Physically Functionalised Gellan Gum Hydrogels for Neural Cell Culture Applications

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Introduction

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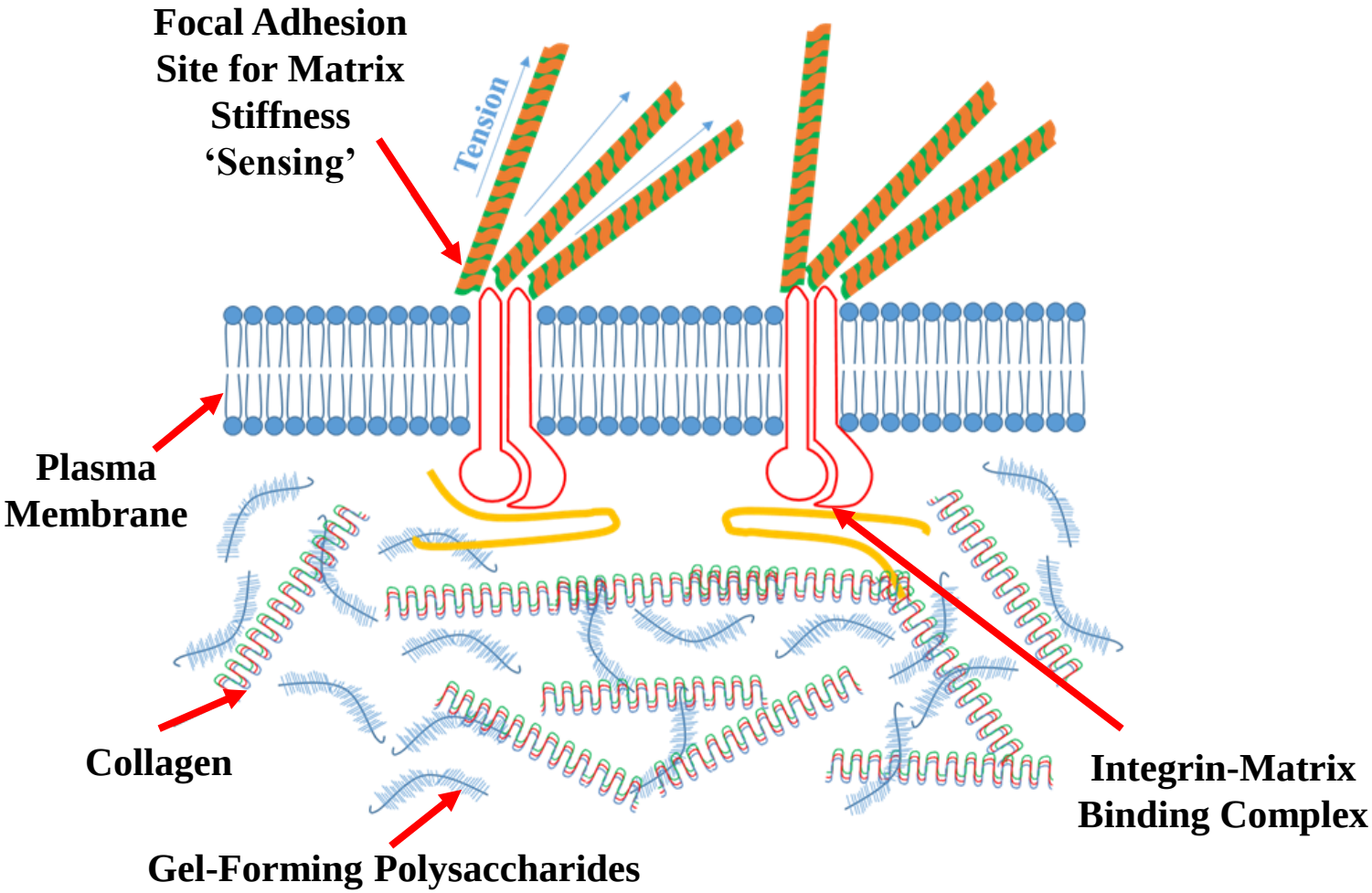
- Dementia is now the leading cause of death in England and Wales
- Consequently, research into probing the mechanisms behind dementia using neural cell culture is widespread
- Progress has been made but is often limited by an inability to accurately model the disease *in vitro* as current methods often employ ‘classic’ 2D monolayer culture on plastic surfaces
- Such strategies are high throughput but don’t allow for accurate replication of the soft, complex microenvironment in which neurodegenerative diseases progress
- This can have a profound effect on cell behaviour and inhibit the ability to replicate disease pathologies and interrogate the underlying mechanisms

The Extracellular Matrix



Replicating the ECM *in vitro*

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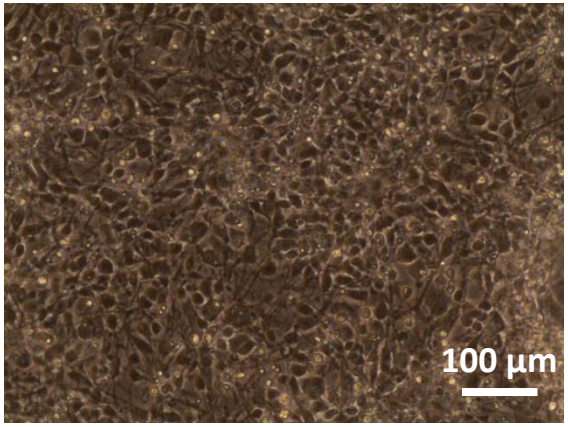
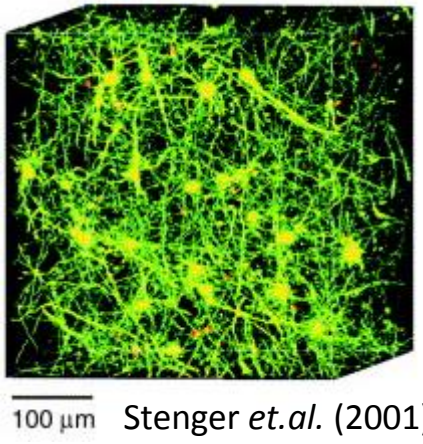
- To overcome issues with monolayer culture, groups often culture cells using *in vitro* 3D cell niches
- Hydrogels are popular tools for this application due to a high water content, low cytotoxicity and ability to replicate microstructural elements of native ECM
- Many food hydrocolloids have been investigated for such applications (gellan gum, alginate, chitosan etc.)





