Development of Antigen Responsive Hydrogels for Accurate Cancer Therapeutic Delivery

Cat 1 Written Report

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Introduction

Abstract

In this study, Polyvinyl Alcohol - Glutaraldehyde (PVA-GA) hydrogel nanoparticles were synthesised using free radical polymerization. Rabbit Immunoglobulin (Rabbit IgG) and Goat Anti-Rabbit Immunoglobulin (GAR IgG) were added as the corresponding antigen and antibody complex for targeted drug release in cancer therapy. The independent variables include the mass of GA used and the presence of the antigen comonomer while the dependent variables include the dye absorption and release of the PVA-GA-Antigen-Antibody hydrogels. It was found that the Polyvinyl Alcohol - Glutaraldehyde - Rabbit IgG - GAR IgG (PVA-GA-AA) hydrogel could absorb more methylene blue dye but less brilliant green and neutral red dye than the Polyvinyl Alcohol - Glutaraldehyde (PVA-GA) hydrogel. When placed in free-flowing antigens, the dye release of methylene blue and neutral red of the PVA-GA-AA hydrogel was higher than that of it placed in normal dye solution while that of brilliant green was slightly lower, indicating successful binding of the antigen-antibody complex outwards to swell. Scanning Electron Microscopy (SEM) and Fourier-Transform Infrared Spectroscopy (FTIR) were also conducted to analyse the hydrogel, which indicated the presence of cross-linked bonds between the GA, PVA and Rabbit IgG-GAR IgG, and the porous structure of a hydrogel with high surface area. In conclusion, the PVA-GA-AA hydrogel shows great potential in targeted drug delivery as a biomedical application.

1. INTRODUCTION

1.1 Rationale / Literature Review

Every single day, there is a huge number of people who lose their lives to cancer globally, which poses a huge threat to the human race. Particularly, colon cancer, which is also known as colorectal cancer, is the third most common case of cancer-related deaths, taking up a whopping 10% of all cancers with about 1.4 million cases diagnosed in 2012 (Lee & Lee, 2017). According to Senapati, Mahanta, Kumar, Maiti (2018), current methods to treat cancer include surgical intervention, chemotherapy or radical therapy. However, these options often result in the damage of healthy normal tissues, as well as adverse side effects like appetite loss, nausea and even cause death. Therefore, due to its poor bio-accessibility, requirement of high doses, elevated toxicity and increased incidence of drug resistance, it is necessary and important for us to develop treatments which are able to specifically target cancer cells (Khan, 2010).

Studies by Hoare & Kohane (2008) have shown that hydrogels are three-dimensional, cross-linked networks of water-soluble polymers. Not only are they able to absorb large amounts of water, their highly porous structure and excellent swelling capacity can also be easily varied according to the cross-linking density in the aqueous environment. As such, hydrogels have a wide range of biomedical applications, such as biosensing, tissue engineering, regenerative medicine, and drug delivery (Souza, Kogikoski, Silva & Alves, 2017). Senapati, Mahanta, Kumar, Maiti (2018) have also found out that these hydrogels can also be created to have a semi interpenetrating polymer network, which causes the polymer network to be partially interlaced on a polymer scale, but not covalently bonded together, by free radical polymerization, which greatly aids in drug release.

Targeted therapies aim to cure cancer through acting on specific molecular targets associated with cancer cells, including biomarkers and antigens created by these cancer cells. Antibodies are large

proteins that bind specifically to a target protein or antigen, which are toxins or foreign substances which induce an immune response in the body, such as the creation of antibodies (Fletcher, Babcock, Murray & Krebs, 2015). Additionally, according to Niu et al (2012), Carcinoembryonic Antigen (CEA) is a commonly used tumor marker in patients with colorectal cancer. It has also been reported that CEA is an extremely reliable and sensitive biomarker for colon cancer, whereby the expression level of this antigen in serum is crucial in the diagnosis of the disease, since there is an exponential increase of up to 44% of CEA in the tissues of patients suffering from severe colon cancer. Rabbit IgG and Goat-anti Rabbit IgG is a corresponding model antigen antibody complex that shows similarity in its secondary and tertiary structure to CEA and is hence used.

