A BIOCOMPATIBLE HYDROGEL SYSTEM FOR ACTIVE PH CONTROL

By

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Abstract of "A biocompatible hydrogel system for ac-tive pH control"

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Potential Hydrogen (pH) of a solution is one of its key characteristics and the ability to control it in a process would lead to increase chances of success. In this study we describe the development of a hydrogel system fabricated from gellum gum and manufactured by crosslinking method. Sodium hydroxide/Hydrochloric Acid was encapsulated into the hydrogels and the release was investigated. The obtained release profile indicated that the hydrogel maintained continuous release of the substance. Further studies indicated that the hydrogel could maintain pH of extreme cases with solution pH of 10 to solutions which are very sensitive like with pH 7. Our work also showcases that these gels can be used for extended periods of time without drastic change in their behavior. By manually adding acid at a fixed time interval to an aqueous solution initially in equilibrium with our hydrogel, our work showed that the solution with hydrogel successfully counteracted with the external acid and maintained a high pH value while the control group (with no hydrogel present) exhibited continuous pH decrease. We were also able to prove using simulation that the release is dominantly Fickian and our calculated diffusion coefficient was 2.23 x $10^{-9} m^2/s$

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2 Introduction

Despite numerous advancements in the treatments for various types of cancers, cancer remains a leading cause of death worldwide [1]. Recent work in understanding the nature of diesease and defining characteristics has revealed a pH-based relationship between malignant and healthy cells. Rapidly growing tumor cells, develop faster than the blood supply.[2][3]. This leads to an increase in the acidity of the tumor microenvironment due to increased production of metabolic acids (carbonic and lactic acids)[4][3].Resulting in a hypoxic microenvironment within the tumor mass[2][3]. As a result, the extracellular pH (pHe) of malignant solid tumors is acidic, in the range of 6.5 to 6.9, whereas the pHe of healthy tissues is significantly more alkaline, 7.2 to 7.5 [5][3]. It is hypothesized that decreased pHe aids tumor invasion by promoting normal cell death and extracellular matrix degradation of the parenchyma surrounding growing Tumors.[5][3][6]

Experiments conducted both *in vitro* and in vivo to test this hypothesis have been promising. [6][7][8]. The *in vitro* study involved studying pH modulation within the rat cells by feeding them sodium bicarbonate ($NaHCO_3$). This bicarbonate therapy resulted in a significant decrease in metastases and an increase in overall survival despite there being no impact on the primary tumor growth [7]. In [7] there was no impact in tumor growth due to a rapid acid production rate of the tumor compared to the bicarbonate fed to the animal and he predicts that if there is a delivery system to provide higher amounts of bicarbonate it can reduce tumor growth [7]. Given that the acid production rate of tumors can be 100 mol/hr/g of tumor weight, oral ingestion of bicarbonate is not a viable solution[9].

Another important application for pH control is production of hydrogen (H_2) which is an important source of renewable energy. Given the minimal changes required to substitute gasoline engine to hydrogen power, it is a promising gasoline substitute. Toyota already has a production car that uses this source of energy[10]. H_2 can be produced by microbial fermentation of organic substrates. The anaerobic fermentation process promises to be an economical and sustainable technology for H_2 production. This process currently lacks the cost efficiency for mass production, however with changes like pH control, cost efficient mass production can be achieved. Experimental data has shown that pH control during the fer-

mentation process can significantly affect production yields[11][12][13][14][15].