Stenger *et.al.* (2001)

Collagen-Gellan Blended Hydrogels for Neural Culture

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- Brain ECM is mostly comprised of linear, unbranched, gel-forming polysaccharides
- Fibrous proteins penetrate the polysaccharide network allowing for cell-matrix binding
- Blended hydrogels of gellan gum and collagen are being investigated as a simple method to replicate this microenvironment

Collagen-Gellan Blended Hydrogels for Neural Culture

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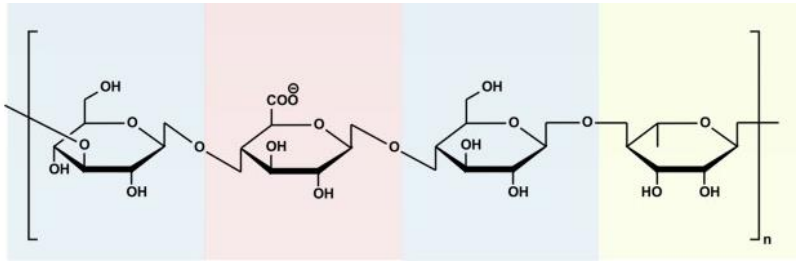
Gellan Gum

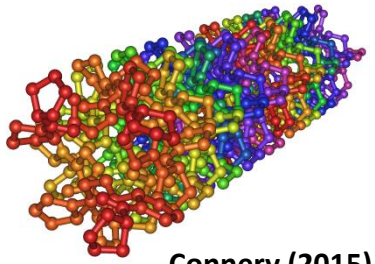
- Linear, unbranched polysaccharide of bacterial origin
- Capable of forming porous hydrogels through ionic interactions
- Could replicate the polysaccharide network in brain ECM



Osmalek *et. al.* (2014)

Collagen

- Fibrous, helical protein found in native ECM
- Contains cellular integrin binding domains
- Could replicate the fibrous element of brain ECM responsible for cell attachment



Connery (2015)

Fabrication of Blended Gels

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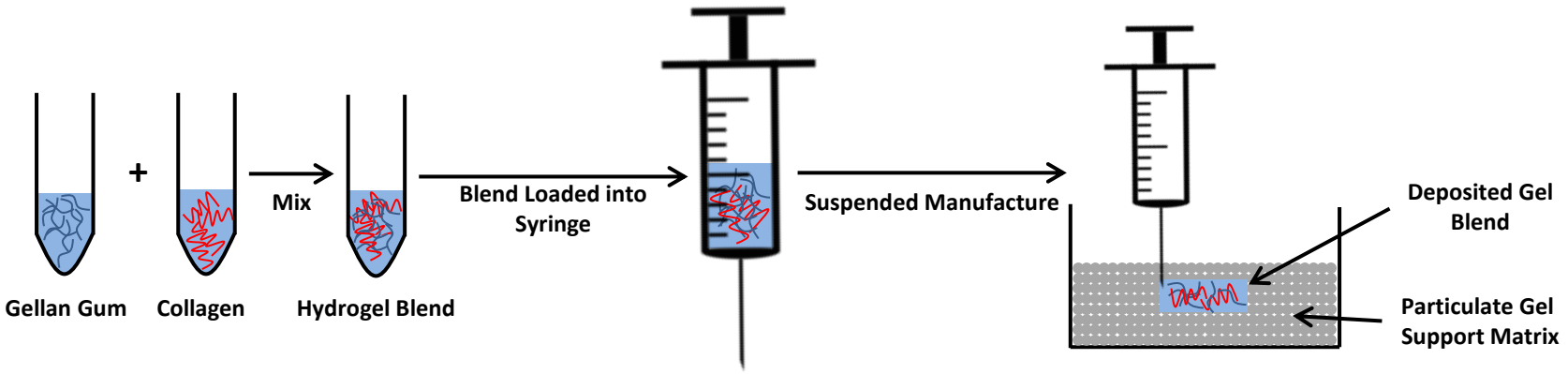