According to Miyata, Asami & Uragami (1999), the swelling and semi-interpenetrating polymer network of hydrogels can be used to create an effective vehicle for the delivery of cancer therapeutics. These scientists developed a 'reversibly antigen-responsive hydrogel', whereby the hydrogel was synthesised with an antibody-antigen complex. The hydrogel was prepared by grafting the antigen, rabbit immunoglobulin G (rabbit IgG), and its corresponding antibody, goat anti-rabbit IgG (GAR IgG), to the polymer network of the hydrogel, in order to induce binding between the antibody and antigen and introduce crosslinks in the network. While in the buffer solution filled with specific free floating antigens, the hydrogel became porous when swollen, thus it could potentially be used for targeted drug delivery, as a drug could permeate through the pores. This complex enables the hydrogel to respond to free floating antigen because of the 'reversible binding' mechanism between an antigen and antibody. When the antibodies from the gel come into contact with free floating antigens, the bonds between the polymerized antibody and antigen dissociate since they are much weaker than that in the antigen, leading the hydrogel to swell and deteriorate, thus releasing any dye it carries. Therefore, this led to the conclusion that antigen-responsive hydrogels for cancer drug and therapeutics release was a feasible option for cancer treatment.

In conclusion, colon cancer is not only deadly, but also extremely common case of cancer (Lee & Lee, 2017). Despite the presence of numerous traditional cancer therapies currently, (Senapati, Mahanta, Kumar & Maiti, 2018) all these pose various drawbacks due to their adverse side effects, poor bio-accessibility and high doses needed (Khan, 2010). Hence, targeted therapy of cancer cells is absolutely necessary due to its biocompatibility and biodegradability, especially through hydrogels which have highly porous structures and excellent swelling capacities (Hoare & Kohane, 2008). Several studies show that antigen-responsive hydrogels for drug release are potential treatment methods, with the use of antigens such as Rabbit IgG as biomarkers to deliver antibodies such as GAR IgG to body tissues without interfering with healthy cells (Niu et al, 2012). On top of that, the loading of dyes into hydrogels as model drugs (Ji, Qin & Feng, 2017) have also proven that they can be easily released due to the dissociation of bonds within the antigen-antibody complex (Miyata, Asami & Uragami, 1999). This research aims to solve the problems with the use of traditional cancer therapy, through the enhancement of the effect of drugs, reduction in side effects, dosages and thus decrease in therapy costs.

1.2 Objectives

This study aims to create a targeted cancer therapy which uses an antigen-antibody complex to locate the antigenic cancer, as well as to release a drug from within the PVA-GA hydrogel nanoparticle

for colon cancer drug delivery.

1.3 Hypotheses

It is hypothesised that the PVA-GA-AA hydrogel nanoparticle can be synthesised, and that it will be able to release an optimum amount of cancer therapeutics.

2. METHODOLOGY

2.1 Materials

Hydrochloric acid (HCl) and acetone was obtained from Honeywell and Merck respectively. Polyvinyl alcohol (PVA) powder was purchased from MP Biomedicals, whereas N-succinimidyl acrylate (NSA) was bought from Tokyo Chemical Industry. Phosphate Buffer Solution (PBS) was obtained from Oxoid, and Goat anti-Rabbit Immunoglobulin (Goat anti-Rabbit IgG) was acquired from Abcam. All other chemicals needed, such as glutaraldehyde (GA), methylene blue, brilliant green, neutral red and Rabbit Immunoglobulin (Rabbit IgG), were procured from Sigma Aldrich.

