Discovering the ideal hydrogel for neural cell culture

F028130 – 22CGC054_CW3

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Word count: 4474

Contents

Abstract

Hydrogel use is very important in bioengineering due to its various applications. It has great flexibility and are generally chemically neutral and unreactive. Moreover, they mostly consist of water and break into nontoxic molecules. They're soft and flexible; these qualities can be controlled by adjusting the hydrogel by implementing different amounts of molecules. Hydrogels are very biocompatible with all types of cells and can be further modified to cater for its need.

Neural cell culture is also very important in bioengineering. The study of neural cell culture is essential to unravel the intricacies of the nervous system. Research in the field improves the understandings of neurotoxicity, regenerative medicine and general neuronal development. The capabilities of neural regeneration are vast. Scientists and researchers hope to achieve advanced knowledge in neuroscience to eventually cure neurological diseases like Parkinson's.

This report aims to assess the current research in hydrogels and neural cell culture for neuronal regeneration, providing an overview of advancements, challenges and further research in this field. The results from the findings will be compared to previous studies conducted and evaluated.

Introduction

Hydrogels

Hydrogels are a type of polymer that can swell and retain a lot of water [1]. They are very flexible due to its water content and tend to remain unreactive. They make for incredible carriers as they are biocompatible, biodegradable, absorb and retain water, and can cater to other desired physical properties e.g., very elastic and strong or soft and viscous.

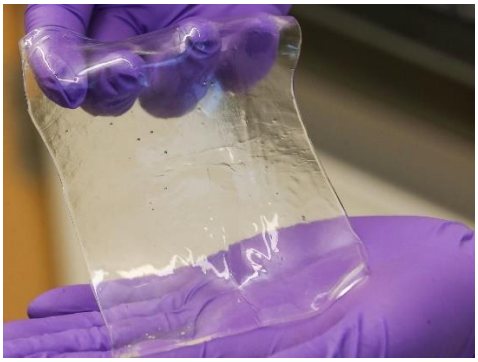

Figure 1 Synthetic hydrogel [2]

Hydrogels consist of several monomers. These are HEMA, HEEMA, HDEEMA, MEMA, MEEMA, MDEEMA, EDGMA, NVP, MA and Vac [3]. One of the methods of preparing hydrogels is by cross-linking. The reaction occurs in aqueous solution. In this, organic hydroxyl groups are used as the cross-linking agent. The formula for the cross-linking ratio, defined as X, is $\bar{M}_c = \frac{M_r}{2X}$ where M_r is the molecular weight of the repeating unit and $\bar{M}_c$ is the average molecular weight between cross-links. The assumption made in this equation is that all cross-linking agent reacts with the polymer.

There are several other methods of preparing hydrogels. This is dependent on how it is going to be used and what mechanical and material properties are wanted. Despite these many potentials of hydrogels, there are still a few challenges to be addressed. Especially in the preparations of them, it is still difficult to get the exact preferred qualities through synthesis and cross-linking. Aside from this, there are still so many fields of research to be explored in hydrogels.

Neural cells and culture

Neural stem cells are multipotent stem cells in the neural system, derived from embryonic stem cells, that can differentiate into neurons, oligodendrocytes and astrocytes [4]. They're located in the subventricular zone or the hippocampus of the human brain.

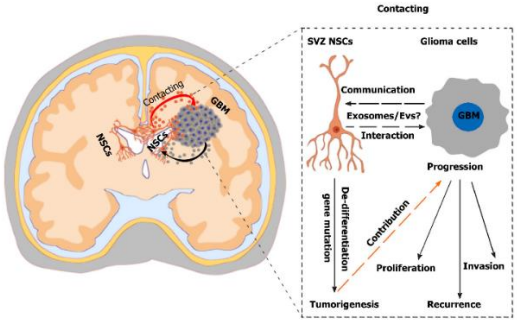

Figure 2 Diagram of the subventricular zone [5]

The process of neuronal cell culture is the preservation of living neurons that have been extracted from animal nervous system tissue (in vivo), from the brain or spinal cord, in an artificial laboratory environment (in vitro) to enable their maintenance and growth [6]. The extracted cells are given nutrients and vitamins for efficient growth. The atmosphere is also controlled for its efficiency, including humidity, temperature etc. Under the right conditions, the cells mature and develop further. However, it should be known that primary neurons in culture do not proliferate; they don't undergo cell division (mitosis). Hence, they're considered to be non-proliferating cells.