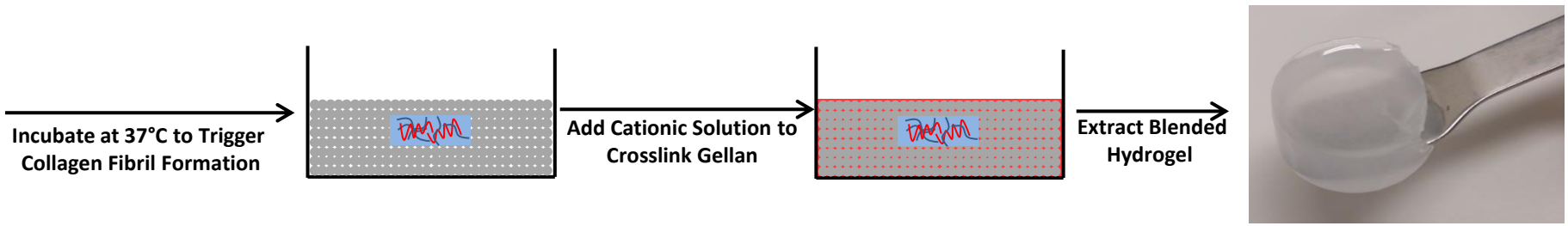

Proof of Concept

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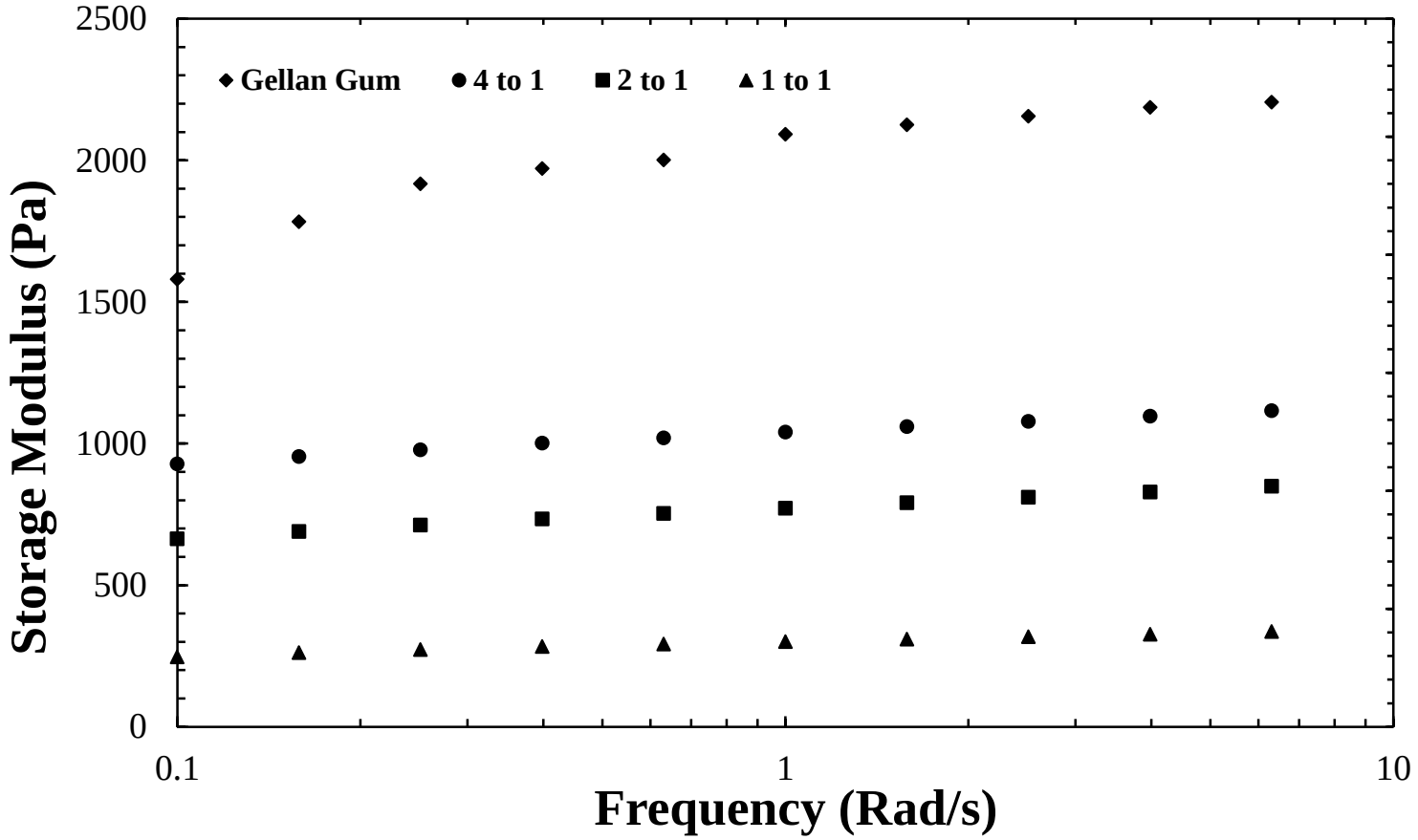
- As an initial proof of concept 3 hydrogel blends were prepared with varying gellan:collagen ratios (1:1, 2:1 and 4:1)
- Both polymers were dispersed at a concentration of 0.5% w/w prior to blending
- The final blends were suspended in particulate gel beds of 0.5% agarose
- Frequency sweeps were conducted to determine the mechanical properties of each blend
- SH-SY5Y neuroblastoma cells were also encapsulated in each blend and cultured for 21 days before a live/dead assay was used to determine cell viability and morphology

Rheology

- The storage modulus of brain ECM is reported as being between 0.1 kPa and 1 kPa
- All blends exhibited moduli within this range at low oscillatory frequencies



