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Research Article

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Synthesis and Analysis of Electrically Sensitive Hydrogels Incorporated With Cancer Drugs

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Abstract

Electric-field sensitive hydrogels are of great interest for many researchers from the aspects of their usage in several biomaterials applications. Controllable drug release under various voltages offers huge benefits for the controlled drug delivery systems. Electrically sensitive polyvinyl alcohol (PVA) hydrogels loaded with methotrexate (MTX) and other compounds were prepared via a solution casting process, and characterized through various techniques. In order to determine if the hydrogels were electro-sensitive or not, bending tests were conducted on the sulfoacetic acid modified hydrogels. It was observed that the prepared samples into strip forms started bending towards the cathode, and this bending was reversible when the polarity of the applied voltage was changed. The drug release study was performed on the MTX-loaded hydrogel strips placed in a sodium chloride (NaCl) solution under three different voltages (e.g., 0V, 5V, 10V, and 20V). Subsequently, the solutions were collected every five minutes in order to determine the drug release behaviors of the hydrogels using an ultraviolet-visible (UV-Vis) spectrophotometer. The test results showed that sulfoacetic acid (SA)-modified PVA hydrogels possess electrical sensitive behavior and kept their electric sensitivity for a long period of time. Also, the results confirmed that the control drug release could be achieved under different electrical voltages. MTT assay results has provided insight about viability of MDA-486 and L-929 cells in the presence of the hydrogels made, also confirmed the results obtained from UV-Vis test.

Keywords

Electro-responsive hydrogels; Release rates; Targeted drug delivery; Breast cancer

Introduction

Because of their advantageous properties such as good biocompatibility, least mechanical irritation to tissues, hydrogels are of great materials to be used in variety of biomedical application such as wound dressing [1], and smart hydrogel applications for which drug delivery [2] artificial muscles, chemical valves, immobilization of enzymes and cells, and concentration of dilute solutions in bioseparation can be given as examples [3]. For about two decades, electric field-sensitive hydrogels, as one type of smart hydrogels, have been the major area of interests for the scientists and engineers who use them in various applications such as sensors, artificial skeletal muscles, chemical valves, and drug delivery systems. By simply adjusting the voltage, the application of an electric field as an external stimulus enables the efficient and accurate release of a drug with good control of both number of molecules released and the release rates [4], which is not possible in other stimuli sensitive drug delivery mechanisms such as thermosensitive, pH sensitive or glucose sensitive. Electroresponsive systems have been developed using hydrogel matrices where pulsatile release profiles are acquired with the on/off application of an electric field [5-8]. There have been a number of reports about electric stimuli responses of hydrogels. One of the earliest studies in 1990 by Shiga and Kurauchi [9] reports on the deformation of polyelectrolyte gels under the influence of an electric field. According to these authors, deformation is related to two substantial changes, one of which is the change of the osmotic pressure caused by the ion concentration difference between the inside and outside of a gel. The other conformational change of the polymer network is the decrease of the polyion concentration. Also, when the electric field is applied to the hydrogels, deformation occurs in three different ways—swelling, shrinking, and bending—which depends on the ion concentration in the gel. While the gel shrinks at low ion concentrations, it swells at high ion concentrations. Moreover, a gel in the shape of a strip exhibits bending behavior [9]. The bending direction is dependent on the type of ion. Polyelectrolyte gels carrying polyanions bend toward the cathode, while polycation gels bend toward the anode. The bending mechanism and swelling behavior of polymer gel is explained by the Flory-Huggins theory of osmotic pressure [10]. According to this theory, when an electric field is applied between the electrodes, mobile ions move toward their counter electrodes, which cause the osmotic pressure π to change at both the anode and cathode sides. If π increases with time, the gel swells; on the other hand, if π decreases with time, the gel shrinks. It has been noted that osmotic pressure on the anode side is $\pi 1$ and that on the cathode side is $\pi 2$. The pressure difference, $\Delta\pi = \pi 1 - \pi 2$, causes the hydrogel to bend toward either the anode side or cathode side, depending on the electrolyte environment and type of ion carried by the gel. From that point, it can be said that if $\Delta\pi < 0$, the gel is a polycationic gel, and if $\Delta\pi > 0$, the gel is a polyanionic gel, which means that polycation gels bend toward the anode, and polyanion gels bend toward the cathode. Figure 1 shows a schematic illustration of this bending mechanism. In addition to these, the bending behavior of a hydrogel differs depending on the environmental conditions. For instance, PVA-PAA gels shrink when they face the positive electrode in a water environment and bend toward the positive electrode. On the other hand, they exhibit a bending property toward the negative electrode side in a basic electrolyte solution [11]. As mentioned previously, for two decades, scientists have been working on the electrical sensitivity of hydrogels and have been trying to enhance the properties by using different polymers and additives. Tanaka [12] showed that a hydrolyzed acrylamide gel collapses in an acetone/water mixture corresponding to an electric field. Yuk and Lee [13] reported that a cross-linked PAA gel showed reversible bending behavior under an electric field in an aqueous NaCl solution. Gong [14] postulated a one-dimensional electro-kinetic model of the

