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PREPARATION AND CHARACTERISATIONS OF ALGINATE-AGAROSE POLYMERIC HYDROGEL FOR POTENTIAL STEM CELL DELIVERY

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ABSTRACT

Polymeric hydrogel is cross-linked material with three-dimensional (3D) structures, capable of retaining water where their softness and smartness properties are highly beneficial in stem cell transportation. Synthetically produced hydrogels can be modified to alter their ability to respond to changes in the external environment. Alginates can transport low molecular weight substances, while agarose is beneficial in cell growth, differentiation and proliferation. The preparation and characterisations of the alginate-agarose (Al/Ag) hydrogels are essential to assess the suitability of components to support cells viability during the transportation period. This study aims to prepare and characterise Al/Ag hydrogels' unique properties for potential stem cell transportation by cells encapsulation. Methodically, Al/Ag hydrogels beads were formed by mixing the different composition of alginate and agarose, dropped into 0.3M calcium chloride solution. An optimized combination of 1.5% alginate and 2.0% agarose ($Al_{1.5}/Ag_{2.0}$) and 1.5% alginate and 0.1% agarose ($Al_{1.5}/Ag_{0.1}$) were selected for characterizations. The swelling test was carried out in 0.3M calcium chloride solution to discover the degree of swelling (DS) with 19.5 swelling ratios for $Al_{1.5}/Ag_{0.1}$ higher than 17.32 swelling ratio for $Al_{1.5}/Ag_{2.0}$. For the degradation test, both optimised samples were observed for 11 days with a degradation rate of 0.486 mg/day for $Al_{1.5}/Ag_{0.1}$ was higher than $Al_{1.5}/Ag_{2.0,}$ with 0.087 mg/day were recorded. The Al/Ag beads were characterised using Fourier Transform Enfrared (FT-IR) Spectrometry and Scanning Electron Microscopy (SEM). From the FT-IR analysis, the spectra revealed an important combination functional group in the Al/Ag hydrogels. The surface morphology of both the samples was porous with different diameters and wrinkled, paper-like rough surface structure. Based on the findings, we suggested that Al/Ag hydrogels' properties can be prepared as stem cells transportation medium where the pore size and molecular interconnections are essential in determining the solute absorption and diffusion.

Keywords: Alginate, Agarose, Polymeric Hydrogels, Stem Cell Transportation

INTRODUCTION

Hydrogels are commonly used as polymeric network materials with three-dimensional (3D) structures capable of retaining water without losing structural integrity. Hydrogels are favoured in biomedicine, as it possesses similar characteristics to soft tissue in a living person [1]. These hydrogel systems include various chemically and structurally responsive moieties and exhibit responsiveness to external stimuli, including temperature, pH, ionic concentration, light, magnetic fields, electrical fields, and chemicals. These smart polymer

hydrogels also can change their structural and volume phase transition as a response to external stimuli resulting in an enormous potential for scientific observations and various advanced technological applications [2]. Significantly, this study intends to characterise hydrogels by analysing the surface morphology and the structural composition of alginate/agarose (Al/Ag) hydrogels. Many studies report the benefits and drawbacks of hydrogels following the characterisation process of various hydrogels. Nevertheless, the existing reports are non–conclusive on alginate–agarose hydrogels' ideal stem cell delivery characteristics. Without detailed characterisation of the constructed hydrogels, the hydrogels' optimum environment to assist in stem cell delivery will be impossible to predict.

Hydrogel is recently one of the smart biomaterials that can provide unique advantages in stem cell delivery. These materials aid the retention of the encapsulated stem cells by providing biological and physical supports. Amazingly, the hydrogel can also be used as semipermeable membranes with interconnected pores that enable mass transport, including the supply of nutrients and removal of waste from the encapsulated cells. Micro-sized hydrogels permit convenient injection compared to bulk hydrogels, and they also have a high surface area that allows the efficient mass transfer of oxygen and nutrients. Hence, micro-sized hydrogels exhibit promising and practical materials for tissue engineering and cell or drug delivery applications and stem cell delivery [3].