Live/Dead Assay

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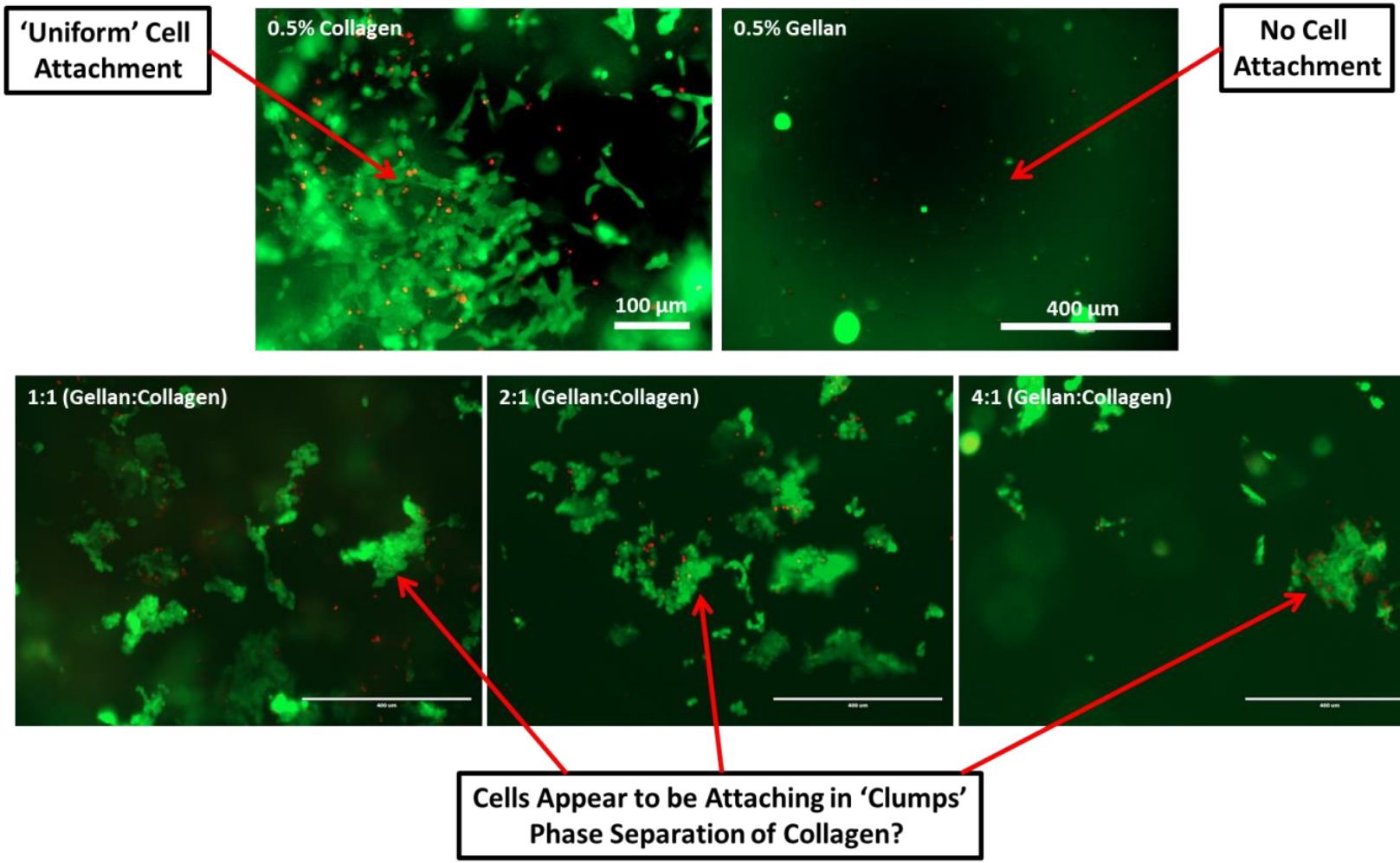

Optimising the Microenvironment

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- Live/dead images suggested incorporation of collagen improved cell attachment, proving the concept of functionalising gellan with collagen fibres

- However, cells seemed to adhere in clumps suggesting fibres did not penetrate ubiquitously through the gellan network

- Perhaps gelation times were an issue?

Optimising the Microenvironment

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- 2:1 and 4:1 ratios of gellan and collagen were prepared suspended in particulate gel beds
- Blends were then incubated at 37°C for 3 different time periods (1 hour, 3 hours and 6 hours) before addition of 100 mM $CaCl_2$
- This allowed the collagenous component to organise into fibrils for different lengths of time prior to gelation of gellan
- Samples were then freeze-dried and analysed with SEM to analyse the resulting gel networks

SEM Results – 0.5% Gellan

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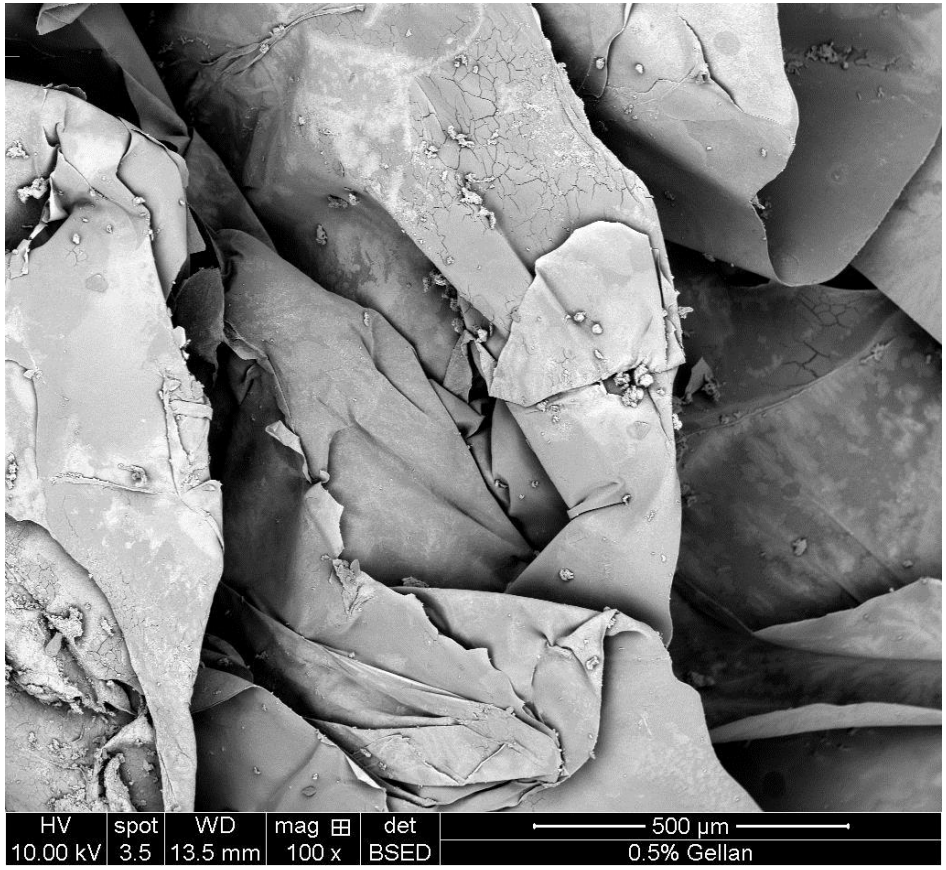