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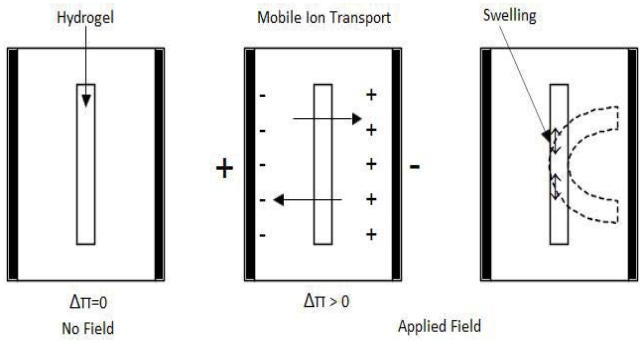

Figure 1: Schematic illustration of bending mechanism. Redrawn from Ref.[9].

contractile phenomenon of polymer gels under an electric field based on Poisson-Boltzmann and Navier-Stokes equations. This model defines the nature of contraction profiles. It was observed that the rate of contraction was linearly dependent on the electric field, and contraction efficiency was inversely dependent upon the cross-linking density. Actuators and skeletal muscle-like materials are other areas where a fast electric response and high mechanical strength properties are required. At this point, PVA based hydrogels are one of the options to use in drug delivery applications because they are known with their biocompatibility and good mechanical properties upon crosslinking. Also modifying with other electrosensitive polymers or chemicals with ionizible groups it is possible to fabricate electrosensitive polymers. Using these methods, PVA has been used in variety of studies with different polymers and modifiers [15-18]. (PVA-PAAc) hydrogel is one of the best IPN hydrogels because of its very strong mechanical properties and electrical-sensitive properties. In 1998, Kim [19] reported on properties of electroresponsive PVA-PAAc IPN hydrogels under an electric field. After producing the IPN hydrogels by UV radiation followed by a freezing-thawing method, they investigated the bending angle measurement under an electric stimulus. Swelling properties and anisotropic deswelling properties were also studied. In 1999, Park [20] investigated the chemomechanical bending behaviors of an ionizable thin film. They demonstrated the time profile deformation and the effect of different pH values on the reversible bending behavior of thin film. In 2002, Kim [21] studied the swelling and bending behaviors of PVA/chitosan IPN hydrogel in a NaCl aqueous solution. They showed that the swelling ratio decreased with an increase in the NaCl concentration. Also, when an electric field was applied, the PVA/chitosan IPN hydrogel moved toward the negative electrode, showing that electrical-sensitive behavior exists. In 2003, Kim [22] also studied the electrical response characterization of chitosan/polyacrylonitrile hydrogel at various concentrations of NaCl aqueous solutions. They demonstrated that the IPN hydrogel showed electrical-sensitive behavior and the equilibrium bending angle (EBA) reached a peak at 0.9 wt% of NaCl concentration. At higher levels, this value decreased. Moreover, with increasing voltage, the EBA increased. Electrical-sensitive hydrogels can be used for various purposes such as artificial muscles, biosensors, chemical valves, and drug delivery. One of the novelest studies in the electroresponsive hydrogel field was done in 2013 by Xiang [23] who studied the electroresponsive behavior of sulfoacetic acid modifying PVA hydrogels. Due to the absence of electrolyte groups in the PVA, it was necessary to introduce some ionizable groups into it, in order to have an electrosensitive hydrogel. These hydrogels were dried and cut into strips. Under a direct current (DC) electric field, displacement was observed and bending was toward the cathode. Also, swelling and mechanical properties were studied. They came to the conclusion that hydrogel swelling increased with a decrease in the ionic strength of the solution. In pulsatile systems, the total amount of drug that is released is an important point. In many cases, the percentage of total drug release reaches a maximum of only 50% of the dose, even after several cycles of on/off switching. Generally, after one or two cycles of on/off electrical stimulation, the drug release is not high and reproducible [24]. Moreover, a controlled drug release and continuous bending property without collapsing under applied voltage are the most important characteristics expected from a drug loaded and electric field sensitive hydrogel. Moreover, as drug release is controlled, it should be also repeatable / reproducible. At this point, mechanical properties of electric field-sensitive hydrogels are very important. Since material is exposed to repeated forces, structure of the hydrogel will weaken and finally collapse. This situation is not desired in applications, especially in drug delivery applications where the destroyed structure hinders the drug release. When hydrogel is used as a drug delivery system, it displays a weak mechanical property and collapses easily. To overcome this problem, hydrogel should be reinforced so that its mechanical properties increase. In 2008, Liu [25] studied montmorillonite effects on the drug-release properties of chitosan hydrogels. Their results showed that montmorillonite-added chitosan displayed a better anti-fatigue property resulting in drug release behavior that is more controllable and lasts longer. The objective of this study was to develop and characterize MTX loaded PVA-based electosensitive hydrogels for drug delivery applications, to investigate the voltage effects on release properties, and to see the effect of released drug amount on cell viability of cancerous and non-cancerous cells. In this present study, poly vinyl alcohol (PVA) is chosen for its good biocompatibility and good mechanical properties so that it can fulfill the basic requirements expected in controlled drug delivery applications. We aimed at producing electric field sensitive, PVA based hydrogels with good release characteristics. The electric sensitivity of the hydrogel was investigated utilizing bending test. For bending test, we used 0.9 wt% of NaCl solution to imitate the body condition because this solution has the same ion concentration as human body. For the characterization of the hydrogels produced, Fourier Transform Infrared Spectroscopy study was performed. Also, for drug release studies, UV-Vis spectrophotometer was used and Methotrexate was chosen as drug in this study. Methotrexate is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug, which is used in the treatment of various diseases such as breast, head and neck, lung, stomach, and esophagus cancers; acute lymphoblastic leukemia; sarcomas; non-Hodgkin's lymphoma; gestational trophoblastic cancer; and mycosis fungoides (cutaneous T-cell lymphoma) [26]. Moreover, thermal behavior studies of MTX suggest that after the removal of the hydration water, anhydrous methotrexate is formed, which also shows a good thermal stability at 127–194°C. The thermal decomposition of anhydrous form of the active substance takes place at 225–320°C [27], which indicates that MTX is an appropriate material to be used in present study in which the heat treatment process was conducted at 80°C. Finally, MTT assay was conducted to investigate MTX effectiveness on cancer cell and also to investigate cytotoxicity behavior of samples fabricated.