The incorporated hydrogel of alginate and agarose have been designed for this study, respectively. Alginate is an essential and standard material that has been used to form hydrogel due to its properties, such as natural anionic and hydrophilic polysaccharide. It has chain-forming heteropolysaccharides, consists of (1-4)-linked β-D-mannuronic acid and α-L-guluronic acid in nature. It can be found in the cell wall and the intercellular matrix of marine brown algae (*Phaeophycota*). Alginates can be readily prepared as a gel form in an aqueous solution by using a divalent cationic cross-linking agent such as calcium ion (Ca^{2+}) provides by calcium chloride ($CaCl_2$) [4].

Meanwhile, agarose is a biocompatible polysaccharide extracted from marine red algae, which contain repetitions of agarobiose (a disaccharide of D-galactose and 3,6-anhydro-L-galactopyranose). The agarose can be prepared by a thermal-reversible process in gel form [5], and it also can be easily dissolved in hot water, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), formamide (FA), N-methylformamide (MFA) and 1-butyl-3-methylimidazolium chloride (BmimCl) [6]. The oxygen and hydrogen in this natural carbohydrate polymer's side groups support the material for its self-gelling characteristic. Besides, hydrogen bonding in agarose hydrogel does not require toxic cross-linking agents like genipin, making this material a biocompatible polymer [7].

Combining alginate and agarose for the construction of hydrogel beads may improve structural integrity and function. Alginate is capable of delivering low molecular weight substances [8]. On the other hand, agarose has been widely used in tissue engineering and regenerative medicine, mainly due to its unique self-gelling characteristic, high water-absorbing capacity, and structure similarity to the extracellular matrix [9]. The features of both components can be incorporated into the composite hydrogel. The strategies may help to overcome the main limitation of hydrogels, which is low mechanical strength. Therefore, it is crucial to study further the alginate-agarose hydrogels to provide a secure medium for the stem cells to be transported, together with the necessary elements for the survival of the cells.

In biomedicine contexts, synthetically produced hydrogels can be modified to alter their ability to respond to changes in the external environment by modifying the physical and chemical stimulus [10]. Moreover, hydrogels' modification can also improve the permeability and degradation rate and diffusion through the hydrogel [11]. By providing a suitable micro-environmental structure, hydrogels can control the encapsulated stem cell's fate and functions [12].

Several similar studies focused on investigating the various changes of stem cell behaviour in a well-controlled microenvironment with hydrogels' aid. In this present study, two commonly used hydrogels, namely alginate and agarose, were combined to produce a composite hydrogel as a transportation medium. Both hydrogels' benefits would produce a structure with better physical and chemical properties than pure alginate or pure agarose alone as a medium for stem cell transportation. This study highlights alginate-agarose (Al/Ag) hydrogels properties to be used for stem cell transportation. This study's positive outcomes may help develop Al/Ag hydrogels as a material for stem cell transportation due to their inevitable benefits.

EXPERIMENTAL

Reagents

The materials used for fabricating the hydrogels were sodium alginate (Sigma-Aldrich), high melting agarose (Bioline, UK) and calcium chloride, $CaCl_2$ (Sigma-Aldrich).

Synthesis of Al/Ag hydrogels

Stock solutions were prepared to precede the process of formation of hydrogel beads. 0.3M calcium chloride ($CaCl_2$) solution was made by dissolving 22.05 g of calcium chloride powder in 500 ml of distilled water. 1.5% alginate stock solution was prepared by adding the 1.5 g of sodium alginate powder to 98.5 ml of distilled water and stirred continuously on a magnetic stirrer at room temperature. 2.0% of high melting agarose solution was prepared by dissolving 2.0 g of agarose in 100 mL of distilled water, heated on a heating magnetic stirrer, until completely dissolved (approximately 96°C). The solution was left to 40°C, before mixing with the respective alginate solution, as agarose has a specific gelling temperature. The pure alginate and agarose hydrogel solutions were loaded into the syringe attached to an 18 G hypodermic needle. The needle's end was adjusted until approximately 30 mm above the 0.3M $CaCl_2$ solution's surface. The agarose-alginate gel solution was then dropped into 0.3M calcium chloride solution while gently being stirred at room temperature for 8 minutes. Al/Ag hydrogels beads were prepared with two different concentrations of agarose and alginate solutions. The first batch was mixed to form 1.5% alginate, 2.0% agarose solution ($Al_{1.5}/Ag_{2.0}$), and the second batch was to form 1.5% alginate and 0.1% agarose ($Al_{1.5}/Ag_{0.1}$). The alginate stock solution was added to the agarose stock solution when it was cooling down around 60°C and cooling down further to 40°C. The same procedure was repeated for the Al/Ag gel solution. It was loaded into the syringe attached to an 18 G hypodermic needle and adjusted until it is approximately 30 mm above the surface of the $CaCl_2$ solution. The beads formed were then filtered into a filter funnel containing a piece of filter paper and thoroughly washed with distilled water three times.