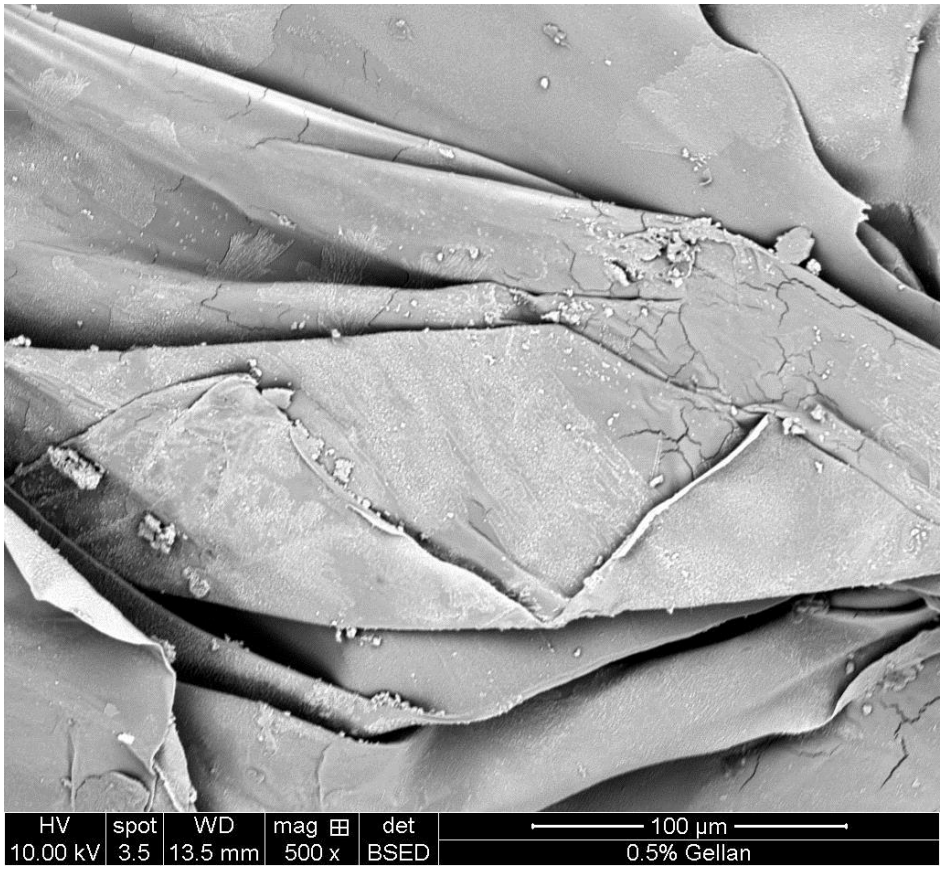

SEM Results – 0.5% Collagen

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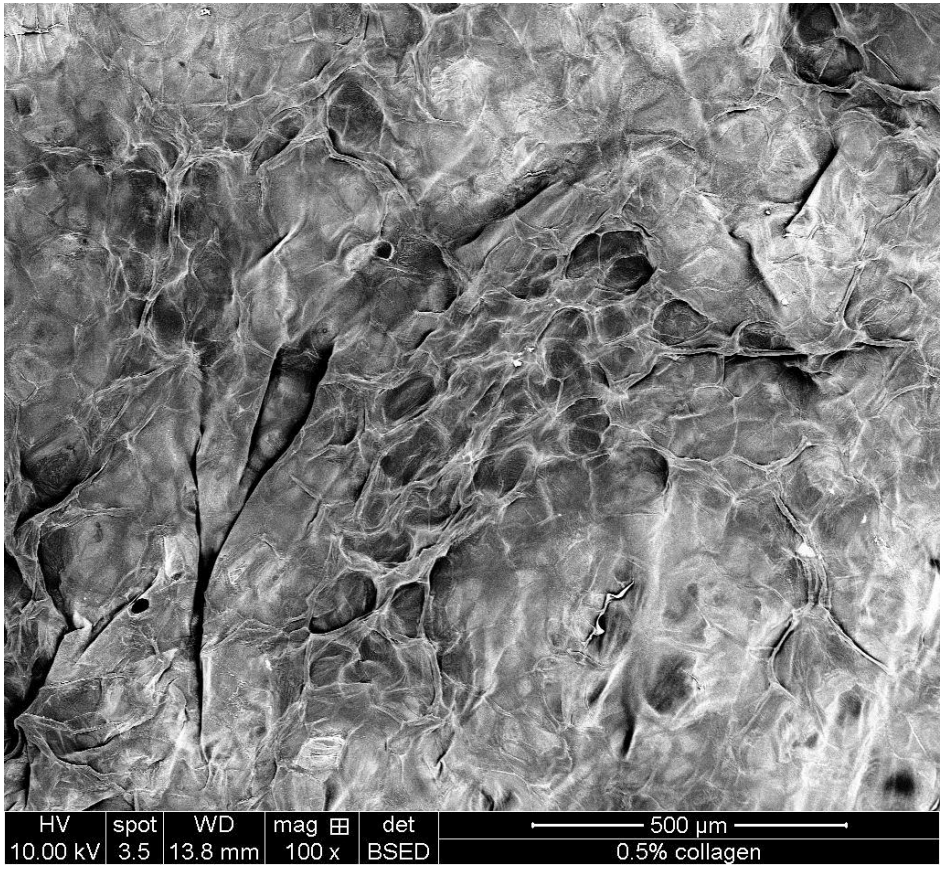