Experiment

Materials

Polyvinyl alcohol (PVA) (average molecular weight of 89,000–98,000), 99% hydrolyzed), sulfoacetic acid (SA, technical grade) and

methotrexate hydrate (MTX) were purchased from Sigma Aldrich Chemical Company. Glutaraldehyde (GA, 25 wt% solution in water) and hydrochloric acid (HCl) were purchased from Fisher Scientific. MDA-486 and L-929 cell lines were purchased from the American Type Culture Collection (ATCC).

Method

Preparation of PVA hydrogels: The preparation method of hydrogels utilized in this work was adopted from Xiang [23]. PVA hydrogels were prepared in the first step of the experiment. Here, 0.75 gram of PVA was dissolved in deionized water for 1 day at 90°C to make a 5.0 wt% PVA solution. After the PVA was dissolved completely at the end of 1 day, the mixing temperature was dropped to 50°C. When the solution temperature reached 50°C, 0.5 ml of a 1 molar HCl solution and 0.75 ml of a 1 wt% GA solution were added dropwise into the PVA solution under vigorous mixing for 1 minute. At the end of 1 minute, this final solution was poured into a petri dish which has diameter of 100 mm and kept in an oven setup at a temperature of 50°C until it dried and became a thin film. The resulting thin film was immersed in deionized water for 3 days to remove any undesired reactants. Later on, the swelled hydrogel thin film was kept in a 40°C oven under a vacuum environment for two days. Finally, PVA hydrogel thin films were generated, producing the PVA-GA hydrogels.

Preparation of SA-PVA hydrogels: Sulfoacetic acid (SA) was introduced to the PVA structure to make it electric field sensitive. When SA is introduced with PVA hydrogel, the carboxylic group (COO–) of SA reacts with the hydroxyl group (–OH) of PVA, so that sulfonic acid group ($–SO_3H$) is generated in the structure, which makes the hydrogel sensitive to an electric field. For this purpose, the hydrogel film was cut into strips in the size of 2 × 0.5 cm. Prepared strips were kept in the 0.06 mg/ml sulfoacetic acid solution for 2 hours to ensure that the PVA hydrogel structure had fully bonded with the SA. As a result, SO_3^- groups were generated in the final structure. At the end of the 2 hours, PVA-SA strips were taken out of the solution and placed in a petri dish and left to air dry for 1 day. Then, they were put back in an oven at 80°C under a vacuum environment and heat treated for 6 hours to increase the mechanical strength. In the next step, the heat-treated PVA-SA strips were placed in deionized water and kept for 4 days. During these 4 days, the water was changed once every 12 hours. Four days later, the strips were put into the oven for the last time under vacuum conditions at 40°C and allowed to dry for the bending test and other further tests and analysis.

Preparation of MTX-loaded PVA hydrogels: MTX was chosen as sample drug and to prepare the MTX-loaded hydrogels, a PVA solution in water was prepared at the same concentration as the previous PVA water solution and heated to 90°C. After mixing for 1 day, the temperature was lowered to 50°C for 1 hour. Then, MTX, which is 10 wt% of PVA powder dissolved in the solution, was added to the solution and allowed to mix for 1 hour. As in the previous PVA hydrogel preparation, 0.5 ml of a 1 molar HCl water solution and 0.75 ml of a 1 wt% GA water solution were added dropwise to the PVA solution under vigorous mixing for 1 minute. SA-modifying tests were performed according to the order performed in the section of preparetion of SA-PVA hydrogels. The final thickness of the strips was measured as 0.19 mm, and final hydrogels were in dimension of 20 mm long × 5 mm wide × 0.19 mm thick. Figures 2 show the images of the samples at every step of the hydrogel prepreation process.