The steps of neural cell division from the central nervous system, are as follows. Firstly, the cells proliferate [7]. They form a cluster of cells and then dissociate from one another. Some of these cells then differentiate and specialise into neurons. They again accumulate into a cluster, dissociate and finally differentiate. This process is repeated several times, whilst the specialised cells are filtered out of the medium.

Neural cells are generally very soft and viscous. This means that it's difficult to carry them as they are. Hence the concept of utilizing hydrogels came about. The proposed idea is to transfer the neural cell culture into hydrogels and deposit the hydrogel into the brain or other desired site. Since hydrogels are biodegradable, there aren't too many concerns with it causing obstructions to other natural processes in the body. Neural cells consist of a cell body containing a nucleus, endoplasmic reticulum, ribosomes, Golgi apparatus, mitochondria, and other organelles that are important for its function [8].

In healthcare, neural cell culture is heavily researched in the hopes of curing neurological diseases like Parkinson's disease. In the field of biotechnology, neural cell culture is researched for developing neural cell implants and related medical devices. As known, the brain is very weak and fragile, yet any damage can be detrimental to the person's wellbeing and health. Hence, it's vital to learn and investigate further about neural cells and neurology.

Research objectives

There are a number of aims for the research of this topic. Firstly, how to synthesize a hydrogel to cater for neural cell culture. Its properties must be biocompatible with neural tissue, and it should be able to carry them and support their growth without too much issue. The properties of the hydrogel can also influence the neural cells which is another factor to consider. The hydrogel must be able to carry growth factors and other nutrients that will support the neural cells' growth. And above all, the neural cells should be able to survive in the conditions and be able to be deposited into the desired region and work appropriately.

Questions to be answered and explored include how hydrogels influence neural cell characteristics and growth, how will hydrogels deposit the neural cells, what nutrients can even be integrated into the hydrogels in order to support the culture of neural cells. The most important question is, are hydrogels the best of transporting neural cells?

Methodology

In this section, the protocols and procedures for preparing the hydrogels and neural cell culture will be discussed. The study involved a series of experiments to investigate the properties of hydrogels for ideal compatibility with neural cells. Analytical techniques will also be explored and broken down.

It should be made aware that all the procedures and analytical techniques will be taken from Jordan Roe [9], a former PhD student from Loughborough University.

Preparing a hydrogel to cater for neural cell culture

In designing a hydrogel, an appropriate monomer, cross-linking agent and method of synthesis must be selected. Firstly, the material properties must cater for the target neural tissues. It must have suitable nutrients for the growth and proliferation of the neural cells. The hydrogel should be applicable for clinical practice; it should be easy to use. And finally, reproducibility should be considered. There should be some possibility to repeat the procedures and manufacture the hydrogels for industrial use.

The study's objective is to create a material much like a living scaffold to support neurons for clinical use. The ideal hydrogel would mimic neural tissue material properties. It would be both biocompatible and biodegradable, degrading into non-toxic products. In this, porosity is also considered to achieve cell migration.

Polymerisation techniques

There are several methods of synthesising hydrogels. The majority of these techniques require crosslinking, which can be done physically or chemically. Ionic interactions, genetically modified protein sequences, and hydrogen bonds all work together to physically crosslink gels. In contrast, the chemical addition reactions, particularly for aldehydes, along with condensation reactions, free-radical polymerisation and enzymatic actions are all used in chemically crosslinking gels. There are also biochemical hydrogels [10]. They utilize biological agents (e.g., enzymes or amino acids) in the gelation processes.