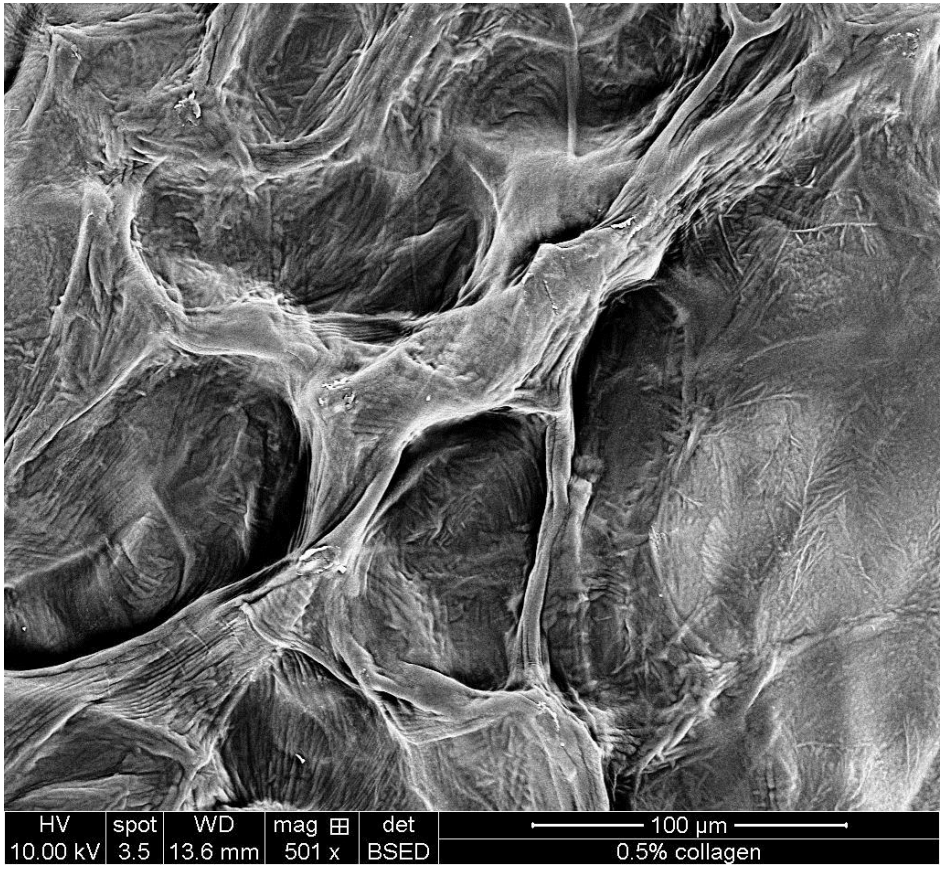

SEM Results – 4:1 (Gellan:Collagen)

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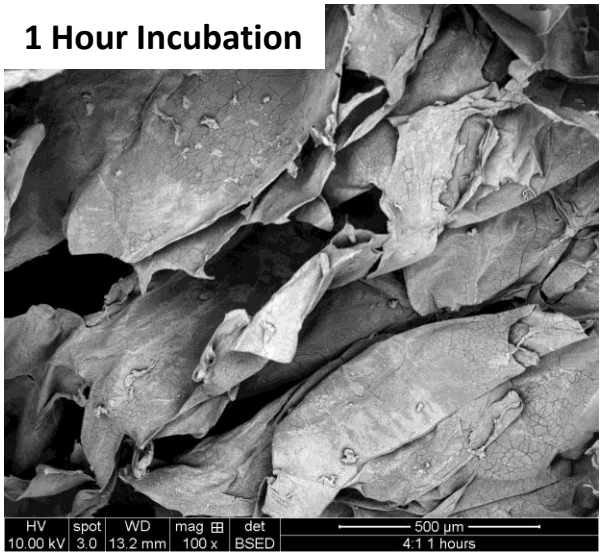
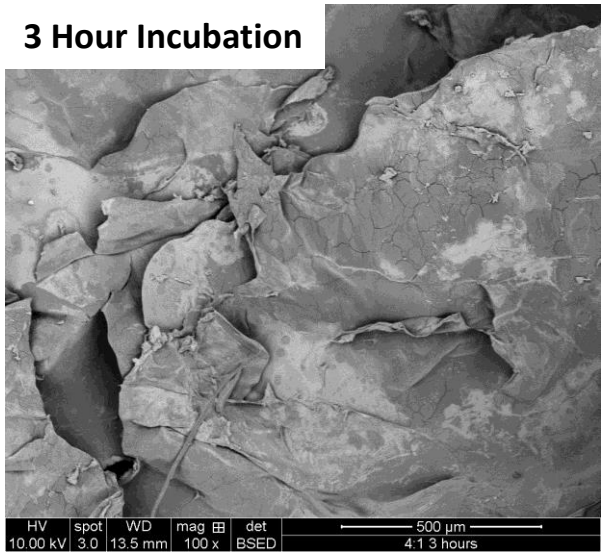
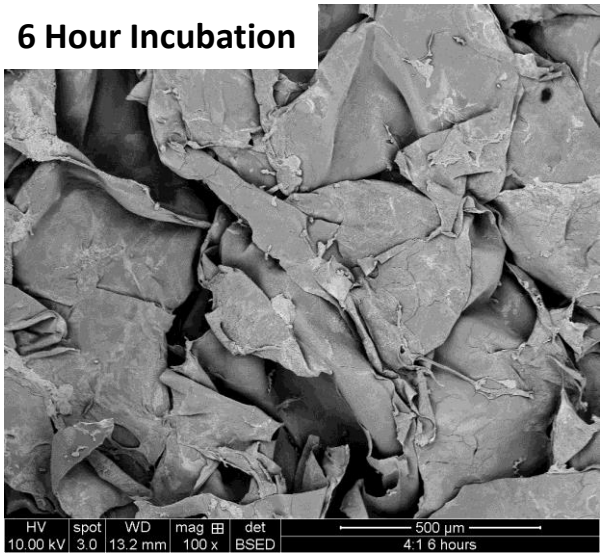
1 Hour Incubation