Swelling ratio

The swelling test was carried out to assess the ability of Al/Ag hydrogels to absorb water. The swelling ratio was measured by incubating the test specimen in 300 μL of deionised

water at 37 °C for a specific time (10, 20, 30, 40, 50, 60, 90, 120, 180, 360 minutes). The Al/Ag hydrogels beads were oven-dried overnight at 40 °C before the swelling test. The dried hydrogel bead was weighed and noted as *Wa*. Hydrogel samples were then transferred to plates containing deionised water for it to return to its swollen state. Excess water was carefully removed using a soft, lint-free paper, and the gels were weighed and noted as *Wb*. The water uptake (%) was calculated using the equation as follows:

$$\text{Degree of swelling (\%)} = [(Wb - Wa) / Wa] \times 100\%$$

Degradation rate

The degradation test was carried out by exposing the hydrogel to the dissolving agent, consisting of sodium citrate and EDTA. The alginate-agarose hydrogels were weighed and placed in a beaker filled with 30 mL of one of the degradation agent concentrations, which was gently shaken. This procedure was carried out until the samples had fully degraded or the degradation course was sufficiently characterised. The time taken for the hydrogel beads to be fully degraded was recorded. The degradation rate was observed on the hydrogel's weight loss starting from day 0 to day 11. The degradation rate was calculated using the following equation.

$$\text{Rate of Degradation} = (A - B) / 11$$

where *A* is the average weight of 1 hydrogel bead at day 0 and *B* is the average weight of 1 hydrogel bead at day 11.

Characterisations of Al/Ag hydrogels

One hydrogel bead from each type of pure alginate, pure agarose and the composites of $Al_{1.5}/Ag_{2.0}$ and $Al_{1.5}/Ag_{0.1}$ were then used for characterisation. Structure elucidation was performed using Fourier transform Infrared Spectroscopy (FT-IR), and surface morphology were analysed using Scanning Electron Microscopy (SEM).

Fourier Transform Infrared (FT-IR) Spectroscopy

The spectral measurements were performed in the Analytical Laboratory of the School of Health Sciences in USM. The middle-infrared of electromagnetic spectra with wavenumbers spanning from 4000–400 cm^{-1} were collected using FT-IR spectroscopy (TENSOR 27).

Scanning electron microscopy (SEM)

Al/Ag hydrogel beads' surface morphologies were observed using Quanta FEG 450 in SEM Lab, School of Health Sciences, USM. The beads were kept in their natural state without freeze-drying. Both samples were examined at a voltage of 5.0 kV in a low vacuum.

RESULTS AND DISCUSSION

Degradation rate and swelling ratio were recorded and compared with a different combination of hydrogel beads concentration. The swelling ratio data analysis of Al/Ag hydrogel beads constructed using a concentration of alginate and agarose solution in 0.3M $CaCl_2$ solution is shown in Figure 1(a). From the result, the data indicated that $Al_{1.5}/Ag_{2.0}$ hydrogel showed less degree in swelling compared to $Al_{1.5}/Ag_{0.1}$ hydrogel at the first 0 to 120 minutes. However, as the time was prolonged to 360 minutes, both batches revealed similarity in the degree of swelling with $Al_{1.5}/Ag_{0.1}$ hydrogel gave 19.5 swelling ratios higher than 17.32 of $Al_{1.5}/Ag_{2.0}$ hydrogel swelling ratio, respectively. Based on the observation, the swelling ratio indicates the gel matrix's stiffness and porosity, primarily affected by the hydrogel matrix's molecular

network. The degree of swelling for $Al_{1.5}/Ag_{0.1}$ hydrogel is higher than the $Al_{1.5}/Ag_{2.0}$ hydrogel. A higher concentration of agarose was found to directly affect the hydrogel matrix's stability as agarose was a non-swelling gel in water. Hayashi et al. [14] demonstrated that agarose present in a double-helical form while forming the gel and the helical segments tend to crystallise. Therefore, the dilute solution-phase molecules seem to make a hard and brittle network in the gel. This formidable network will result in a strong elasticity against the swelling effect in the water. In contrast, a higher concentration of alginate in the hydrogel matrix will tighten the cross-linking molecules, significantly influencing the hydrogel swelling behaviour [15].