Most commonly used lab methods that allow pH control are titration and addition of buffer compounds. Both are primarily used for laboratory purposes as they neither economical or practical to be used for applications described above. Most commonly used lab methods are titration and addition of buffer compounds. Titration works by knowing the exact pH of the solution and adding the right amount of counter solution (base or acid depending on whether pH needs to be increased or decreased) to the media[16][17]. This involves external interaction with the media at all times, to both measure its pH and add solution, which is often difficult to accomplish. Buffer solutions on the other hand are a mixture of weak acid and base thus if the solution gets very acidic or basic it counteracts the changes by reacting with the new ions to bring it back closer to the original pH[18][19]. This is only effective if there is a drastic shift in the pH or a vast quantity of the buffer is needed to be effective which makes it very expensive or impractical for use. We found out that gellan gum hydrogel encapsulated with an acid or base is an excellent candidate for pH regulation applications. These hydrogels provide an excellent solution because their versatility allows them to be applicable in highly harsh basic/acidic pH and in sensitive pH range (6-8). Being able to engineer them allows to regulate pH through a control activation, this means that even a limited quantity of these gels could be highly effective.

From cell encapsulation to stretchable electronics, hydrogels have successfully exhibited its versatility in wide array of applications [20][21]. The most common application of hydrogels is drug delivery, due to its high water content and biocompatibility [22]. Drug molecules are mobile and free to move in and out the aqueous network formed by the polymeric chains inside the hydrogel. Gellan gum hydrogel is a natrual biocompatible polysaccharide and has been approved by Food and Drug Administration (FDA) as a food additive [23]. Numerous publications have demonstrated its excellent capability as a drug deliverer to achieve sustainable release [24][25][26]. Moreover, gellan possesses tunable mechanical properties given credit to two separate yet relevant thermal-activated mechanisms, namely coil to helix transition and solution to gellation transition [27][28].

3 Materials and procedure

3.1 Materials

The following materials were used in the experiments described and presented in this paper. Deionized (DI) water was filtered through Millipore filtration system in all experiments requiring water. 99% pure calcium chloride ($CaCl_2$) dehydrate was used for preparation of gels and was purchased through Chem-Impex. Gellan Gelrite 50% sodium hydroxide (NaOH) solution and 25% hydrochloric acid (HCl) solution was purchased through Sigma Aldrich.

3.2 Preperation of hydrogel

Using a new 50 mL pipette, 20 mL of the deionzied water was transferred into a 50 mL beaker. 500 mg of gellan gum powder was added to the beaker and then vigorously stirred using a glass rod to ensure that no clumps of gellan gum are formed. After stirring for about 10-15 seconds, glass rod was removed and the solution was heated at boiling temperature for 6-7 seconds. Solution was brought back to ambient temperature and stirred again. This process was repeated until all air bubbles initially present have been removed and the mixture became transparent.

Separately, 8.3 mg of dry $CaCl_2$ powder was weighed out and sealed in a plastic vial [29]. Following that, 5ml of DI water was mixed with NaOH or HCl solution, whose amount was designed by individual experiment, and added to the vial. The vial was manually placed on a vortex mixer to ensure the homogeneity of the solution. Removed from the mixer, the solution in the vial was then poured into the beaker with the hydrogel mixture and stirred again for about 10 seconds. Once homogeneous, solution was transferred into a plastic petri dish, sealed with lid and placed inside a refrigerator for final cooling. After about an hour, solid hydrogel was formed and ready to be used for experiments. This procedure produces 2% w/v and 3mM $CaCl_2$ gellan hydrogel with varying NaOH or HCl concentration depending on the experiment requirement. Using an 8mm biopsy punch, individual hydrogel samples were punched out of the petri dish. This resulted in cylindrical samples of size 8mm diameter and 10 mm height, which is shown in Figure 1B. All experiments described below used the cylindrical samples.

Mechanical characterization of the gellan hydrogel was performed on an MTS Bionix table-top test system with axial-torsional load frame. Young's modulus of the hydrogel was acquired by uniaxial compression test and determined to be 7 kPa at ambient temperature.