2.2 Apparatus

Soxhlet Apparatus, FTIR Analysis Machine, Reflux Condenser, Glass Beaker, Glass Rod, Measuring Cylinder, Cuvette, Magnetic Stirrer, Sieve, SEM Machine, Centrifuge Machine, UV-vis Spectrophotometer

2.3 Variables

The independent variables of this study include the mass of Glutaraldehyde used and the presence of the antigen comonomer when synthesising the hydrogel. The dependent variables include the dye absorption and release of the varying PVA-GA-Antigen-Antibody hydrogels. Lastly, the controlled variables are the concentration of Rabbit IgG antigen used in the complex, the mass of PVA used, and the volume of hydrochloric acid added. The positive control of this study are the PVA-GA hydrogel nanoparticles while the negative control are the PVA-GA-AA hydrogel nanoparticles with 0 μg/ml of antibody.

2.4 Project Protocols

2.4.1 Synthesis of PVA-GA Hydrogel Nanoparticles

7.38g of PVA powder was dissolved in 100ml of deionised water at 80ºC in a glass beaker. The PVA solution was stirred until it cooled to room temperature, before 10ml of hydrochloric acid as catalyst and 0.05g of glutaraldehyde was added for cross-linking to occur. The resultant reaction mixture was then stirred at 350 rpm at 80ºC for 12 hours. Then the obtained polymers were washed, filtered off, dried and extracted using acetone as the solvent in a Soxhlet apparatus. After drying in air, the polymer was blended and fractionated using sieves to obtain hydrogels of varying sizes.

2.4.2 Synthesis of Antigen Co-monomer

Rabbit IgG was chemically modified by dissolving 1mg of it and 0.004mg of N-succinimidyl acrylate (NSA) in 100ml of phosphate buffer solution (PBS) of 0.02M and pH 7.4. The reaction was

stirred at 36ºC for one hour introduce vinyl groups into the Rabbit IgG. Afterwards, this was purified by centrifugal filtration to remove the PBS before adding it to the PVA solution.

2.4.3 Dye Loading

10mg of PVA-GA-AA hydrogel was measured before loading 10ml of methylene blue, brilliant green and neutral red dye solution into the centrifuge tube. The centrifuge tubes were then left to shake on the orbital rocker overnight for 2 days at 30 rpm. Next, the solutions were centrifuged to separate the hydrogel nanoparticles from the dye solution before pouring the supernatant into a cuvette to record the dye concentration to calculate the dye absorbance.

2.4.4 Dye Release

All supernatant in the centrifuge tubes were decanted, leaving only the hydrogels which absorbed any dye solution. 10ml of PBS was then measured and loaded into each centrifuge tube, before leaving them to shake on the orbital rocker overnight for 2 days at 30 rpm. For the dye release in free-flowing antigen, 9.5ml of PBS and 0.5ml of GAR IgG was added to the other half of the centrifuge tube. Again, the solutions were centrifuged to separate the hydrogel before recording the concentration of the solution to calculate any dye release.