Furthermore, the degradation rate was higher for $Al_{1.5}/Ag_{0.1}$ at 0.486 mg/day than the $Al_{1.5}/Ag_{2.0}$ hydrogel at 0.087 mg/day, as shown in Figure 1(b). As mentioned above, alginate was combined with agarose to construct hydrogels due to its several excellent characteristics, such as high porosity, mechanically resistant, chemically and physically inert, and strongly hydrophilic [16]. However, a high concentration of agarose content in $Al_{1.5}/Ag_{2.0}$ hydrogel resulted in lower degradation capacity under an optimised 100mM citrate+100mM EDTA as the dissolving agent. This is because agarose can only be degraded in the presence of agarase or when the temperature is higher than the melting point of 80-90°C [17]. Increasing temperature will not be a feasible and practical approach as the high temperature will influence cells survival as the hydrogels will only denature a few degrees above 40°C [18]. However, after optimising the hydrogel component, the $Al_{1.5}/Ag_{0.1}$ hydrogel bead was successfully dissolved using 100mM citrate + 100mM EDTA between 4-12 minutes. The present finding was also in good agreement with data obtained from another study that suggested that the speed of degradation for alginate hydrogels increases for higher sodium citrate concentration [19] with a minimum concentration of 0.1% agarose 0.3 M of calcium chloride.

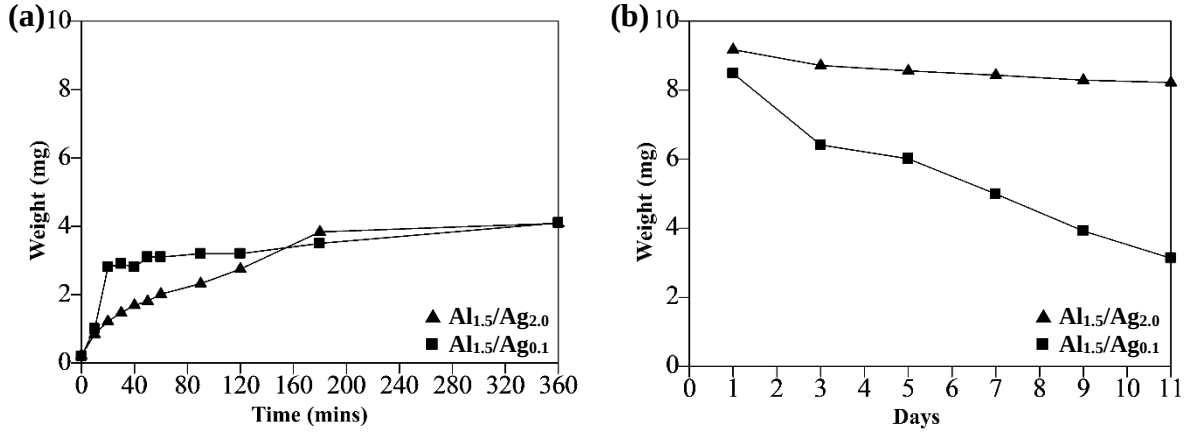

Fig. 1 Graphical presentation of (a) swelling ratio analysis and (b) degradation rate analysis of Al/Ag hydrogel beads in a combination of $Al_{1.5}/Ag_{2.0}$ as batch 1 and $Al_{1.5}/Ag_{0.1}$ as batch 2.

Structure elucidation by Fourier Transform Infrared (FT-IR) Spectroscopy

Based on the spectra obtained, the peaks formed by all four samples, pure 1.5% alginate, pure 2.0% agarose and the composites $Al_{1.5}/Ag_{2.0}$ and $Al_{1.5}/Ag_{0.1}$ hydrogel are as shown in Figure 2. The FT-IR analysis was carried out to detect the specific absorption of peaks formed from the functional groups' chemical bonding in Al/Ag hydrogel. Most of the peaks represent the predominant region of 3000 cm^{-1} and 3500 cm^{-1}, 1500 cm^{-1} and 2000 $cm^{-1,}$ and relatively minor vibratory peaks below 1000 cm^{-1} indicating weaker binds of C-O stretching. All four samples, 3000 cm^{-1} and 3500 cm^{-1}, revealed O-H groups, involving H bond in the region. All

four samples portrayed relatively similar stretching of the peaks. The specific functional groups for the respective peaks are shown in Table 1.

