TEKNOFEST

AEROSPACE AND TECHNOLOGY FESTIVAL

BIOTECHNOLOGY INNOVATION COMPETITION

PROJECT DETAIL REPORT

TEAM NAME

widyadharma

PROJECT NAME

Injectable Polysaccharide-based Self-healing Hydrogel as the carriers for NSC's in Alzheimer Disease Treatment.

APPLICATION ID

#60250

CATEGORY

Idea Category

Project Detail Report

1. **Project Summary (Project Description) :**

Alzheimer's disease (AD) is a physical disease that was caused by accumulation of amyloid-β (Aβ) and hyperphosphorylation of Tau which triggers neurodegeneration. Until now, only symptomatic treatments exist for this disease with low efficiency to hold the progression of the disease. Multiple researches have shown that stem cells had potentials to treat AD by reducing neuron cell loss which were linked and caused by AD. But there is still some challenge regarding a reliable approach for transporting stem cells to the injury site. Based on this case, we try to innovate an idea by using injectable polysaccharide-based self-healing hydrogel as the carriers for NSC's in Alzheimer Disease treatment. Self-healing hydrogels is a hydrogel that exhibits the ability for self-repair, similar to living tissues, and injectable properties induced by shear-thinning and stimuli responsiveness. Polysaccharides are one of the most abundant natural macromolecular polymers which have several advantages compared to synthetic polymers in biomedical applications. These combinations of the unique delivery ability of self-healing hydrogels with polysaccharide as its base, through reversible chemical covalent bonds method (Schiff base) could enhance the ability of the hydrogels to deliver the neural stem cells, as well protecting the cells from external forces that could damage the cells. The injection of the self-healing hydrogel for NSC's delivery will most likely follow the same procedure as the in-vivo experiment, which is around the hippocampus area. The innovative aspect of the hydrogel is the improvement of the hydrogel stiffness which will be around ~700 Pa, making it more suitable for neural tissue engineering. This project is still an idea-based project, that's why the estimation of the cost and project scheduling is based on the previous research with the same topic. Chitosan as polysaccharide-based is a material that is easy to gain and the estimated time for production for the self-healing hydrogel is around 3-5 months. This project is aimed for patients with Alzheimer's disease due to various previous research that has discussed the benefits of NSC's as a method of treating Alzheimer's disease. Some of the risk of this project includes using a chemical crosslinking method via Schiff bases between polymers or macromolecules that could trigger UV rays, which could cause inhibition at the injection time for hydrogels to reach into deep tissue by photopolymerization and the failure of NSC's to carry out the differentiation process due to the widespread migration of NSC's throughout the hippocampus that triggers autoimmune. The solution could be by changing the main materials for the hydrogels and transplanting nerve precursor cells (NPC's) in experimental autoimmune encephalomyelitis (EAE).

2. **Problem :**

According to (Boese *et al.,* 2020) Alzheimer's disease (AD) is a physical disease that affects the brain. This disease occurs due to accumulation of amyloid-β (Aβ) and hyperphosphorylation of Tau, a microtubule-associated protein that responsible to maintains neuronal microtubules in a normal state of condition of the body (Barbier *et al.,* 2019), which triggers neurodegeneration. This formation and deposition of Aβ plaques occur in the brain *parenchyma* as well as in the *cerebral vasculature* (Yiannopoulou & Papageorgiou, 2020). AD will cause the neurons to experience damage, lose their connection with each other, and eventually lead to the neuron's death. This disease is often found and increases exponentially in people aged 65 years and over (Vally & Kathrada, 2019). Until now, only symptomatic treatments exist for this disease with the purpose to counterbalance the neurotransmitter disturbance: 3 cholinesterase inhibitors and memantine. This treatment aimed to block the progression of the disease by using therapeutic agents to interfere with the pathogenic steps responsible for the clinical symptoms. Currently, some of the approved drugs

for the symptomatic treatments of AD include cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and the NMDA receptor antagonist memantine. However, not only these drugs had poor efficacy to hold the progression of the disease (Salomone *et al.,* 2012), they are also accompanied by various side effects (Casey *et al.,* 2010).