In research, chemically crosslinking gels are preferred as their mechanical properties can be controlled since it's affected by the crosslinking density. This also affects the porosity; therefore, the swelling of the hydrogel and biocompatibility are also controlled. However, porosity should be carefully monitored as they must prevent the early migration of cells and nutrients.

After considering all the factors of hydrogel synthesis, photoinitiated free radical polymerisation was used in the study to produce hydrogels. It should be noted that they were modified further with magnetic nanoparticles later in the study. With the use of ultraviolet or visible light, the monomers polymerised with the crosslinking agents (in a solution). Free radicals were seen due to the photoinitiation that took place. Each molecule responded to differently to the different parts of the absorption spectrum. The photons emitted from the light source excited or dissociated the photo-initiator molecules into high-energy free radicals. This started the polymerisation of the molecules. The product was a 3D crosslinked network inside the vessel that was used to contain the solution.

However, due to the free radicals, the solution had some level of cytotoxicity. Some additional cell-characterization techniques were necessary to ensure biocompatibility as UV radiation poses considerable risks to cells. One potential idea to prevent this is by using the UV lamp at low intensity. The hydrogels can also be sterilized before the cell culture to remove any residual photo-initiators and other harmful compounds.

The ideal monomer

As mentioned earlier, there are several monomers used to synthesize hydrogels. Each monomer has different characteristics and properties to one another. Figure 3 shows some of the common monomers used in the preparation of hydrogels.

Table 1
MONOMERS FOR PREPARATION OF HYDROGELS

Monomer	Name	Abbreviation	Chemical structure
I	Hydroxyethyl methacrylate	HEMA	$CH_2=C(CH_3)COOCH_2CH_2OH$
II	Hydroxyethoxyethyl methacrylate	HEEMA	$CH_2=C(CH_3)COOCH_2CH_2OCH_2CH_2OH$
III	Hydroxydiethoxyethyl methacrylate	HDEEMA	$CH_2=C(CH_3)COOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$
IV	Methoxyethyl methacrylate	MEMA	$CH_2=C(CH_3)COOCH_2CH_2OCH_3$
V	Methoxyethoxyethyl methacrylate	MEEMA	$CH_2=C(CH_3)COOCH_2CH_2OCH_2CH_2OCH_3$
VI	Methoxydiethoxyethyl methacrylate	MDEEMA	$CH_2=C(CH_3)COOCH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$
VII	Ethylene glycol dimethacrylate	EGDMA	$CH_2=C(CH_3)COOCH_2CH_2OOCC(CH_3)=CH_2$
VIII	*N*-vinyl-2-pyrrolidone	NVP	$CH_2=CHNCOCH_2CH_2CH_2$
IX	Methacrylic acid	MA	$CH_2=C(CH_3)COOH$
X	Vinyl acetate	VAc	$CH_2=CHCOOCH_3$

Figure 3 Monomers used for preparing hydrogels [3]

There are a few categories for the monomers used in hydrogel synthesis. They can be classified as homopolymers, copolymers, semi-interpreting network (semi-IPN) and interpreting network (IPN) [10]. Homopolymers only contain one type of monomer in their polymeric chain. Copolymers have two monomers where at least one of them are hydrophilic. Semi-IPNs form when a polymer penetrates a crosslinking agent (without forming any further chemical bonds). IPNs form with the collision of two polymers.

The study resolved that the use PHPMA and PEGMA hydrogels were the most ideal for the experiment. PHPMA, also known as poly[N-(2-hydroxypropyl) methacrylamide], is a heterogeneous hydrogel [11]. It has a porous structure and a large surface area, ideal for cell interactions. Moreover, its viscoelastic properties are similar to that of neural tissue. A different study concluded that PHPMA hydrogels had the ability to promote tissue repair and axonal regeneration in rat spinal cord. The hydrogel showed to aid in cell growth, angiogenesis and axonal growth.