Table 1 The FT-IR data of functional group in alginate, agarose and Al/Ag hydrogels beads

Samples	Wavenumber (cm^{-1}) Functional group			
	O-H	C=O	C-C	C-O
Alginate	3331	1637,1368	1216	1027
Agarose	3328	1637	1357	1073
$Al_{1.5}/Ag_{0.1}$	3325	1636,1426	–	1034
$Al_{1.5}/Ag_{2.0}$	3331	1636,1334	–	1043

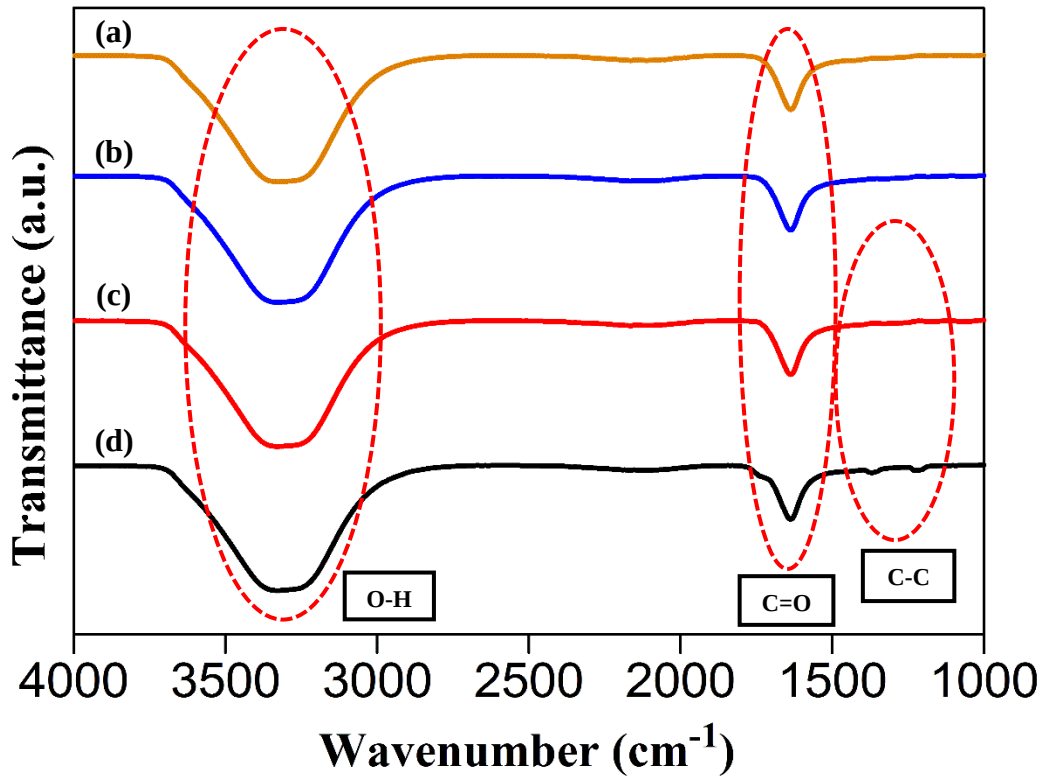

Fig 2. FT-IR spectra with the range of 4000-1000 cm^{-1} for (a) $Al_{1.5}/Ag_{0.1}$, (b) $Al_{1.5}/Ag_{2.0}$ (c) pure 2.0% agarose and (d) pure 1.5% alginate hydrogel beads.