To some extent, the mammalian brain can repair itself throughout life with neurogenesis (Boese, Hamblin and Lee, 2020). Neurogenesis begins with the proliferation of adult neural stem cells (NSC's) and then develops into neural progenitor cells (NPC) and finally migrates and differentiates into neurons or glia (Lazarov *et al.,* 2010). Injuries that occur in the central nervous system (CNS) due to Alzheimer's disease can stimulate neurogenesis (Liu *et al.,* 2018). Unfortunately, neurogenesis decreases significantly with age until it reaches a condition where it could not happen again (Sorrels *et al.,* 2018). This limitation could be answered by transplanting NSC's to help reduce neuron cell loss associated with Alzheimer's disease (Boese, Hamblin and Lee, 2020). Stem cells have advantages for being able to be genetically engineered in vitro and have a high migratory capability after transplantation into the brain, allowing them to gain a way to distribute neurotrophic factors or to improve gene expression and possibly altering the disease progression (Wang *et al.,* 2015). Several preclinical studies have shown that paracrine effects on transplanted NSC's induce neurogenesis and synaptogenesis-type therapy in the CNS (Boese, Hamblin and Lee, 2020). Injections with NSC's in the hippocampal region in transgenic K 3xTg-AD mice showed increased synaptic density in the hippocampus associated with brain-derived neurotrophic factor (BDNF) secretion (Blurton-Jones *et al.,* 2014).

Stem cell-based replacement therapies have shown potential to treat a neurodegenerative disease, but there is still some challenge regarding a reliable approach for transporting stem cells to the injury site (Wu *et al.,* 2010). A soft and biocompatible hydrogel is a favourable candidate to provide a 3D microenvironment that has physicochemical properties similar to the extracellular matrix for tissues for the differentiation and engraftment of transplanted NSC's (Wei *et al.,* 2016). However, the current conventional injection hydrogels that were used to encapsulated cells tend to get disrupted by the cell properties, such as interface pulling, which lead to failure in structural integrity of the hydrogels (Talebian *et al.,* 2019). Without proper delivery methods, stem cells could be easily damaged by external and shear forces during the injection process that could deteriorate the cell's functionality.

3. Solution:

A soft and biocompatible hydrogel that could maintain the shape of the NSC's during the injection process is needed to protect the stem cells from external and shear forces during the injection process. Based on this case, we try to innovate an idea by using injectable polysaccharide-based self-healing hydrogel as the carriers for NSC's in Alzheimer Disease treatment. Self-healing hydrogel is the specialized type of hydrogels, enhanced in the self-healing properties of the hydrogels such as the ability to restore the morphology and mechanical properties after being damaged (Cheng *et al.,* 2019). Compared to typical hydrogels, self-healing hydrogels may regain their initial structures and functions upon injury due to dynamic/reversible links in its hydrogel network thus eliminating the need for additional external treatments to repair it (Tu *et al.,* 2019). In addition to this case, hydrogels with self-healing properties have some appealing characteristics such as short invasive injection operations without gel fragmentation and may also be incorporated as bulk gels while retaining structural and functional integrity in specified regions for a certain length of time(Tseng *et al.,* 2015; Cheng *et al.,* 2019; Tu *et al.,* 2019).

Earlier study about this case examined the biological functions of self-healing hydrogels with different chemical compositions and rigidity. Self-healing hydrogels is a hydrogel that exhibits the ability for self-repair, similar to living tissues, and injectable properties induced by shear-thinning and stimuli responsiveness (Tu *et al.,* 2019). These properties made the self-healing injectable hydrogels able to directly deliver cells via transplanting parts of the cell-loaded hydrogel into the lesion site, which will self-heal into integrally connected structures and filling the area. (Wei *et al.,* 2016). Self-healing hydrogel has been engineered with a wide range of specific functions and applications such as tissue engineering, wound healing, and the cell/drug delivery area. In the current study, the long-term survival of NSC's in composite hydrogels with the same rigidity and different self-healing properties were investigated systematically (Cheng *et al.,* 2019). To name a few, such as the carrier for C212 myoblasts for cardiac cell therapy (Dong, Zhao, Guo and Ma, 2016), mouse chondrogenic cells (Yan *et al.,* 2017), sustainable controlled drug release ability on tumour cells (G. Chang *et al.,* 2015), and NSC's (Tseng *et al.,* 2015; Wei *et al.,* 2016).