3.3 Release experiments

3.3.1 Release rate and mechanism experiments

To understand the release mechanism of our hydrogel, we used NaOH embedded hydrogel and the amount of total released NaOH were collected for the first 30 minutes. Eight experiments were carried out in total with NaOH embedded hydrogel. We used various masses of NaOH solution in the preparation stage, including 50 mg (n = 1), 200 mg (n = 6) and 527 mg (n = 1). One cylindrical hydrogel sample described in section 3.2 was put into a beaker with 15 mL of deionzied water and the pH of the water was measured every five minutes. The initial pH value of the deionized water was also taken individually for each experiment and it was 7. The solution in the beaker was constantly stirred by magnet stirrer to provide a 'perfect sink' condition around the hydrogel.

The acquired pH values need to be converted into mass of released substance. The general equation is

$$M_{t,X} = \mathrm{MMass}_X \cdot V \cdot (c_{t,X} - c_{0,X}) \tag{3.1}$$

where $M_{t,X}$ is the time-dependent released mass of a substance X which could be either an acid or a base, MMass_X is its molar mass, V is the volume of solution that the hydrogel is submerged in and $c_{t,X}$ and $c_{0,X}$ are time-dependent and initial concentrations of substance X, respectively. For NaOH, since its molarity to OH^{-1} is 1:1, the concentration difference can then be expressed as a function of pH

$$c_{t,X} - c_{0,X} = [10^{-(14-\mathrm{pH}_t)} - 10^{-7}] \tag{3.2}$$

taking into the initial pH of DI water, where pH_t is the time-dependent pH value of the solution. The same argument was carried out for other release experiments where the expression for concentration difference is slightly different than equation 3.2 depending on the pH range the experiment was conducted.

3.3.2 Active pH control experiments

Active pH control capability of our hydrogel was demonstrated by utilizing six beakers in total, three out of which served as the control group without the presence of hydrogel. Each of the beakers was filled with 15ml of deionzied water and its initial pH was artificially calibrated to 10.5 by addition of NaOH solution. One sample hydrogel was put in three non-control beakers at the same instance. Once the hydrogels were introduced, pH of the solution was measured every 5 minutes. Each time before measuring the pH, solution was swirled to ensure homogeneity.

4 Results and discussion

4.1 Release rate and mechanism

In order to successfully characterize the diffusion mechanism inside the hydrogel, simulations were performed as validation methodology to experimental results. Numerical simulations were carried out using finite element software Abaqus, in a three-dimensional mass diffusion analysis governed with Fickian-type diffusion model. Taking advantage of the cylindrical symmetry, CAX4 axisymmetric elements were utilized and only half of the middle cross section plane was modeled. The boundary condition was identical to the experiment setup, with the NaOH concentration on all the surfaces of the hydrogel to be zero at all times. The initial condition was to assume that NaOH was initially uniformly distributed inside the hydrogel. Leaving the only unknown material parameter to be the diffusion coefficient for NaOH inside the hydrogel. Our prediction for the diffusion coefficient is carried out as follows: assume that the ions (Na^+, OH^-, etc.) are spherical with a well defined radius, then we can apply Stokes-Einstein relation which gives an analytical expression of the diffusion coefficient for a spherical particle through a liquid

$$D_0 = \frac{k_B T}{6\pi\eta r} \tag{4.1}$$

where k_B is the Boltzmann's constant, T is the temperature, η is the viscosity of the liquid and r is the radius of the particle. The D_0 here is often treated as the reference diffusion coefficient which is valid for an ideally diluted solution. We further assumed that the motion of ions was Brownian and that the viscosity of fluid inside the hydrogel has the same value

as pure water, which is 8.9×10^{-4} Pa·s at room temperature 25°C. This assumption fails for most drugs which has a radius in the same order of magnitude of that of gellan gum. However in our case, the diffusive substances are OH^- ions which are extremely small. The radii of an OH^- ion and a Na^+ ion are approximately 0.11 nm and 0.1 nm respectively while the radius of the double helical backbone of crosslinked gellan gum chain is approximately 15 nm [30][31]. The significant difference in the size makes the polymer chains not an effective obstruction to the Brownian motion of OH^- ions, hence justifying our assumption. Another evidence that supports our assumption can be found in this journal article where it summarized correction to reference diffusion coefficient in hydrogels using various approaches such as free volume theory, hydrodynamics and obstruction [32]. Because of those additional mechanisms involved in the diffusion process, the real diffusion coefficient in gel, D_g, is often smaller than D_0.