Specifically, for alginate, the peaks that appeared at 1637 and 1368 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations of carboxylate salt ion, respectively. The peaks correspond at 1216 and 1027 cm^{-1} representing C-C and C-O stretching. On the other hand, the agarose spectrum displayed typical bands at 1637 cm^{-1}indicated the C=O stretching (amide I) of O=C-NHR functional groups. The absorption peak at 1357 cm^{-1} is attributed to the C-C stretching, and 1073 cm^{-1} is assigned to C-O stretching from the glycosidic bonding

[20,21]. The peak that displayed at 1200 to 1370 cm^{-1} which belongs to C-C stretching in both alginate and agarose was the only evidence before the mixture. However, these peaks are absent in the Al/Ag hydrogel mixture, indicating that interaction has occurred to form a cross-linked network. These results show good miscibility between alginate and agarose, which is likely caused by the formation of intermolecular hydrogen bonding between the amino and hydroxyl groups in both hydrogels [21]. From the spectra obtained, the composite of alginate and agarose beads possess similar absorption peaks to pure alginate and pure agarose beads. Reason being, when two or more polymers are added together, the absorption peaks formed is the reflection of its component [22].

Typically, the synthesis of alginate/agarose hydrogel can be formed from the electrostatic interaction, which involves ionic cross-linking. In this reaction, the presence of Ca^{2+} ion from $CaCl_2$ as divalent cations cooperatively interact with the block G monomers from alginate to form ionic bridges. However, the blocks M monomers of alginate formed weak junctions with divalent cations. In contrast, the interactions between blocks G monomers and divalent cations form tightly held junctions [23], leading to the so-called egg-box structure [24]. The gelation speed is too fast to be controlled because of calcium chloride's high solubility in an aqueous solution. Therefore, the mixture of alginate/agarose in $CaCl_2$ solution can be stabilised due to the presence of agarose that formed hydrogen bonds between amino and hydroxyl groups of the mix. During the agarose transition from solution to the hydrogel, it decreased in temperature. The alginate chains can still retain within agarose networks and form hydrogels within a given time at room temperature [25]. The linear polymer structure of agarose consisted of repeated units of alternating 1,3-linked β-$_D$-galactose and 1,4-linked 3,6-anhydro-α-$_L$-galactose mixture gave the ability to form strong gels at even low concentrations. Moreover, agarose is a reversible gelling agent, which solidifies or liquefies cooling or heating. Thus, no is specific counter ions or additives were needed to cause gelation [20].

Surface morphology analysis by Scanning Electron Microscopy (SEM)

The SEM images of pure 1.5% alginate and $Al_{1.5}/Ag_{2.0}$ hydrogels are shown in Figure 3 and 4, respectively. The surface microstructures of 1.5% pure alginate hydrogel bead in Figure 3(a) revealed rougher, more compact network structures with heterogeneous pores than the $Al_{1.5}/Ag_{2.0}$ hydrogels mixture beads, as shown in Figure 4(a). In the mixture of $Al_{1.5}/Ag_{2.0}$ hydrogel beads, the pores formed more homogenous. They had an inter-connectively, well-defined porous structure with a relatively higher number of pores than the pure alginate sample. The previous finding by Zhang et al., they reported that both components of alginate and agarose in the hydrogel structure were found to produce more refined 3D constructs with improved consistency on porosity and interconnectivity a higher number of pores. Based on the SEM findings, pure agarose had a smooth surface texture. Nevertheless, when agarose was significant in the composite agarose/hyaluronic acid, the surface morphology was more homogenous and porous [26].

Based on the observation of the SEM images in Figure 3(c) and (d), the 1.5% pure alginate had been gelled and showed porous structure due to the formation of a network from alginate chains polyguluronate moieties and form coordination bonds with the cation Ca^{2+}. Meanwhile, the rough, compact structure on the bead's surface has similar morphology to freeze-dried sodium alginate that has been reported by Ayarza *et al.*, where the surface architecture of sodium alginate observed under the SEM appears compact, rough with fewer pores. There was also apparent morphological variation between sodium alginate and calcium alginate in which calcium alginate had a more porous surface than sodium alginate [27].