4. Method:

Based on the explanation above, self-healing hydrogel could be a potential candidate for neural stem cells (NSC's) delivery. Preparation for this kind of hydrogel can be through reversible chemical covalent bonds and physical non-covalent interactions (Tu *et al.,* 2019). The existence of reversible covalent bonds exhibits stable and slow dynamic equilibriums, while physical non-covalent interactions show fragile and rapid dynamic equilibriums (Liu & Hsu, 2018). Due to these advantages, chemical covalent cross-linking with reversible imine bonds (Schiff base) were more preferable as the method to create the self-healing hydrogels. Schiff base, often known as a reversible imine bond is a dynamic covalent bond produced by the reaction of amine with a carbonyl or aldehyde group, which is a crucial element that offers the hydrogel self-healing capacity through the uncoupling and recoupling of imine bonds in the hydrogel networks. (Tu *et al.,* 2019).

NSC's then will be encapsulated with the self-healing hydrogel to increase the efficacy of the NSC's after implantation in the targeted site (Tseng *et al.,* 2015). The encapsulation provides an environment for the NSC's to proliferate, and protection from the external environment that could damage the cells (Tseng *et al.,* 2015). After the production of the self-healing hydrogel, the next step is the injection of the self-healing hydrogel. As stated in the problem section, the point of NSC's therapy is to increase neurotrophic factors secretion such as brain-derived neurotrophic factor (BDNF) which were an important neuroprotective factor derived from NSC's (Liu *et al.,* 2020). This addition of BDNF increases the possibility to improve the gene expression and neurogenesis and thus possibly alter the AD progression. In particular, BDNF is generated by neurons and is abundant in the cerebral cortex and hippocampus, which are the regions that are critical for brain learning and memory (Liu *et al.,* 2020). Most of the experiment of NSC's delivery as AD treatment is delivered to one of these areas, which has a more significant possibility to increase the BDNF in the brain and could give more impact to disrupt the disease progression in the brain. For example, research conducted by Zhu *et al.,* 2020, showed injection of NSC's for AD treatment into the bilateral hippocampus of a transgenic mouse showed a significant improvement in spatial learning and memory of the mouse. Injection of the self-healing hydrogel for NSC's delivery will most likely follow the same procedure. So far, the injection of self-healing hydrogels for NSC's has only been experimented with in-vivo procedures in primary animals and there haven't been any clinical trials in humans yet.

5. Innovative Aspect :

Hydrogel as a crosslinked polymer network is a highly versatile method with good mechanical stability (Tu *et al.,* 2019). In this idea we proposed a self-healing polysaccharide-based hydrogels, both as the main compound and the crosslinker agent inside the hydrogel (CEC-l-OSA). In nature, polysaccharides character is hydrophilic, which also provides an advantage of creating the polysaccharides-protein complex, because it can act as a stabilizing agent (Bealer *et al.,* 2020). Polysaccharides are one of the most abundant natural macromolecular polymers that have several advantages compared to synthetic polymers in biomedical applications (Yang *et al.,* 2016). Also, polysaccharides are constructed from monomeric sugars linked together by O-glycosidic linkage. This glycosidic linkage has an ability to store material, compose structural components, and act as a protective material. (Bealer *et al.,* 2020). Polysaccharides are commonly used as a carrier for therapeutic agents and tissue scaffolds due to their high chemical reactivity, polyfunctionality, high biocompatibility and biodegradability and their similar macromolecular properties to the natural extracellular matrix (ECM) which could increase cell adhesion, spreading, and proliferation (Nikolova & Chavali 2019). A hydrogels-based polysaccharide is easy to form either alone or in combination with other substrates with unique physical and chemical properties (I. Kwiecień, *et al.,* 2018). Furthermore, hydrogel-based polysaccharide has properties that are sensitive to changes such as temperature, pH, or ionic intensity changes, qualifying them as a viable biomaterial in biomedicine (W. Wei *et al.,* 2017; Dang *et al.,* 2017).