Fourier transform infrared spectroscopy: In infrared (IR) spectroscopy, IR radiation is utilized and passed through a sample. While some of it is absorbed by the sample, the rest passes through the sample, which means the IR radiation has been transmitted. The resulting spectrum shows the molecular absorption and transmission, and these spectrums generate a molecular fingerprint of the sample. By using Fourier-transform infrared spectroscopy (FT-IR), a preferred method for IR, it is possible to identify unknown materials from the existing functional groups to determine the quality or consistency of a sample, and ascertain the amount of components in a mixture [28]. Attenuated total reflectance (ATR) spectroscopy which is one of the FT-IR techniques was used to characterize the structure of the prepared hydrogels. ATR is the most commonly used FT-IR sampling tool enabling the characterization of both solid and liquid samples.

Bending test: The aim in performing a bending test was to observe the electric field-sensitive bending behavior of the hydrogels. Firstly, a solution tank was designed for the bending test. Carbon electrodes were placed in the tank at a distance of 30 mm from each other. 45 ml of 0.9 wt% sodium chloride solution was used for the external solution in this test, and samples, cut into thinner strips, to be used in the bending test were placed in that solution in different locations in order for them to swell until they reached equilibrium. Then, certain voltages —0V, 5V, 10V, and 20V— were applied on the same strip and the bending properties of the strip were observed. Also, the voltage effect on bending magnitude was observed by simply increasing the voltage. The placement of solution tank and electrodes are in shown in Figure 3.

UV-VIS spectrophotometer: In the tests in this study, the Hitachi-2900 spectrophotometer was used. For this study 0.9 wt% of NaCl solution was used as blank control sample. Disposable square cuvettes with a pathway of 1 cm were used as sample holders.

Drug release test: In electric field-sensitive drug release testing, prior to placing the hydrogel strip into the test setup, the sample was kept in the same solution as the external solution in the test tank. Once the hydrogel reached equilibrium swelling, it was put into the tank filled with 45 ml of 0.9 wt% NaCl solution to begin testing, and under four different voltages — 0V, 5V, 10V, 20 V—, samples was

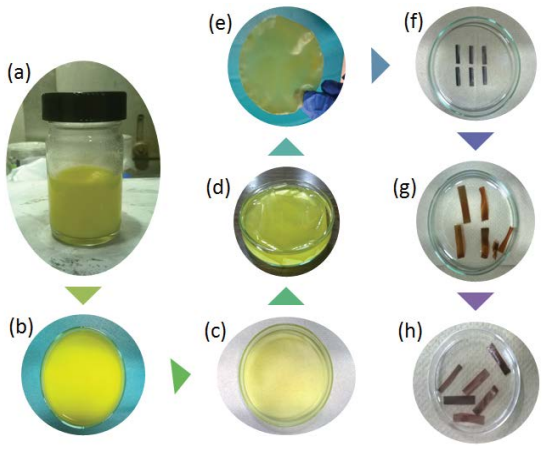

Figure 2: Steps in producing electric field-sensitive MTX-PVA hydrogel strips: (a) Cross-linked solution after addition of GA, (b) solution poured into petri dish, (c) hydrogel after drying at 50°C, (d) hydrogel thin film kept in deionized water, (e) hydrogel thin film after drying at 40°C, (f) hydrogel strips baked at 80°C, (g) strips kept in deionized water, and (h) final strips dried at 40°C.

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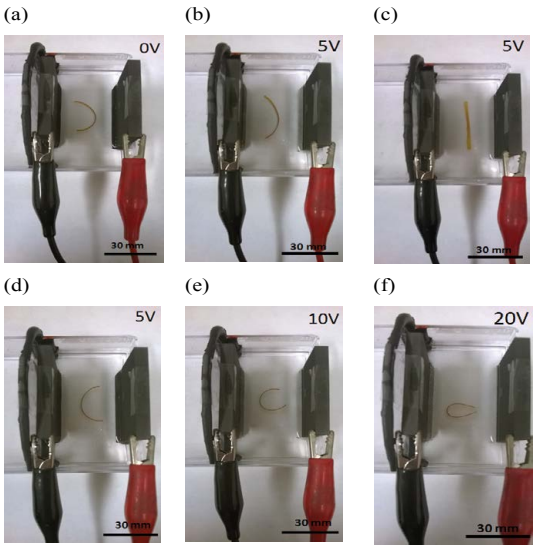

Figure 3: Response of SA-PVA hydrogel to applied electric fields: (a) hydrogel sample under 0 volt (V), (b) through (d) hydrogel strip under 5V, (e) hydrogel strip under 10 V, (f) hydrogel strip under 20 V. Black alligator is anode and red alligator is cathode.

started to be collected. First, 3.2 ml of solution was collected from the external solution as voltage was applied. A 3.2 ml sample was taken from the area around the hydrogel strip every 5 minutes. The syringe used to remove the solution was placed close to the shrunken surface of the hydrogel, in order to increase the chances of extracting more of the released drug. This process was continued for 60 minutes, and 12 samples were obtained at the end of the drug release test. Later on, 3.2 ml of the sample solution was poured into a cuvette of the UV-Vis spectrometer and results were obtained. Absorbance values of the samples were utilized to interpret the results. Low absorbance indicates that the amount of released drug was low, and high absorbance indicates that the amount of drug released into solution increases.