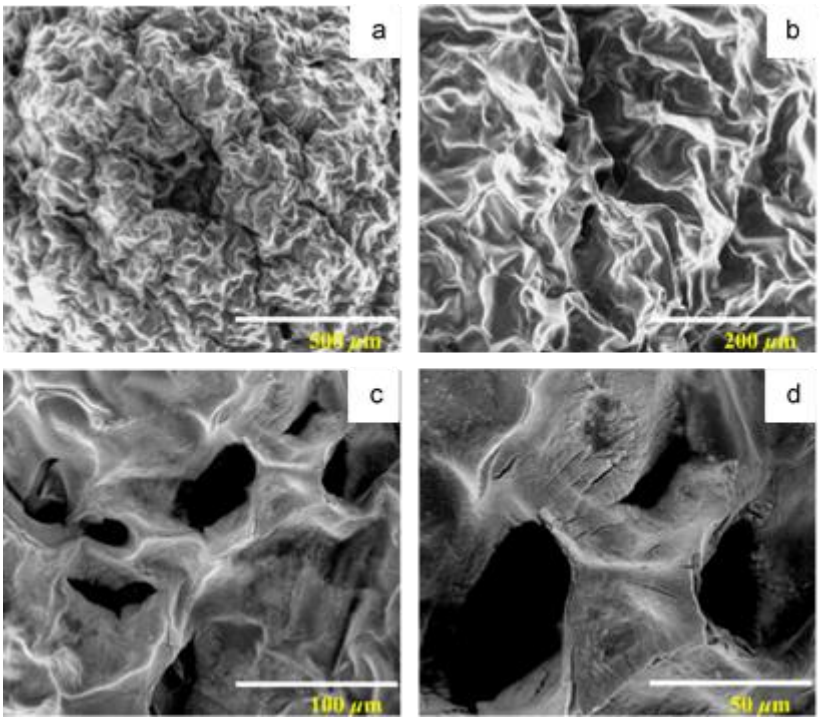

Fig. 3 SEM images of pure 1.5% alginate at (a) 200x, (b) 500x, (c) 1000x and (d) 2000x magnifications.

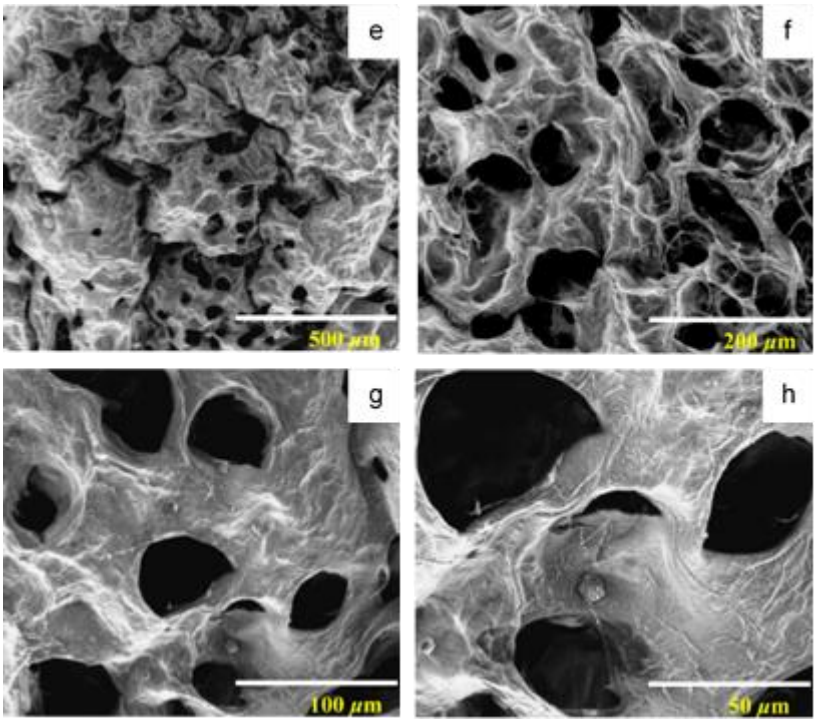

Fig. 4 SEM images of $Al_{1.5}/Ag_{2.0}$ hydrogel at (e) 200x, (f) 500x, (g) 1000x and (h) 2000x magnifications.

The present study has also performed a similar approach to analyse the 2.0% pure agarose sample. However, the SEM images could not be captured due to their low resistance

to the electrons' heat. Thus it shrunk too fast before an image of the desired magnification was obtained. In order to obtain a more producible image and to protect the gel from destruction due to heat, freeze-dried hydrogels can be used during this morphological testing. In several studies, a freeze-drying technique was used to analyse hydrogels; however, this technique should be noted to lead to changes in the agarose beads' surface morphology. It was reported that the surface topography was irregular in nature [28].