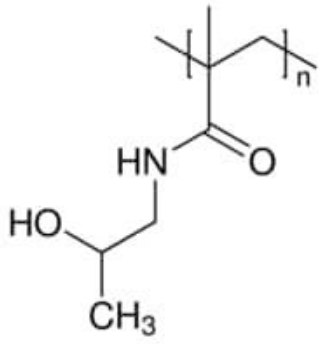

Figure 4 Skeletal structure of PHMA [12]

PEGMA is known as monoacrylated poly (ethylene glycol). The PEG backbone alone doesn't support cell attachment, but integrated with biological ligands, the level of cell interactions can be controlled [13]. PEG networks are formed using radiation crosslinking.

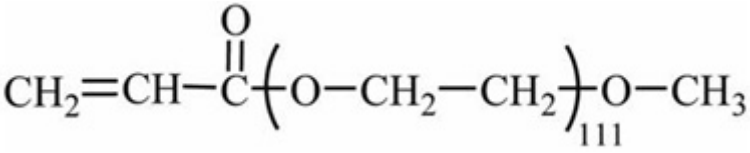

Figure 5 Skeletal structure of PEGMA [13]

Synthesis of PHPMA and PEGMA hydrogels

The synthesis of the hydrogels started with the preparation of 2-hydroxypropyl methacrylate (HPMA) monomer [9]. 7.5mL of 0.097 mol 1-amino-2-propanol was added to 90mL dichloromethane solvent in a 250mL flask with a magnetic stirrer. The temperature was set to -5°C and the acid, 0.04mol

methacryloyl chloride was added to the mixture in drops of 4.6mL; dichloromethane was also added as drops of 10mL to the mixture. The mixture was stirred overnight with the magnetic stirrer. The product was filtered to remove 1-amino-2-propanol hydrochloride, and then recrystallized at -18°C to produce the desired HPMA monomers.

PHPMA and PEGMA hydrogels were synthesised through the free radical polymerisation of HPMA monomers in a nitrogen atmosphere. In this, the photoinitator was 0.2mg of Irgacure 184. The resultant liquid was cured with UV light for 2 hours at room temperature.

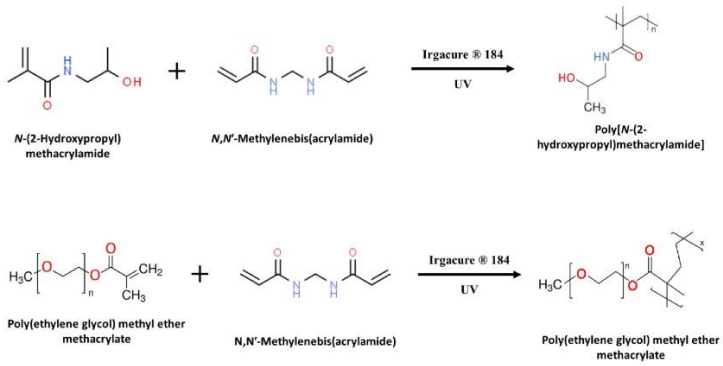

Figure 6 The reactions forming PHPMA and PEGMA hydrogels respectively [9]

Neural cell culture

The primary objective of neural cell culture is to preserve and develop living neurons that have been isolated from animal nervous system tissue (in vivo), such as the brain or spinal cord, in a synthetic laboratory setting (in vitro). There are two types of cells in the nervous system: nerve cells and glial cells. Nerve cells are concerned with the functions of communication within the body, while glial cells are more for support.

Before the procedure is explained, aseptic techniques should be investigated to prevent any contamination. The cell culture was done in a class II laminar flow cabinet. All equipment was sterilised prior to the experiment with either ethanol or by using an autoclave machine (for larger apparatus). The hydrogels were sterilised with deionised water and then sterilised further using UV. The cell cultures were sterilised with ethanol.