Cell Viability Assay by MTT Assay: MTT assay was performed to provide insight into the release profile of the drug carrier and its efficiency against cancer treatment. L-929 fibroblast cells and MDA-486 breast cancer cells were used for the cytotoxicity test. Cell viability was measured depending on the reduction of 3-[4,5-Dimethylthiazol-2-yl]-2,5-Diphenyltetrazolium Bromide (MMT). In this assay, 6 different hydrogel samples were used, which are SA-modified PVA hydrogel, MTX loaded SA-modified hydrogel, and 4 MTX loaded hydrogels from release studies under 0V, 5V, 10V, and 20V. Once the supernatants were saved for day 1, day 4 and day 7, 10^4 cells were incubated with these supernatants at 1:1 and 1:25 dilutions in 96-well plates for 3 days. Later, 20 µl of MTT was added to each well of 96-well plates. The MTT was kept for 6 hours in the wells. Later on, MTT was replaced with 200 µl of 10% SDS and maintained at 37°C overnight. Finally, 150 µl of SDS was transferred from each well, and optical density studies were carried out.

Results and Discussions

Results of bending tests

Bending test was performed to see whether the produced hydrogel strips are electric field sensitive. It can be seen that while the strip was bent toward anode due to internal stress before applying voltage, it started bending toward the cathode after applying voltage (Figure 4). Eventually, it can be seen that the strip changed its direction to the cathode side, and tips of the strip moved close to each other. It was observed that at the moment the strips placed in the tank, they showed significant bending capabilities. They also exhibited an increase in bending speed corresponding to the increased voltage. When voltage was increased from 0V to 5V and then from 5V to 10V and finally from 10V to 20V, the bending of the hydrogel progressively increased, and at further voltage levels, two tips of the strips touched each other. This resulted from shrinking/swelling or bending of the gel inside the external solution. The images in Figure 3 indicate that SA has successfully modified the PVA hydrogel, meaning that the hydrogel film produced is sensitive to an electric field.

Results of FTIR spectra analysis

The FTIR test was performed on the prepared samples to identify existing and new generating bonds. FTIR results of the PVA powder show that at 3328 cm^{-1}, a very strong and broad peak is given to the hydroxyl (-OH stretching vibration) group, and at 2945 cm^{-1}, another strong peak is given to C-H asymmetric stretching. Other peaks are attributed to CH-OH bending at 1427 cm^{-1}, C-H bending at 1337 cm^{-1}, C-O-C stretching vibration at 1098 cm^{-1}, and C-C stretching at 854 cm^{-1} (Figure 4A). Figure 4b shows the FTIR spectrum of PVA hydrogel including glutaraldehyde. As can be seen, the absorbance at 1098 cm^{-1} (C-O-C stretching vibration) increased, compared to the IR spectrum of the PVA powder. This indicates that glutaraldehyde was crosslinked with PVA, so that the peak at 1098 cm^{-1} was increased. The spectrum of MTX added to PVA hydrogel is shown in Figure 4c. This spectrum indicates a new kind of bonding. The peak at 1644 cm^{-1} indicates the presence of N-H bending (primary amines). Also, the peak at 1602 cm^{-1} indicates the presence of aromatic hydrocarbons (C-C stretch (in-ring)), which belong to the MTX chemical structure having a primary amine group, tertiary amine group, carboxylic group, and secondary amide group. Also, aromatic groups (in-ring) are present in the structure. Figure 4d shows the IR spectrum of SA added to the PVA hydrogel. It can be observed that there is a new peak formed at the wavelength of 1232 cm^{-1}, which is the absorption band of the sulfonic acid group (–SO_3H). Also, the peak value of 1731 cm^{-1} indicates the formation of an ester (–COO–). From the result, it can be concluded that SA reacted with the PVA hydrogel, and PVA hydrogel was modified successfully. Heat treatment effects on SA-MTX-PVA hydrogels were investigated by FTIR, and the spectra of them are shown in Figure 4d and 4e. From these spectra, it can be observed that heat treatment caused the –OH peak to be deeper compared to the sample without heat treatment. This was due to hydrolysis of the ester bonds from the heat, meaning that carboxylic esters hydrolyzed to the parent carboxylic acid and an alcohol. When ester bonds were hydrolyzed, –OHs were generated, so that depth of the peak increased.

Results of drug release tests

The samples collected every 5 minutes under 0V did not give any absorption peak during the 60 minutes. It indicates that the hydrogel structure can keep the drug inside while there is no voltage applied. "Due to the absorption values of 0 obtained at all time periods, only the absorption value belonging to 5V, 10V, and 20V were compared in two separate graphs (Figures 5 and 6). From Figures 5, it can be clearly seen that the curve belonging to 5V exhibited the lowest release property.