Combining alginate and agarose as a biocomposite hydrogel for potential cell delivery applications has proven that these two polymers improved mechanical properties compared to pure agarose or alginate [25]. The resulting hydrogel was homogeneous and stable with the cross-linking agent as the critical factor in the gelation process's kinetics and the resulting gel's structural characteristics [27]. Thus, this characterisation has been proven as the composite hydrogel had more pores than pure alginate, shown in Figure 4 (f, g). When diffusion of solutes occurs through the pores, it decreases the cross-linking between the molecules, leading to increased pore size over time. As the pore size increases, larger substances can diffuse through the hydrogels. This allows various sized particles to diffuse into the hydrogels to maintain the vitality of the cell. These particles may include oxygen, nutrients, and other essential chemicals that ensure the cells' survival within the hydrogel for a specific period [29].

The bead's size depends on the hypodermic needle and force used, resulting in bigger or smaller droplets. However, in this experiment, gel-beads of approximately similar measures were produced and used for SEM [21]. In order to obtain macroporous structures, a phase separation has to take place during the network formation process so that the formation of additional cross-links fixes the two-phase structure formed. After polymerisation occurs, the diluent was removed from the network, leaving a porous structure within the highly cross-linked polymer network. To form a polymer network with an interconnected pore structure, absorption or desorption of water occurs through the pores through convection. The porous structure causes water inside the hydrogel to allow free diffusion of some solute molecules, while the polymer serves as a matrix to hold water together [30].

In this study, several limitations were encountered. Firstly, it should be noted that morphological study and chemical structure elucidation alone is not entirely sufficient to prove that the composite of alginate and agarose will be a viable transport medium for stem cells. Other characterisation methods, including mechanical testing, degradation and swelling tests, are essential to study the pure alginate and agarose hydrogels and compare their properties to the composite hydrogel. One of the most common tools used for mechanical testing is the Universal Test Frame. This machine can be used to test for tensile and compression strength [31]. A universal dynamic Rheometer can be used to test the hydrogels' thermal behaviour and determine the gel point [32].

Next, in this study, only the Al/Ag hydrogel was characterised without cell encapsulation. Thus only its benefits and drawback were noted without considering the stem cells factor, or vice versa. In order to assess its full potential as a transport medium, it is important to encapsulate the stem cells into the pure hydrogels and composite alginate-agarose hydrogel. This step is essential to study the survival of these cells over some time. The study can also be extended by manipulating hydrogels' microenvironment, such as pH, temperature, and concentration of nutrients/gasses, to analyse the cell behaviour in various conditions. A previous study concluded that stem cells' shape was fostered by the microenvironment and the composition of the surrounding adhesion proteins, which closely

relates their functional biological activity to direct or maintain cellular identity in vivo [33]. In another study, the hydrogels' biocompatibilities were evaluated in relation to the thermo-responsive properties, and there was no cytotoxic effect on cell metabolic activity from the hydrogel system. The study concluded that this hydrogel's thermo-responsive property provides easy management during clinical practice and application as a dressing system [34]. The various testing that involves manipulating the environmental contents and conditions is vital for the durability and survival of both the stem cells and hydrogel.

Future studies should be encouraged to be conducted in sterile conditions in the pursuance of transforming this composite hydrogel as a stem-cell transportation medium. The materials and methods used have to be sterilized, and the surrounding environment has to be maintained in a sterilized condition. A few key areas also need to be investigated in further details. The sterility of the surrounding environment, using suitable medium and techniques in the storage of hydrogels encapsulated with stem cells may need to be adequately addressed. The knowledge gained from this study may enhance the quality of the research to compare the pure and composite hydrogels used as a transport vehicle.

CONCLUSION

The gathered data suggested that the pore size and molecular interconnections are essential in determining the solute absorption and diffusion in and out of the hydrogels. The condition ensures the viability of cells encapsulated and further confirmed the potential application for stem cell transportation. In short, Al/Ag hydrogel bead constructs possess better properties than pure alginate or pure agarose to serve as a critical component in stem cell transportation. Nevertheless, further studies on morphology, structural bonds, and physical strengths have to be conducted by analysing the hydrogels with encapsulated stem cells. A similar approach may then be used to manipulate different variables to assess the cells' viability in different microenvironments.

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