Chitosan is one of the many materials that was chosen to create a self-healing hydrogel for cell delivery. Chitosan is a polysaccharide produced from alkaline deacetylation of chitin which is the main component of the protective cuticle of various crustaceans (crabs, shrimp, lobster) and cell walls of various fungi (Ambore, 2013). Chitosan has good biocompatibility, biodegradability, and low toxicity, inexpensive so it is widely used in the biomedical field (Tseng *et al.,* 2015; Zhao *et al.,* 2018). In the biomedical field, chitosan can be used as a scaffold, gel, membrane, micro/nanoparticles for its application in tissue engineering, both soft and hard tissues. Literature also indicated that chitosan as a biomaterial could promote nerve regeneration (Tseng *et al.,* 2015). However, chitosan still has weaknesses, one of which is its weak hydrophilicity (solubility in water) which limits the variety of its use (Yang *et al.,* 2016). Therefore, many researchers use chitosan derivatives, namely N-carboxyethyl chitosan (CEC) in order to maintain the properties of chitosan suitable for biomaterials as well as to have good solubility properties as seen from the addition of hydrophilicity (Yang *et al.,* 2016).

Sodium alginate (SA) is a high molecular hydrophilic polysaccharide extracted from brown algae and is a biocompatible, biodegradable, and non-toxic biopolymer (Sun and Tan, 2013; Lyu *et al.,* 2020). SA has often been used for biomaterials in the form of drug-delivery systems and cell carriers for tissue engineering because of its ability to be easily modified both chemically and physically so that it has various functions and properties (Sun and Tan, 2013). Sodium Alginate derivatives can be obtained by carrying out an oxidation process to obtain oxidized sodium alginate (OSA). In the study of Florczyk, Kim, Wood and Zhang (2011), SA was oxidized in order to modify the degradation and covalently grafted biomaterials formed with growth factors to enhance cell adhesion and proliferation in biomaterials.

A research conducted by Wei, Z., Zhao, J., Chen, Y. M., Zhang, P., & Zhang, Q. already reported self-healing polysaccharide-based hydrogels as injectable carriers for NSC's with the same materials (CEC-l-OSA) in 2016. The researchers managed to create a hydrogel that had similar mechanical strength as natural brain tissue with well supported retention,

proliferation, and differentiation of NSC's. The result showed that NSCs could proliferate vigorously over 5 days under 3D and 2D cultures which are shown by the higher expression level of neuronal marker, b-III tubulin than the control group. However, the research still showed a lower glial marker GFAP than the control group, which indicates that the CEC-l-OSA hydrogels could promote NSC's differentiation toward neurons more effectively than toward glial cells. This problem may occur from the stiffness of the hydrogel which is around ~500 Pa. Based on the research of Tseng *et al.,* (2015), the ideal stiffness for self-healing hydrogels for neural tissue engineering is around 0.7-10kPa. Thus by this problem, we propose another idea using the same materials but by tuning the composition to receive the ideal stiffness for neural tissue engineering, thus creating it more suitable for NSC's delivery for AD.

6. Applicability:

NSC's already showed promising results as one of the treatments for AD. NSC's therapy and its various delivery methods are already being applied in vivo with primary animals as the disease models (Zhu *et al.,* 2020) and the result showed some significant cognitive improvement and the increase of neural markers. Research regarding the applicability of self-healing polysaccharide-based hydrogels as injectable carriers for NSC's already showed some promising results as one of method of NSC's delivery which has already proven by it's in-vivo experiment (Tseng *et al.,* 2015; Wei *et al.,* 2016). In the future, this method holds some promising advantages, as stated in the solution, to be the carrier of NSC's therapy to combat AD.

7. Estimated Cost and Project Scheduling:

This project is still an idea-based project, that's why the estimation of the cost and project scheduling is based on the previous research with the same topic. As stated in the innovation section, the main material for the self-healing hydrogel is the polysaccharide, which are CEC linked by OSA. Chitosan itself is a popular material for biomedical applications due to its biocompatibility. biodegradability, low toxicity, and inexpensive and easy to gain due to chitosan sources that could be gained from various crustaceans exoskeleton waste (Tseng *et al.,* 2015; Zhao *et al.,* 2018). Based on the paper written by Özogul, Hamed, Özogul and Regenstein, (2019), crustacean shells, heads, and tails account for around 50–70% of crustaceans and are frequently thrown without further processing, posing a severe environmental threat. By extracting these valuable chemicals (chitin) might not only reduce the environmental effect of the waste, but also generate value-added goods that are also socially helpful.