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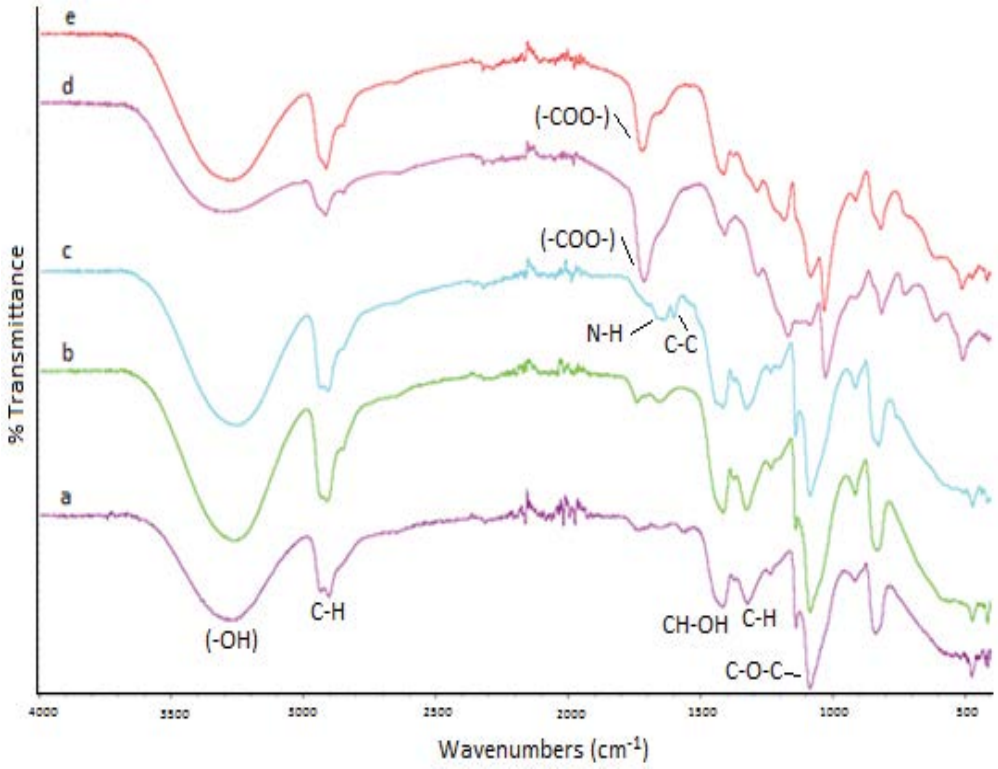

Figure 4: FTIR of spectra: (a) PVA powder, (b) PVA hydrogel, (c) MTX-loaded PVA hydrogel, (d) SA modified MTX-loaded PVA hydrogel without heat treatment, and (e) SA modified MTX-loaded PVA hydrogel with heat treatment.a

Moreover, the huge gap between the curves of 5V and 10V shows the stronger voltage effect on drug release behavior. However, when the voltage was increased to 20V, the release amount did not show a large difference, indicating that after a certain voltage, drug release capability will not change significantly. Another conclusion is that drug release first increases and then starts decreasing when it reaches a peak. From Figures 5, it can be seen that curves reached their peaks earlier with increasing voltage. While the 5V curve made the peak at 35 minutes, the 10V curve made it 25 minutes, and the curve belonging to 20V had a peak at 15 minutes. These results indicate that the power of the stimuli increased the released amount of drug every 5 minutes. It also caused a higher amount of drug to be released in a shorter period of time. Knowing this behavior is very important in applying this system in terms of good control of drug release. By changing the voltage level, it is possible to control the release time and the amount of the released drug. To compare the cumulative absorption of samples on which different voltages applied, Figure 6 was created. The effect of voltage change on MTX release can be clearly seen. According to this chart, it can be definitely concluded that increasing the voltage value after 10V did not change the released amount of drug significantly. However, a slight increase can still be seen in the absorbance. On the other hand, the curve belonging to 10V exhibited a higher total amount of released drug compared to the 20V curve. The reason for this is the smaller number of samples collected from the 20V test. The reason for this is that the evaporation of water with the temperature in the tank was caused from the high voltage. Also, decreasing the amount of external solution because of both evaporation and drawn water every 5 minutes might have caused a slower diffusion rate of ions in the solution, and also, the number of ions must have decreased.

Results of MTT assay

Figure 7 shows images of cancer cells after MTT assay. Figure 7A and B shows cell in obtained from wells containing medium only. As shown, in the medium, not containing any supernatant, numerous cancer cells are seen. However, from Figure 7C and D, it's obvious that supernatant collected from the PVA + MTX sample reduced the number of the cells drastically. Since there was no release study performed on it, the sample kept all the drug in its structure, so that it could kill the cancer cell. On the other hand, the sample on which 20 volts was applied could not reduce the cell number as much as the PVA + MTX sample did Figure 7E and F. Because, during released under 20 volts, most of the drugs were released, the hydrogel structure did not release much drugs in the period of supernatant collection for MTT assay. For control purposes, images from SDS-loaded wells were also taken (Figure 7G and H). Results indicate that the PVA + MTX sample reduced the cell number almost as much as did the SDS. The *in vitro* study provided an estimation about drug release profiles of samples and also the drug concentrations necessary for effective treatment. Figures 8 and 9 show the MTT assay results for the breast cancer cell, MDA-486, at two different concentrations. Results indicate that with increasing voltage, the released amount of the drug increased, so that the number of living cells decreased. As shown, the PVA + MTX sample was the most effective one for cancer cells because the percentage of living cells is shown as being the lowest. On the other hand, the sample on which 20V was applied exhibited the highest percentage of cells, meaning that after applying voltage, most of the drug was released in release study and the sample became less effective in MTT assay due to the lower amount of drug remained in