3. RESULTS

3.1 FTIR Spectrum

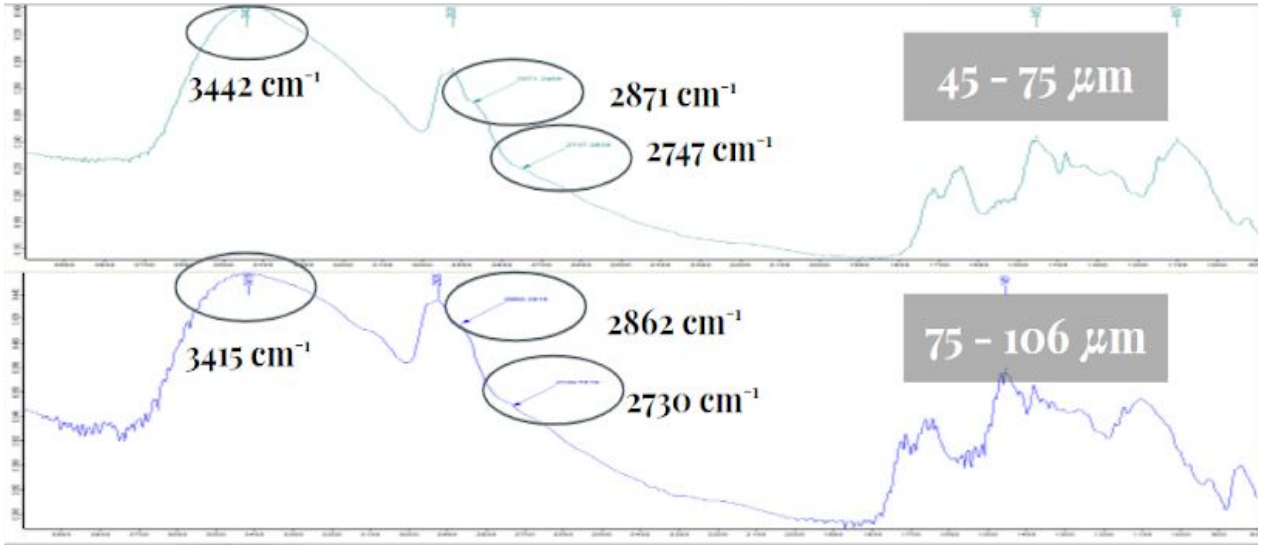


Figure 3.1.1: FTIR spectrum of PVA-GA hydrogel of size (a) 45-75 µm (b) 75-106 µm

For the peaks from wavelength 2000 cm^{-1} to 4000 cm^{-1}, the peaks of interest are shown at 3442cm^{-1} which shows a hydrogen bond, as well as at 2871 cm^{-1} and 2747 cm^{-1} which shows the GA crosslinking the duplet CH_2. The overall graph of FTIR spectrum for both hydrogel sizes are roughly similar, with more pronounced peaks for the hydrogel nanoparticles of size 45-75 µm.

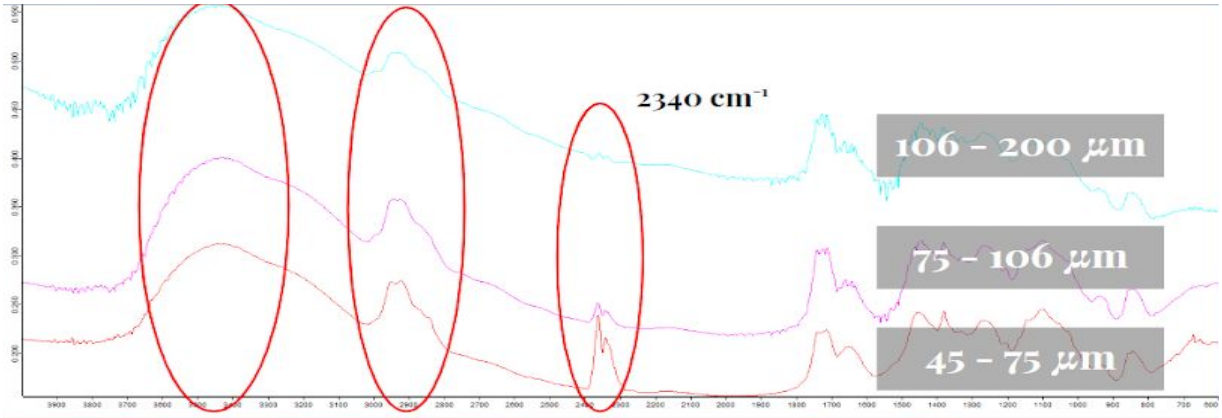


Figure 3.1.2: FTIR spectrum of PVA-GA-AA hydrogel of size (a) 45-75 μm (b) 75-106 μm (c) 106-200 μm

For the peaks from wavelength 2000 cm^{-1} to 4000 cm^{-1}, there is an additional observable peak of interest at approximately 2340 cm^{-1} showing the crosslinking of the antigen-antibody complex with PVA using GA, thus indicating successful synthesis of PVA-GA-AA hydrogel nanoparticles. This peak is more pronounced for the hydrogel nanoparticles of size 45-75 μm.