Cell culture procedure

Firstly, the growth medium used was Dulbecco's Modified Eagle's Medium (DMEM), supported with fetal bovine serum (FBS). FBS is the most employed serum due to its high levels of growth factors and gamma globulin [14]. It's considered the most ideal serum for promoting cell growth. DMEM is also a promising media for the growth of various cells including smooth muscle cells, glial cells, fibroblasts and most relevant, nerve cells [15].

Penicillin-streptomycin was also added to DMEM which provides further protection against microbial infections. L-glutamine was also added to the mixture in the form of GlutaMAX™ [16]. This is an amino acid that aids in cell proliferation. The use of GlutaMAX™ instead of L-glutamine directly meant that there was a slow release of L-glutamine. This is to prevent a large build-up of toxic ammonia hence the process was more controlled.

The DMEM solution naturally contained phenol red, a pH indicator. With the natural release of lactic acid from cells, visible colour change was observed, from red to orange/yellow. The produced lactic acid is either removed or converted to glucose through natural cycles.

In the study, SH-SY5Y cells were used. This is a cell line derived from SK-NH-SH neuroblastoma cell line which was first established in 1970 to be from human bone marrow [17]. The cells were cryopreserved and taken out when required. The cryovials were stored at -80°C and then placed in liquid nitrogen storage.

The cells were sub-cultured to maintain high proliferation. In this, the growth media was aspirated, and the apparatus were sterilised with phosphate buffer saline (PBS) to remove residual growth media. The flask was put into an incubator at standard conditions to allow cell detachment. The solution then underwent centrifugation to form cell pellets. The cells were resuspended in growth media before analysis took place.

Cell culture analysis

The cells were counted using a haemocytometer. In this, the suspension was mixed with trypan blue stain to highlight the cells. The cells wouldn't absorb trypan blue as such they appeared as white circles under a light microscope. The cells viable for the counting had to appear bright and within the borders of the quadrant. Since there are 4 quadrants, the total cell count was divided by 4 to find the mean average cell.

In the study, a LIVE/DEAD™ cell imaging kit was used to distinguish between live cells and dead cells by measuring cytotoxicity and cell viability (by assessing esterase activity within the cell and plasma membrane integrity) [18]. This is done through two-colour fluorescence that is part of the kit. A solution of Calcein AM dye and Ethidium Homodimer-1 (EthD-1) was then used to further distinguish the live cells from the dead cells. Fluorescent images were taken to identify the viable cells which appeared as green in the screening.

Cell culture in hydrogels

The PHPMA and PEGMA hydrogels were modified to optimise cell adhesion. A thin crevice was created to facilitate the cell suspension and increase its interactions with the gel. The cells were directed to the walls of pores as they had larger surface area. Proliferation and cell attachment was encouraged in these regions.

PEGMA hydrogels were cultured with SH-SY5Y cells for 4 days. A large population of cells were observed, occupying the hydrogel surface. The cells were spherical and suspended. Results showed signs of cell proliferation. UV treatment was used to prevent bacterial infections and sterilise the suspension. However, one result showed the growth of fungal spores which can be resistant to UV. It was suggested that a fungicide could potentially be added to the medium to prevent the occurrence of the spores.

Results

The initial examination of the study showed the presence of apoptotic and necrotic cells around the hydrogels. The conclusive analysis determined that the reduction of cell viability was linked to the presence of the hydrogels. A further investigation was conducted to remove the hydrogel material through dialysis. After this, the SH-SY5Y cells were shown as a monolayer culture. It was suggested that the spherical cells initially observed were unfavoured to the cell growth environment. After longer conductions of dialysis, larger cell populations were discovered. However, the results attained were unsatisfactory.