3 Hour Incubation



6 Hour Incubation



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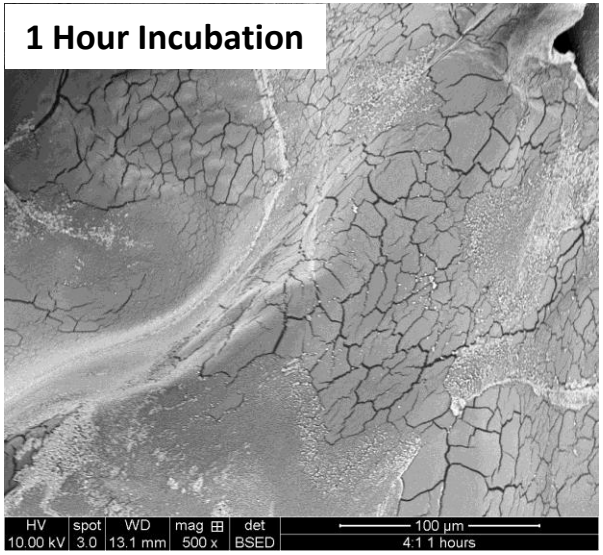

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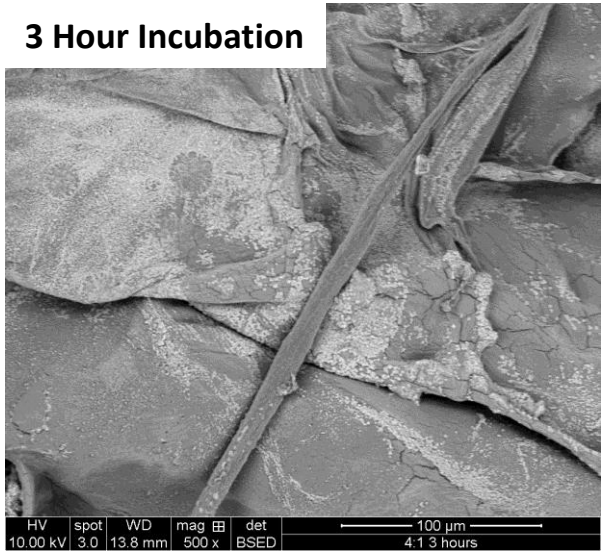

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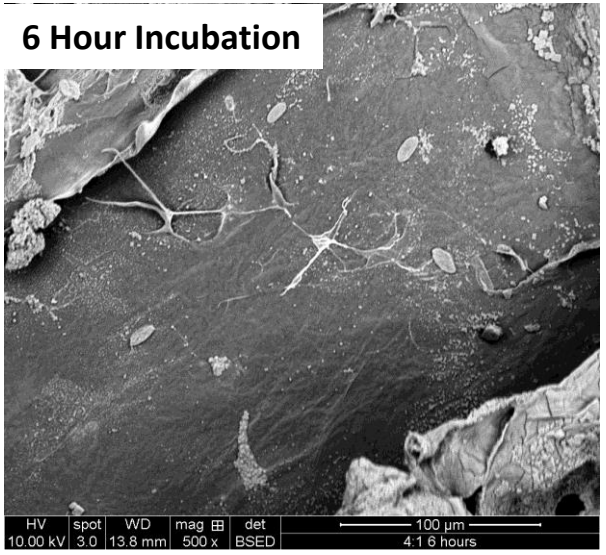

SEM Results – 2:1 (Gellan:Collagen)

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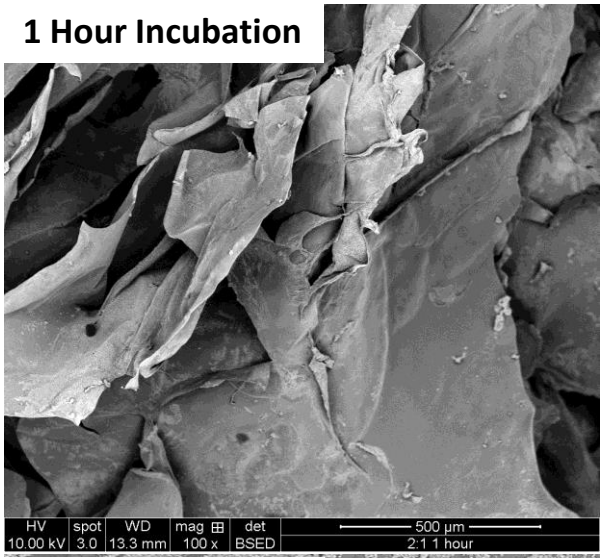
1 Hour Incubation



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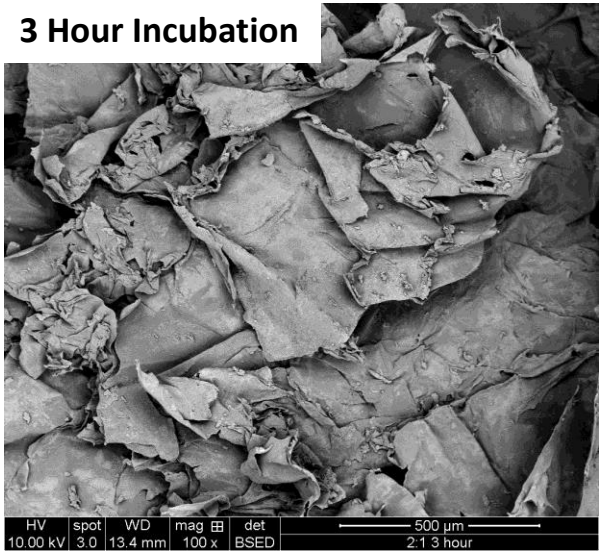