3.2 SEM Scans

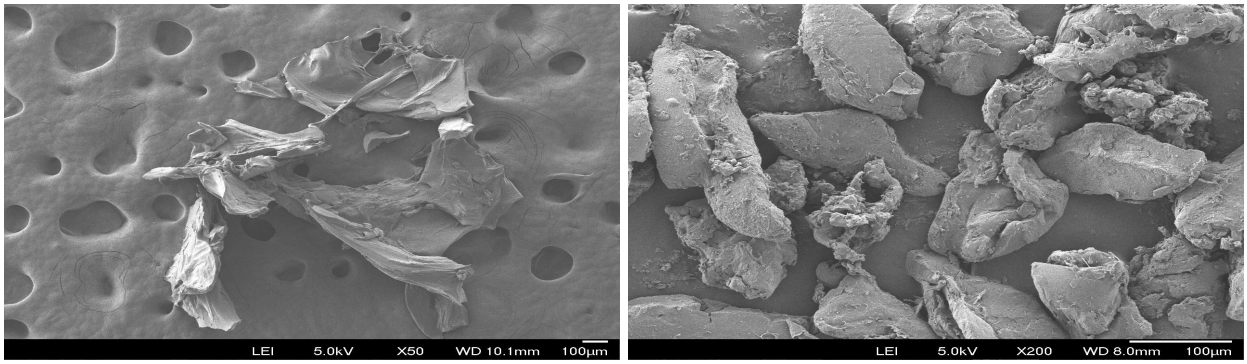


Figure 3.2.1: SEM images of PVA-GA-AA hydrogel, magnified (a) 50x, (b) 200x

From (a), it can be observed that the PVA-GA-AA hydrogel nanoparticle has a porous structure and uneven surface, thus increasing its surface area to allow for the absorption and release of dye, as well as drugs. Upon closer look, (b) shows how the hydrogel nanoparticle swells up upon absorbing dye.

3.3 Dye Absorption

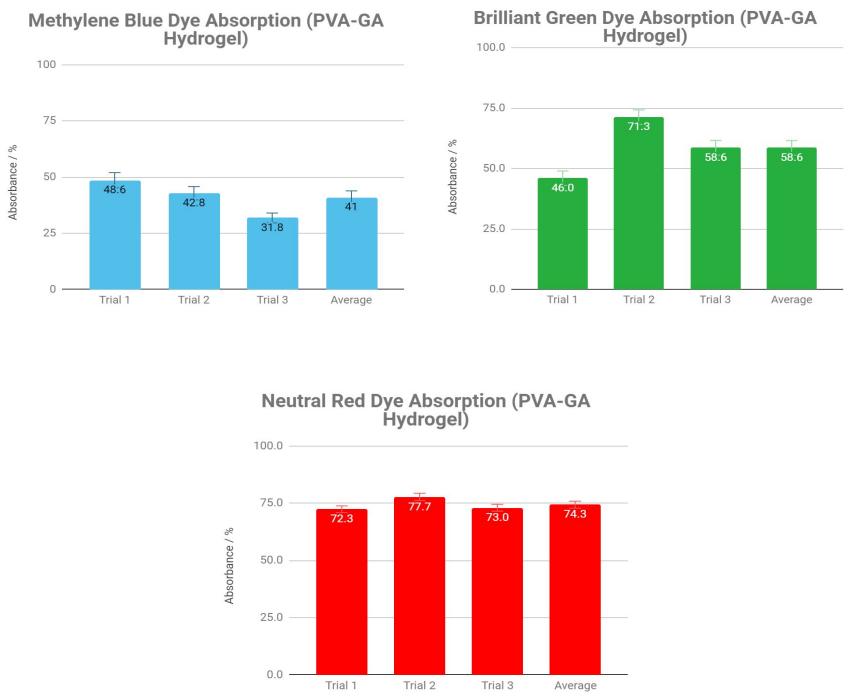


Figure 3.3.1: Dye Absorption of PVA-GA hydrogel with (a)Methylene Blue (b)Brilliant Green (c)Neutral Red

The PVA-GA hydrogel showed increasing dye absorption capacity, with an average absorbance of about 41.0% for methylene blue dye, 58.6% for brilliant green dye and 74.3% for neutral red dye.