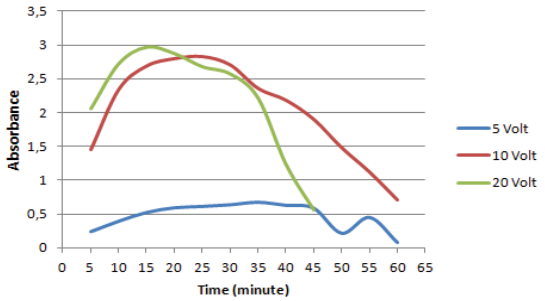

Figure 5: Comparison of absorption curves acquired every 5 minutes during 60 minutes.

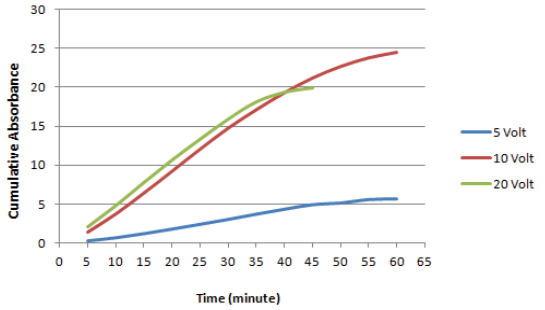

Figure 6: Comparison of cumulative drug release curves belonging to three different voltage values.

the structure. Also, when dilution is made 1:25, it is seen that overall living cell percentages increased for hydrogel samples compared to 1:1 dilution. However, for both dilutions, it is obvious that there is an inconsistency between living cell percentages of each sample obtained from different supernatant from day 1, day 4, and day7. Results of the MTT assay using L-929 cells are shown in Figures 10 and 11. Again, results show a similarity with results of the MTT assay using MDA-486 cells. As seen, however, if results belonging to MDA-486 and L-929 are compared, it can be clearly seen that MTX is more effective on MDA-486 than it is on L-929. Living cell percentage values from PVA sample (SA-modified hydrogel crosslinked with glutaraldehyde) in 1:1 dilution exhibited low cell viability for the first day release (day 1) (Figure 12). However, for day 4 and day 7, cell viabilities were seen to increase. These results suggest that glutaraldehyde is toxic to body at low dilutions only for first day it is placed to body. After first day release, living cell percentages were seen to be more than 70%, indicating that hydrogels stopped releasing toxic substances into body after 1 day. On the other hand, 1:25 dilution gives better results for supernatants from all 3 days. As seen in Figure 13, living cell percentages were around 100, meaning that for 1:25 dilution, samples did not show any cytotoxic effect. Although the percentages of living cells show consistency among the six samples, the released amounts of the drug on days 1, 4, and 7 show inconsistency. It was expected from hydrogels to release the drug at maximum amount for day 1, and the minimum amount for day 7. The result expected from day 1 was supposed to show the lowest living cell percentage due to the highest release of the drug. Likewise, day 4 was expected to show a higher number of cells compared to day 1, and day 7 was expected to show a higher number compared to day 4. However, results collected from days 1, 4, and 7 exhibit inconsistent changes. This situation is thought to have resulted for several reasons: non-homogenous dispersion of drug in the gel, different thickness cuts from the sample, and different cross linkage percentages.

Conclusions

This study was aimed at fabricating electric field-sensitive PVA hydrogels for controlled drug delivery purposes. For this purpose, non-electrolyte PVA was modified by sulfoacetic acid, so that the structure was introduced with a negative charge. ATR analysis results showed that all the expected peaks are present in the spectra meaning that crosslinking and sulfoacetic acid modifying steps were successful. Also, due to negative charges, hydrogel strips became electrosensitive and exhibited bending behavior in the presence of the applied voltage. Bending test results showed that the hydrogel strip was bent toward

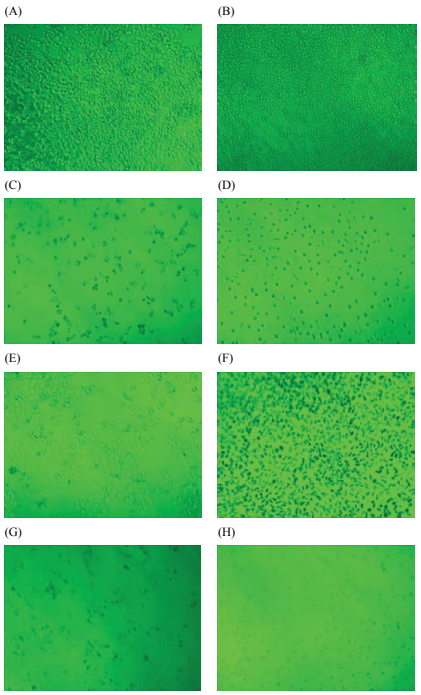

Figure 7: Images of (a) MDA-486 cells in medium only, (b) L-929 cell in medium only, (c) MDA-486 cells in supernatant of sample PVA+MTX, (d) L-929 cells in supernatant of sample PVA+MTX, (e) MDA-486 cells in supernatant of sample PVA+MTX+ 20V, (f) L-929 cells in supernatant of sample PVA+MTX+20V, (g) MDA-486 cell in SDS, (h) L-929 cell in SDS.