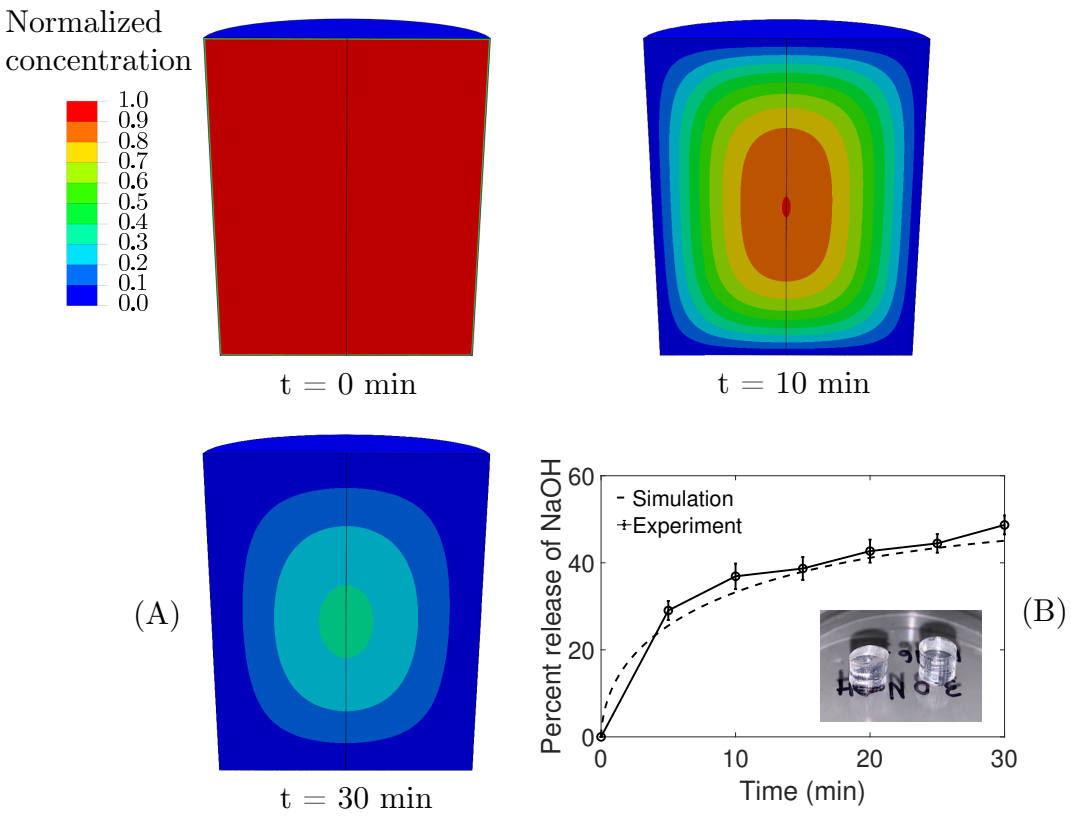

Figure 1: (A) Cross-sectional view of a simulated cylindrical hydrogel. Normalized concentration of initially uniformly distributed NaOH is shown at $t = 0$, 10 and 30 minutes. (B) Comparison of percent release between simulation and experiments over time (with hydrogel picture inserted).