Based on the production on the research of self-healing hydrogel for NSC's delivery method (Tseng *et al.,* 2015; Wei *et al.,* 2016) showed that the production of the self-healing hydrogel and the test characterization test will probably take around 3-5 months to complete including the time to write the research paper. The team will work into categories based on the timeline that had been created, which are administrative, research (including searching for reference, contacting laboratorium, and ordering the materials), and finishing (including writing the final research paper and preparing for publishing the paper). The work will be divided equally among the team members, which will be supervised by the supervisor. The team timeline could be visualized using this table,

No.	Activities	First month				Second month				Third month				Fourth month				Fifth month			
1.	Preparation of the tools and materials	■	■	■	■																
2.	Synthesis of the self-healing hydrogel					■	■	■	■	■	■										
3.	Characterization test of the self-healing hydrogel								■	■	■	■	■								
4.	Data analysis											■	■	■	■	■	■				
5.	Project report																■	■	■	■	■

8. Target Audience of the Project Idea (Users):

Injectable polysaccharide-based self-healing hydrogel was designed to be a carrier for NSC's in Alzheimer's Disease treatment. This project is aimed for patients with Alzheimer's disease. Alzheimer's Disease (AD) is a physical disease that was caused by accumulation of amyloid-β (Aβ) and hyperphosphorylation of Tau which triggers neurodegeneration (Boese *et al.,* 2020). Actually, there are many kinds of neurodegenerative diseases, but this product is more specifically for people with Alzheimer's disease. This specification was made because there have been many studies discussing the benefits of NSC's as a method of treating Alzheimer's disease. In addition, according to the World Health Organization (WHO), Alzheimer's disease is the most common form of dementia and can contribute to 60-70% of cases.

9. Risks:

One of the risks of self-healing hydrogel is underlying from the process of how it is made. Self-healing hydrogels were designed using a chemical crosslinking method via Schiff bases between polymers or macromolecules. Chemical cross-linking through covalent bonds will introduce organic compounds that can trigger UV rays. UV rays could cause inhibition at the injection time for hydrogels into deep tissue by photopolymerization (Yan *et al.,* 2017). In addition, the dynamic network formed by different crosslinking mechanisms has an impact on the application of the injected self-healing hydrogel. For example, excessive acid could break the acylhydrazone in the self-healing hydrogel bond and thus the gel-sol transition, which can lead to hydrogel failure. That's why the condition of the acidity around the area of the injection needs to be one of the primary concerns. For another case, in the study of Ding *et al.,* 2013, cross-injected glycol chitosan (GC) hydrogel was made by crosslinking di-benzaldehyde macromolecules bounded by linear polyethene oxide derivatives (OHC-PEO-CHO). The function of the cross-injection GC is as an implant deliverer for antitumor drugs and cells. However, as a classical chemical crosslinked hydrogel, dynamic covalent bonded GC gels are still quite brittle and affect their application in tissue applications that demand certain mechanical strength as a scaffold. That's why in this experiment we are using different materials as the backbone of the hydrogels such as chitosan to limit the possibility of negative effects of the self-healing hydrogel application.

The imperfection of self-healing hydrogel in maintaining mechanical strength could also have an impact on the state of the injected NSC's. Although transplantation of NSC's exerts a therapeutic effect by directly activating the lost cells, it rarely produces significantly different neurons. The failure of NSC's to carry out the differentiation process due to the widespread migration of NSC's throughout the hippocampus that triggers autoimmune (Baek, Kang & Ra, 2011). However, this risk can still be overcome by transplanting nerve precursor cells (NPCs) in experimental autoimmune encephalomyelitis (EAE), to activate the

immunosuppressive effect. Immunosuppressive effect works to lower the immune system on neuroprotective (Joyce *et al.,* 2010). The first indication of the immunosuppressive effect of NPCs came from rat experiments with EAE. It was shown that intraventricular transplantation of NPCs reduces brain inflammation and severity in clinical disease (Park *et al.,* 2013). Therefore, the principle to fully utilize the neuroprotective properties for NSC's in the clinical setting is by early intervention, which could prevent/slow the disease progression and reduce the need for cell replacement later in life.

10. Resources :

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