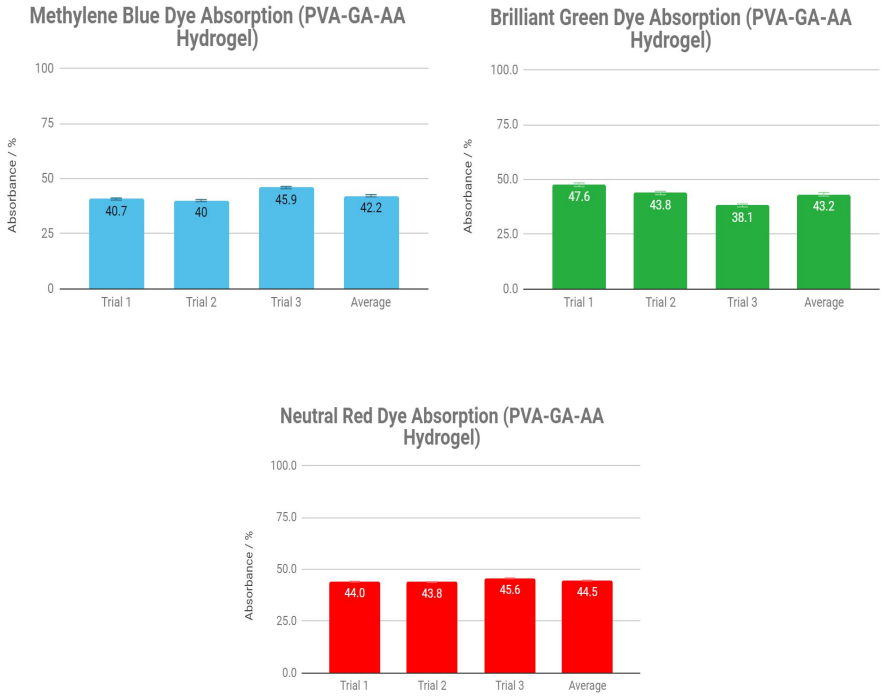


Figure 3.3.2: Dye Absorption of PVA-GA-AA hydrogel (a)Methylene Blue (b)Brilliant Green (c)Neutral Red

The PVA-GA-AA hydrogel showed average dye absorption capacity for all 3 dyes, with an average absorbance of 42.2% for methylene blue dye, 43.2% for brilliant green dye and 44.5% for neutral red dye. The PVA-GA-AA hydrogel absorbed less brilliant green and neutral red dye, and it absorbed slightly more methylene blue dye than the PVA-GA hydrogel.