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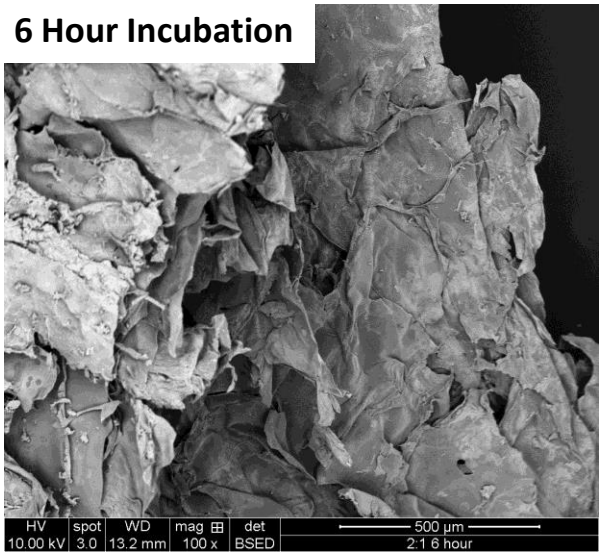

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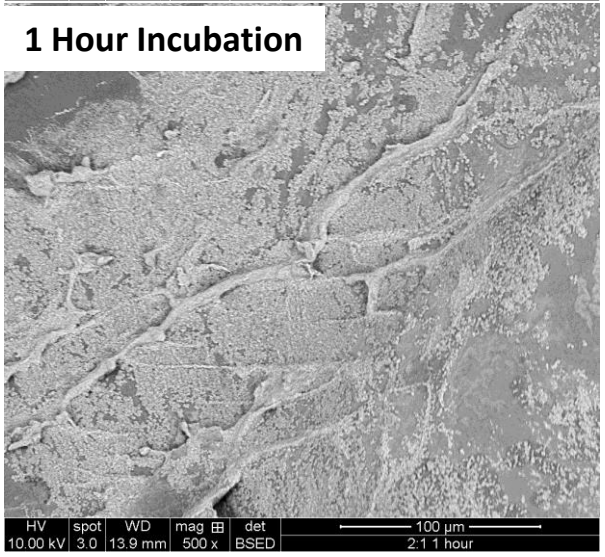

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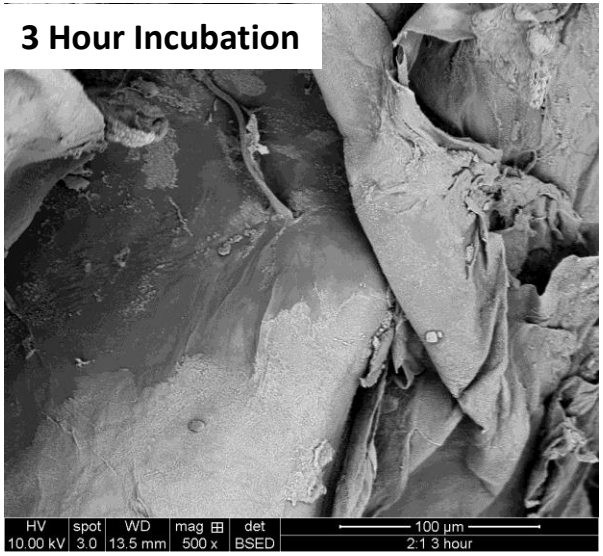

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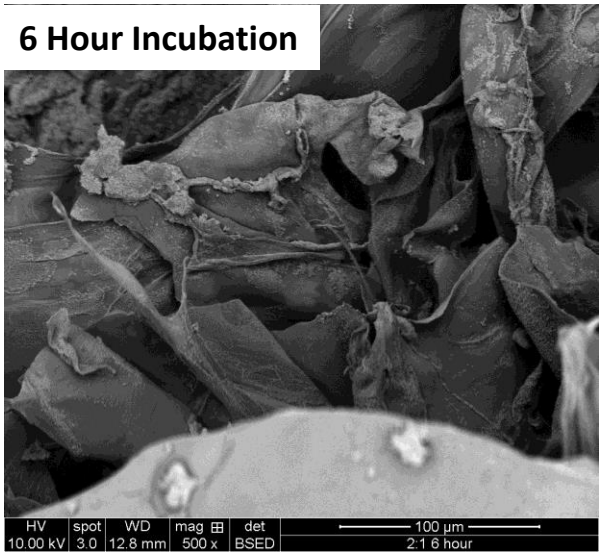

Current Conclusions/On-Going Work

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- Blending 0.5% gellan with 0.5% collagen creates a soft matrix that can better reflect the mechanical properties of brain ECM.
- Live/dead results suggests cells can adhere to blended gels but not to un-modified gellan. Further optimisation is required to achieve more uniform cell adhesion within the whole matrix.
- SEM images could potentially demonstrate increasing incubation time prior to gellan crosslinking results in better formation of collagen fibrils within the matrix but this is difficult to quantify.
- The proof may be in the cell behaviour with the effect of 1, 3 and 6 hour incubation on actin expression now being investigated. This could give us a more quantitative analysis of how cell adhesion can be optimised in the blended gels.
- Once optimised, blended gels will be evaluated for neural cell culture using iPSC-derived neurons.

Acknowledgements

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