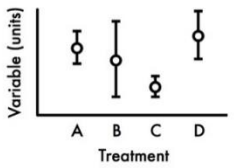


Figure 3. Legends can briefly describe the experiment, answer the question, and even include statistics if you so choose (unlike a manuscript figure legend).

Do As and Bs respond differently to X?

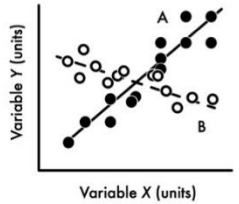


Figure 4. Label elements instead of relying on annoying keys that are default on most software. Add pictures of A and B if they are actually things (e.g., icons of rat with, without brain).

Are medians of treatment A and D different?

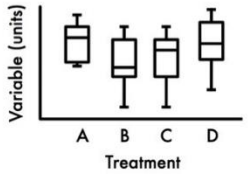


Figure 5. Don't be tempted to reduce font size in figure legends, axes labels, etc. This is because viewers are probably *most* interested in reading your figures and legends.

Conclusions

Conclusions should not be dry restatements of your results. You want to guide the reader through what you have *concluded* from results, and you need to state why those conclusions are interesting (i.e., don't assume reader will guess). These first several sentences should refer back to the burning issue mentioned in the introduction. If you didn't mention a burning issue in the introduction, fix that.

A good conclusion will also explain how your conclusions fit into the literature on the topic. E.g., how exactly does your research add to what is already published on the topic? It's important to be humble and generous in this section, partly because authors of previous literature may still be alive and even attending the conference. You can also display your appreciation of others' input by citing conversations you have had (with pers comms).

Finally, you want to tell readers who have lasted this long what might be done next and who should do it. E.g., are you currently taking the next logical step, or should another person with different skills follow up on your amazing result? It's OK to put a bit of personality into this ending because viewers expect posters to be personal (and if you're not actually standing there to convey your enthusiasm, your poster text should be doing that *for* you).

If you have a graphical way to express the next step of your hypothesis, by all means include it in this section. For example, you might make a graph with hypothetical data that shows an expected result in a future experiment. That's something you normally don't show in a traditional manuscript, but it's totally fine for a poster.

If you're curious, this poster has 683 words. Aim for 500 words. If you are above 1000 words, your poster will be annoyingly long to everyone except your collaborators.

A well designed poster retains plenty of white space separating edges of text boxes, graphics, and tables. You also want space between your text and edge of box. Without white space a poster will looked cramped and uninviting.

Literature cited

Bender, D.J., E.M Bayne, and R.M. Brigham. 1996. Lunar condition influences coyote (*Canis latrans*) howling. *American Midland Naturalist* 136:413-417.

Brooks, L.D. 1988. The evolution of recombination rates. Pages 87-105 in *The Evolution of Sex,* edited by R.E. Michod and B.R. Levin. Sinauer, Sunderland, MA.

Scott, E.C. 2005. *Evolution vs. Creationism: an Introduction*. University of California Press, Berkeley.

Society for the Study of Evolution. 2005. Statement on teaching evolution. < http://www.evolutionsociety.org/statements.html >. Accessed 2005 Aug 9.

Acknowledgments

We thank I. Güor for laboratory assistance, Mary Juana for seeds, and Herb Isside for greenhouse care. Funding for this project was provided by the Department of Thinkology. Note that people's titles are omitted (titles are TMI).

Further information

More tips (and templates) can be found at "Designing conference posters":

http://colinpurrington.com/tips/poster-design

OARDC OR EXTENSION IDENTIFICATION HERE

Title of the Research Study

Presenter name, Associates and Collaborators

INTRODUCTION

This editable template is in the most common poster size (48" x 36") and orientation (horizontal); check with the conference organizers for specific conference requirements regarding exact poster dimensions.

Writing Style:

The writing style for scientific posters should match the guidelines for the university. Use the Editorial Style Guide at **http://go.osu.edu/Vrg** for general guidance with academic titles, names of campus buildings, the correct way to refer to the campus, etc.

Copyright and Intellectual Property Guidelines

In today's world, just about everything is copyrighted, whether it carries the copyright symbol © or not. Moreover, under today's law, materials are protected by copyright as soon as they are completed. Copyright applies broadly to all creative pieces whether written on paper, sculpted in stone, found in cyberspace or created on videotape. Please visit **http://go.osu.edu/Vrh** for more information.

AIM

How to use this template

Highlight this text and replace it with new text from a Microsoft Word document or other text-editing program. The text size for body copy and headings and the typeface has been set for you. The text boxes and photo boxes may be resized, eliminated, or added as necessary. The references to the department, college and university, including the logo, should remain.

Head 3, to label the table below

[illegible]

METHODS

Text

Be sure to spell check all text and have trusted colleagues proofread the poster. In general, authors should:

- **Use the active tense**
- **Simplify text by using bullet points**
- **Use colored graphs and charts**
- **Use bold to provide emphasis; avoid capitals and underlining**
- **Avoid long numerical tables**

Authors should re-write their paper so that it is suitable for the brevity of the poster format. Respect your audience. As a general rule, less is more. Use a generous amount of white space to separate elements and avoid data overkill. Refer to Web sites or other sources to provide a more in-depth understanding of the research.

Head 3, to label the table below

[illegible]

Head 3, to label the table below

RESULTS

Images

Images must be 72 to 100 dpi in their final size, or use a rule of thumb of 2 to 4 megabytes of uncompressed .tif file per square foot of image. For instance, a 3x5 photo that will be 6x10 in size on the final poster should be scanned at 200 dpi.

We prefer that you import tif or jpg images into PowerPoint. Generally, if you double click on an image to open it in Microsoft Photo Editor, and it tells you the image is too large, then it is too large for PowerPoint to handle too. We find that images 1200x1600 pixels or smaller work very well. Very large images may show on your screen but PowerPoint cannot print them.

Preview

To see your in poster in actual size, go to view-zoom-100%. Posters to be printed at 200% need to be viewed at 200%.

Printing and Laminating

CommTech Printing Services can print and laminate your research poster. To place your order, contact us by phone at 330-202-3508 or send an e-mail to **warren.119@osu.edu**. Plan ahead; allow at least seven business days for Printing Services to complete the order. Other dimensions are available; the charge is by square foot. Contact Printing Services for specific pricing information.

Head 3, to label the table below

CONCLUSIONS

We have created this template with scientific researchers in mind. We encourage any comments or suggestions so that we can continue to update and improve this template. E-mail **brown.3384@osu.edu** with suggestions.

BIBLIOGRAPHY

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4. [illegible]
5. [illegible]
6. [illegible]

ACKNOWLEDGEMENTS

Check to make sure you've acknowledged partner and funding agencies, either with text or with their logos.

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Useful Links

https://colinpurrington.com/tips/poster-design

https://lantsandlaminins.com/scientific-posters/

https://www.the-scientist.com/careers/poster-perfect-42000

Any Questions?

janders@liverpool.ac.uk