3.4 Dye Release

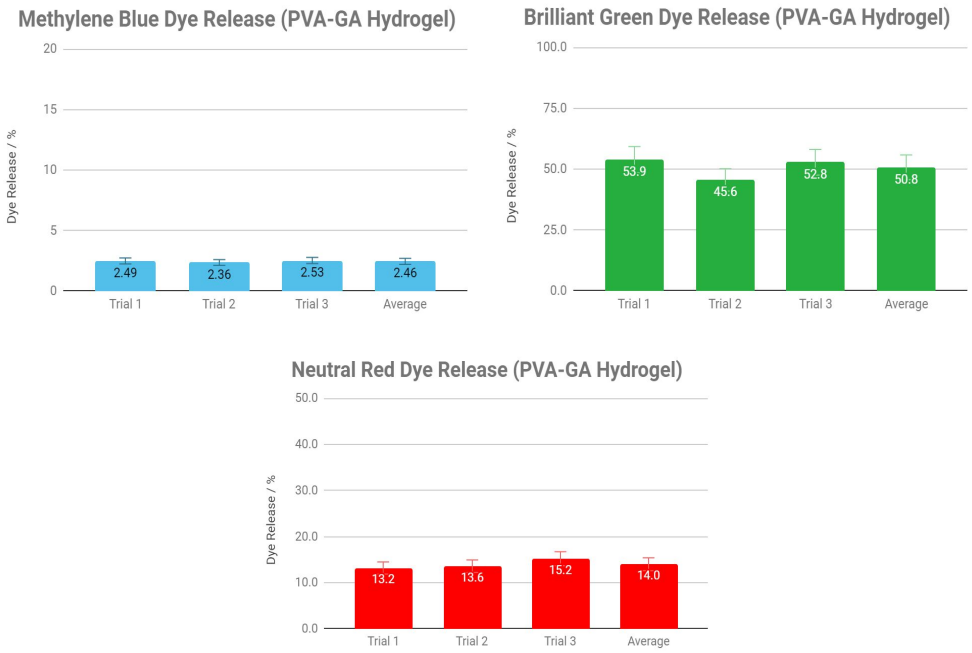
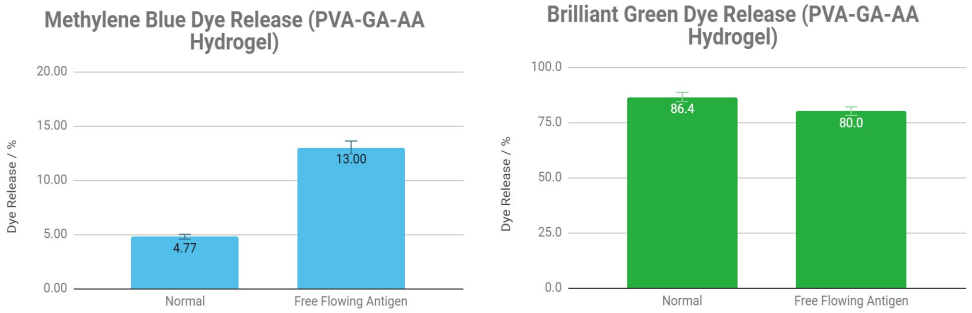


Figure 3.4.1: Dye Release of PVA-GA hydrogel with (a)Methylene Blue (b)Brilliant Green (c)Neutral Red

The PVA-GA hydrogel showed low dye release capacity for methylene blue and neutral red dye, with an average release of 2.46% and 14.0% respectively. For brilliant green dye release, it showed average dye release capacity with an average release of 50.8%.



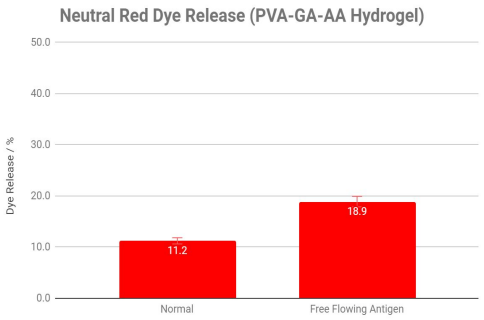


Figure 3.4.2: Comparison of Dye Release of PVA-GA-AA hydrogel in Free-Flowing Antigens with (a)Methylene Blue (b)Brilliant Green (c)Neutral Red

When comparing the dye release of PVA-GA-AA hydrogel with the presence of free-flowing antigens are a biomarker, it was found that it mostly released more dye than that without the biomarker. For methylene blue, the hydrogel released 13.0% of dye as compared with 4.77% of dye in normal conditions, while for neutral red, it also released 18.9% of dye which was more than 11.2% of dye without the free-flowing antigens. However, for brilliant green, the presence of free-flowing antigen resulted in the hydrogel releasing only 80.0% of dye, which is slightly lower than 86.4% of dye normally.