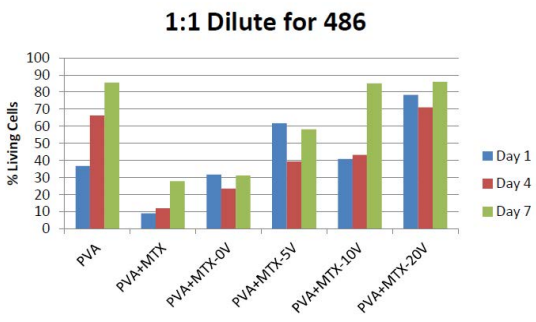

Figure 8: Living cell percentages for 1:1 dilution of MDA-486. PVA refers to SA-modified PVA hydrogel, PVA+MTX refers to MTX loaded SA-modified PVA hydrogel, and PVA+MTX-(0V, 5V, 10V, 20V) refers to MTX loaded SA-modified hydrogels after release study under 4 different voltages of 0V, 5V, 10V, 20V.

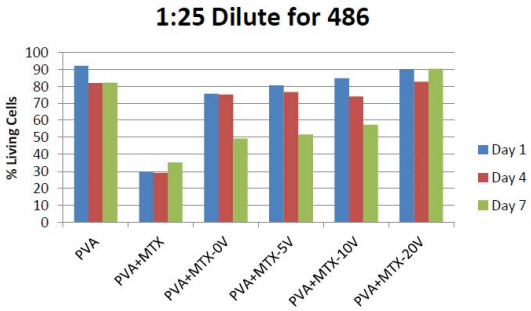

Figure 9: Living cell percentages for 1:25 dilution of MDA-486. PVA refers to SA-modified PVA hydrogel, PVA+MTX refers to MTX loaded SA-modified PVA hydrogel, and PVA+MTX-(0V, 5V, 10V, 20V) refers to MTX loaded SA-modified hydrogels after release study under 4 different voltages of 0V, 5V, 10V, 20V.

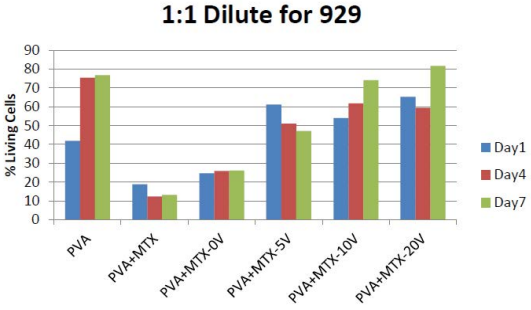

Figure 10: Living cell percentages for 1:1 dilution of L-929. PVA refers to SA-modified PVA hydrogel, PVA+MTX refers to MTX loaded SA-modified PVA hydrogel, and PVA+MTX-(0V, 5V, 10V, 20V) refers to MTX loaded SA-modified hydrogels after release study under 4 different voltages of 0V, 5V, 10V, 20V.

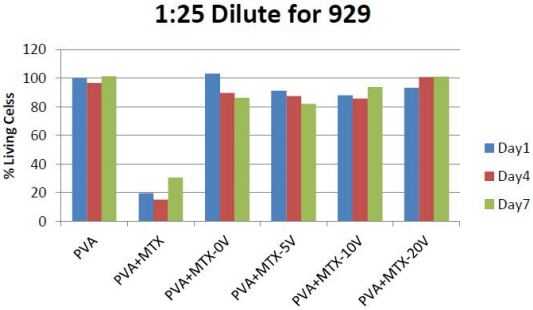

Figure 11: Living cell percentages for 1:25 dilution of L-929. PVA refers to SA-modified PVA hydrogel, PVA+MTX refers to MTX loaded SA-modified PVA hydrogel, and PVA+MTX-(0V, 5V, 10V, 20V) refers to MTX loaded SA-modified hydrogels after release study under 4 different voltages of 0V, 5V, 10V, 20V.

the cathode corresponding to the applied voltage. Bending test results also support the results of ATR analysis. According to UV-Vis results, increase in applied voltage led more amount of drug to release, which suggests that under the various applied voltages, a controlled drug release was achieved. MTT assay results showed that by increasing the voltage, the amount of drug released also increased; therefore, the structure containing a lesser amount of drug showed the lowest effectivess, which was the sample applied with 20 volts. MTT results has also confirmed the results obtained from drug release study. Cell viability increased with increasing voltage due to lower amout of drug remained in the structure during release drug release test. Moreover, MTT assay showed that MTX is more effective on breast cancer cells meaning that it shows selective toxicity aganist breast cancer cells. This study may open up new possibilities for the controlled drug delivery applications.

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