To increase the number of adherent cells, the idea to remove the dense outer layer of hydrogels was investigated. A reduction in monomer and crosslink density was also introduced, while increasing the nutrient-rich solvent part of the solution. One of the new batches of hydrogels showed greater promise than the previous one in cell attachment and growth. The resultant conclusion was that the second of three batches of the PHPMA hydrogel was the greatest candidate for neural cell culture.

Neural cell viability in the PHPMA hydrogel

The results showed that PHPMA hydrogels were quite promising for neural cell attachment and growth as opposed to PEGMA hydrogels. In particular, the PHPMA hydrogel that was modified to have lower surface density, lower monomer and crosslink density and a larger solvent volume. In this section, "PHPMA F2" from Jordan Roe's thesis will be explored in detail [9].

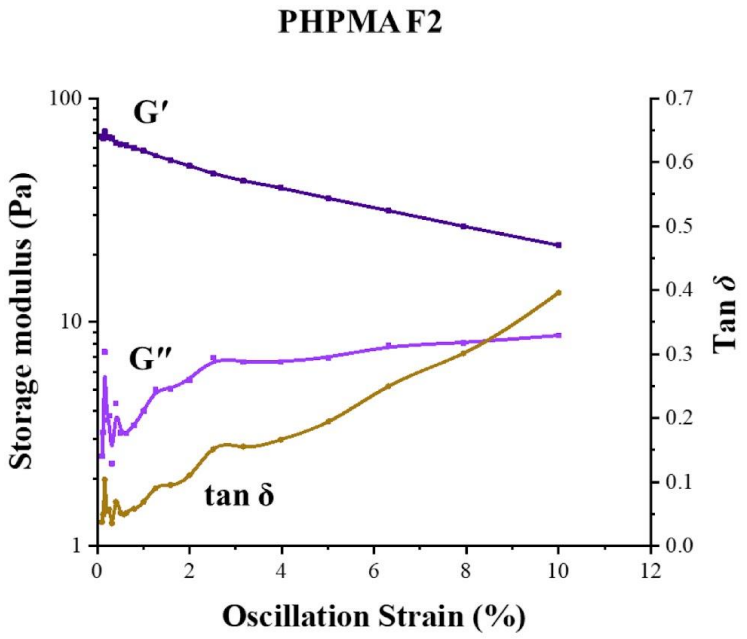

Figure 7 Oscillatory rheological test for the subject hydrogel measuring the elastic modulus (G'), viscous modulus (G") and loss tangent (tanδ) [9]

The main testing done to the hydrogel was related to stress and strain, and rheology. In rheology, hydrogels are investigated to determine their characteristics [19]. It assesses whether a hydrogel is more like a solution or like a gel. Therefore, factors like elasticity and viscosity are analysed.

The storage modulus refers to the ability to elastically store energy [20]. The higher the storage modulus, the more difficult it is to break down the polymer. Therefore, ideally in this scenario, the storage modulus should remain relatively low.

The loss tangent (tan δ) is the ratio of energy lost to energy stored [21]. It is defined by $tan\delta = E'' / E'$ where E" is the loss modulus (energy lost), and E' is the storage modulus (energy stored). In hydrogels, a low loss tangent may be preferred as it would indicate a high degree of elasticity in the hydrogel, with low energy dissipation.

The elastic modulus is concerned with the elasticity of the hydrogel. As implied from the data (fig. 7), the elastic modulus slowly decreases as the oscillation strain increases. The viscous modulus measures the viscosity of the hydrogel. As the data shows, the viscous modulus gradually increases.

To conclude the results, the second hydrogel was made with the properties shown on figure 7. The storage modulus, loss tangent, viscous modulus and elastic modulus remained relatively low in comparison to the other hydrogels that were tested. It should be noted that the third generation PHPMA hydrogel had a lower storage modulus than the second generation ("PHPMA F2") did. Since the second generation hydrogel showed the most promise, it was the one to be researched.