4. DISCUSSION

4.1 Observation of PVA-GA Hydrogel Nanoparticles formed

From Figures 3.1.1 and 3.1.2, it can be confirmed that the GA crosslinks the PVA polymer through the peaks at about 2863cm^{-1} and 2750cm^{-1}(Elizabeth et al, 2006) showing that the PVA-GA structure has been successfully formed. Whereas the discrepancy in the peak at about 3450cm^{-1} between the 2 graphs indicates the presence of OH^- bond in the product. This can be accorded to the intentional addition of less GA, to ensure ample cavities within the hydrogel for H_2O in the structure.

From Figure 3.2.1, the surfaces of the hydrogel of different sizes are similar and display similar physical properties, such as water absorption capabilities. There are 2 distinct shapes of hydrogel formed, namely in the form of the particle and strand. The water absorption capabilities between the 2 types differ greatly, with the particle being significantly more water absorbent. Upon closer observation, the similarity in physical structure and property shows that the strand is unreacted PVA, explaining the phenomenon.

From Figures 3.3.1, 3.3.2, 3.4.1 and 3.4.2, the PVA-GA Hydrogel Nanoparticles formed is highly absorbent and shows significant dye absorption and release capabilities. Hence, it shows its potential for usage in drug loading and release when applied to cancer treatment.

4.2 Comparison between PVA-GA and PVA-GA-AA Hydrogel Nanoparticles

The hydrogel nanoparticles shows 2 distinct types, namely strands and particles. The strands are unabsorbent, while particles of smaller size, specifically 45nm to 75nm are most effective for PVA-GA-AA for dye absorption and release.From the FTIR spectrum, a previously unobserved peak at 2340cm^{-1} is seen in the PVA-GA-AA hydrogel nanoparticle, showing that the addition of Rabbit IgG and GAR IgG has disrupted the structure and bond formation in the PVA-GA hydrogel, affecting the physical properties henceforth observed. The peak is significantly larger for the FTIR spectrum of particle size of 45nm to 75nm, showing that the bonds between PVA-GA and the antibody-antigen complex are more

pronounced at smaller particle sizes. Hence, largely PVA-GA-AA hydrogel nanoparticles were used in the characterisation of the product thereafter.

From the bar graphs of dye absorption and release, the water absorption capability of the PVA-GA-AA hydrogel nanoparticles is higher than that of thePVA-GA hydrogel nanoparticles. Hence, this shows that the addition of the antigen-antibody complex increases the effectiveness of the hydrogel nanoparticles for drug delivery, as more drugs can be absorbed and released with the PVA-GA-AA hydrogel nanoparticles.

4.3 Behaviour of PVA-GA-AA Hydrogel in Free-Flowing Antigen

From Figures 3.3.1, 3.3.2, 3.4.1 and 3.4.2, PVA-GA-AA hydrogels are effectively absorbent and when placed in a PBS solution with free-flowing antigens, the increase in dye release shows the antigen-antibody binding mechanism is effective and bind outwards, disrupting the hydrogel structure and releasing drugs at a more specific location. When comparing to the dye release of the normal PVA-GA hydrogel, it can also be seen that the use of an antigen-antibody complex in free-flowing antigens is generally more effective in more targeted dye release. The structure of the hydrogel can be seen to be more porous and opens up to release the drugs it absorbed.

5. CONCLUSION

This study has shown that the PVA-GA-AA hydrogel is equally effective as the PVA-GA hydrogel, which is widely used in the biomedical industry, in absorbing and releasing dye as a model drug. When placed in an environment with free-flowing antigens, which in this case is Rabbit IgG, to model the body of a patient suffering from colorectal cancer, the antigen-antibody complex has a tendency to bind outwards and cause the pores to expand, thus releasing any dye loaded within. However, to maximise the dye absorption and release of the hydrogel, it has to be optimised to increase the amount of GA used so as to enhance the cross-linking density and thus sorption capacity of the hydrogel, As such, this concludes that the PVA-GA-AA hydrogel is potentially feasible as a cancer treatment method, using Rabbit IgG as the antigen and biomarker.

6. CITATIONS

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