Discussion

This section aims to evaluate the results of the studies involving the cultivation of neuronal cells in hydrogels. This section will also compare the results of the thesis [9] to other studies.

Interpretations of the results

As mentioned earlier, the conclusions made from the results indicated that a hydrogel for neural cell culture should have a low storage modulus. This would mean that the hydrogel should be relatively easy to break down. This idea supports the conclusions that were made in the thesis wherein it was suggested that the hydrogels should be slightly weaker in density to allow the cultivation of the neural cells. Therefore, it could be argued that the hydrogel should have properties closer to that of solution rather than gel.

Other properties of the ideal hydrogel include elasticity and porosity. Having a good level of porosity would mean that the hydrogel would have a larger surface area, allowing direct contact for greater cell attachment. The hydrogels should very slightly inelastic to maintain the soft nature of the hydrogel. If the hydrogels are too elastic, then they'd become rigid and dense. In contrast, if the hydrogels are too inelastic, they'd become too fragile and won't be able to carry the cells, the very purpose of the hydrogels. With fragility in mind, the storage modulus also shouldn't be too low.

Finally, the hydrogels should be very rich in solvent. The solvent consists of several nutrients that aid in the proliferation of the neural cells. It is an aqueous solution with minerals and ions that provide nutrition for cell growth. These can include growth factors, amino acids and particularly, PBS. Essentially, the solvent contains all the nutrients and substances of the growth media.

Other studies

In bioengineering, there have been several studies on synthesising hydrogels for cell culture, namely for neural cells. The research is still an ongoing topic as the perfect hydrogel has not yet been discovered. There are also many applications of hydrogels in various fields, hence there will be interest in hydrogels for a very long time.

Along with this, the studies of neural cells can be explored. There may be a more suitable region to extract the cells as opposed to the SH-SY5Y cells. Perhaps the extraction of neural stem cells might be a more efficient source as they can differentiate to any desired cell.

NeuroGel

One of the derivatives of PHPMA hydrogels is NeuroGel [22]. It's made by integrating the peptide, arginylglycylaspartic acid (RGD), into PHPMA hydrogels. The pore diameters are large enough to allow cell migration along with other macromolecules. Along with this, studies also showed that the hydrogels had similar viscoelastic properties to that of neural tissue [23]. The hydrogels also exhibited signs of conductivity due to the presence of the peptide bond.

The hydrogel proved to be beneficial in the facilitation of the cells. The experiment showed signs of tissue development, axonal growth and angiogenesis. The presence of wound-healing cells was also observed. Moreover, the hydrogel seemed to have reduced the cases of necrosis, the death of body tissue.

Another study was done where adult cat spinal cords were repaired with the use of NeuroGel [24]. With the use of confocal microscopy, showed that with the application of the hydrogel, scar formation was prevented in the healing process. The gel provided a suitable environment for the growth of myelinated fibres and angiogenesis was observed. Axonal regeneration was also observed with an electron microscope. There was a reduction in the physical damage to the distal caudal part of the spine. Neurologic assessments concluded that the spinal cord was also functioning properly. Therefore, NeuroGel was deemed a suitable candidate for neural cell proliferation.

Bone marrow-derived mesenchymal stem cells

Bone marrow-derived mesenchymal stem cells (BMSC) are retrieved from the bone marrow of adults. They can differentiate into several types of cells including bone cells, cartilage cells, fat cells, and neural cells. As such, they've shown promise in neural regeneration.

A study was conducted to determine the efficiency of BMSCs for neural regeneration [25]. The evaluations were done by assessing the electrophysiology (studying the electrical properties of tissue) and histomorphometry (measuring the morphology of tissue) of the nerve regeneration. The experiment was to find how effective BMSCs are for the nerve regeneration in rat sciatic nerves; these are nerves originating from the spinal segment of rats.

The research concluded that BMSC implantation was beneficial for the nerve regeneration. In this, BMSCs had several purposes. Firstly, the BMSCs aided in the production of neurotrophic factors; substances that control proliferation and differentiation in the nervous system [26]. Additionally, some differentiation into artificial Schwann cells was observed.

However, there were some limitations to the study. The study was investigated on rats, research on human cells would be very different. Many factors would have to be considered including the morphology, genetic changes, cell adhesion etc. Therefore, the study isn't accurate for applications in human.

Future research

While the field of hydrogels and neural cell culture has many findings, there are still many limitations to the field. The most important factor to consider is biocompatibility. Majority of the studies done were on animal cells, this is inaccurate for human cells as there would be a difference in biocompatibility and other characteristics.

The hydrogels need to be catered for neural cell regeneration. As mentioned previously, they need to have a low storage, elastic and viscous modulus. This is to ensure a good environment for the cell culture. The hydrogel should also be rich in solvent with growth factors and other nutrients for cell growth. The research can be done further to explore more suitable properties of the hydrogel for more efficient neural cell culture and regeneration. Further investigations on cell adherence also needs to be explored.

There can be a lot more to investigate in the type of neural cell used for the regeneration as well. While the use of SH-SY5Y cells was proven to be worthwhile in the study, it's a lengthy process to prepare them. As such, a more efficient method is yet to be discovered. There might be other cells that proliferate and differentiate more efficiently than the subject cells.

Furthermore, other factors could be integrated into the procedure to obtain better results. For instance, the growth media could be improved by adding additional growth supplements (e.g., FBS), neurotrophic factors (for the differentiation of neural cells), antioxidants (to protect neural cells from oxidative damage), cytokines (e.g., interleukin, TNF-α etc.), and antibiotics (to prevent contamination).

Conclusion

In conclusion, this study investigated the potential of hydrogels for neural cell culture and how that might affect various applications in regenerative medicine and neuroscience. The concept of implementing a relatively soft hydrogel deemed as a viable candidate for neural regeneration. The hydrogel with the growth media proved to be biocompatible with the cells. And showed signs of cell growth, differentiation and proliferation.

The use of SH-SY5Y cells, derived from cells in human bone marrow, was ideal for the culture. They are used in various fields of neuroscience as they possess the ability to differentiate into various neuronal cells under the right culture conditions. They have the quality of maintaining their neuronal properties for long periods of time. They are capable of proliferation in different environments. Hence, they're a great candidate for the subject of neural cell culture.

While the study showed good promise, there are still many limitations. There is still so much to be researched before the hydrogels can be used in clinical applications. Allergies could be a factor to consider in future research; some immunological responses may not adhere to the manufactured hydrogels. Moreover, the human immune system may recognise the foreign neural cells as pathogens and as such, may attack them, defeating the whole purpose of their repair.

Hydrogel industrialisation for broad use adds a new level of complexity. It is crucial to carefully consider all the safety implications of using hydrogels because they have a lot of potential in a variety of medicinal applications, including the cultivation of brain cells. It is essential to undertake thorough evaluations to ensure that the hydrogel materials and culture conditions are both safe for the human body.

In conclusion, further investigations need to be done in order to commence the public use of the hydrogels and the cell cultures in the restoration of neural tissue. The methodology may require modifications to create hydrogels adequate for clinical usage. Further testing will also need to be done. Firstly, to test the biocompatibility with the subject culture as well as natural cells in the body. The material, physical and viscoelastic properties will additionally require advanced investigations.

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Appendix

Meeting minutes

This section gives the dates where I met with my supervisor, Dr Helen Willcock.

1. 2nd February 2023 – Careers' information (brief assessment on scheduling meetings)
2. 15th February 2023 – Lab induction for hydrogel synthesis
3. 21st February 2023 - Lab induction for neural cells (with Tim Coles)
4. 15th March 2023 – Review and update

5. 20th March 2023 – Planning for writing