But given our experiment condition with very low polymer volume fraction which is lower than 5%, the ratio between diffusion coefficient in a gel D_g due to polymer chain hindrance and reference diffusion coefficient D_0 is very close to 1. Hence, we used the reference diffusion coefficient predicted by equation 4.1 in the simulation. Figure 1(A) shows the normalized NaOH concentration in the middle cross section of a hydrogel sample at different time steps. Half of the sample is shown here for better visualization. The aggregate percent release from the sample in the simulation was calculated by summing up the diffusion flux at all the surfaces throughout 30 minutes. The simulation result is plotted with the experiment for this percent release in Figure 1(B) where it is normalized against 50% of the total NaOH inside the gel, as we only have a maximum release of 50% which will be discussed shortly after. Excellent agreement is achieved between simulation and experiment, with error bars

being small. Although there might be other factors affecting the diffusion of ions such as the electrostatic force between polymer chains and ions, this agreement reveals that Fickian diffusion is the main release mechanism in our case.

After the observed Fickian release, the gels reach an equilibrium condition and no more release is observed. The amount of release is a function of difference in concentration of OH^- ions in the gel and outside. To prove this, we filled 3 beakers with different concentrations of NaOH, initial pH of 7, 9 and 10. Next, we added hydrogels made with 1.8mg of NaOH to each of the beakers, pH inside the gel was calculated to be 13.0. After 36 hours the pH of all three solutions were observed to be 10.5 and the pH inside the gel was 12.950, 12.953, 12.967 respectively. The reason this is only a small difference between pre and post gel pH is due to the non-linear nature of pH scale.

Other than demonstrating Fickian type release, hydrogels also demonstrate ability to actively turn on and off to maintain a consistent solution pH. figure below shows experimental results to demonstrate this effect, X-axis is time measured in minutes, after adding gel to the beaker and y-axis is aggregate amount of NaOH released in milligrams. Starting with a solution of pH 10.48, we introduced hydrogel with pH 13.0 into it. Data was measured every 5 minutes until 80 minutes. Concentrated HCl (25% by wt) was pippeted 4 times over next 60min, amount added was : 500ml, 200ml , 200ml and 500ml as indicated in the figure. The pH of the solution over the first 20 minutes rose to 11.3 and stabilized, however after first batch of acid was administered there is yet another 5% release, indicating hydrogels ability to actively control the pH of the solution. However this trend does not continue forever, after about 50% release gels do not demonstrate any more release regardless of the pH difference. As a result at the end of 80 minutes, pH of the solution ultimately drops to 7.3 while the pH inside the gel was still at pH 11.0.

Amount of NaOH released was measured by observing the pH change of the solution and then converting it to mass using method described in 3.3.1. Overall release witnessed after 80 minutes is observed to be 0.9mg or 50% $\pm$10%, this suggest a threshold release amount after which no further release will occur regardless of the concentration difference.

4.2 Release Threshold

As demonstrated from the experiments above there is a threshold release amount which is why the total release is restricted to 50% and the same reason why even at equilibrium concentration in the gel and in solution are not equal. We believe this is due to ionic bonds between gellan and NaOH ions.

Typically when ramping down the temperature, the random coil-like gellan polymer chains in aqueous environment will self aggregate and form a fixed double-helical shape. This coil-helix transition establishes the initial mechanical strength for gellan.[33] Further reducing the temperature causes the subsequent formation of additional physical crosslinks between polymeric chains in the presence of ionic salts[34]. Because of the anionic nature of gellan hydrogel, the positive ions in those salt such as calcium chloride tend to bind the polymer chains together and form ionic crosslinks. Even though sodium ions and calcuim ions are both present, due to the divalent nature of calcium ions they are likely to form bonds.[35] These crosslinks greatly enhance the mechanical strength of gellan, especially for divalent salts[36]. Qualified with such desired properties, gellan hydrogel is used as a carrier for acid and base release.

pH of the solution while creating the hydrogel has significant affect on the gel and its physical properties.[37][38][39] [40] hydrogels produced at neutral pH tend to have a more tightly bounded structure whereas gels produced at low pH were found to have more permeable structure[4]. Given the changes observed in physical properties of the gel simply by changing the pH of the solution, suggests that presence of extra ions interact with gellan molecules and form bonds. This could be happening because of formation of hydrogen bonds which bind excess ions with the gellan molecule. This would lead to reduced amount of ions available for release.

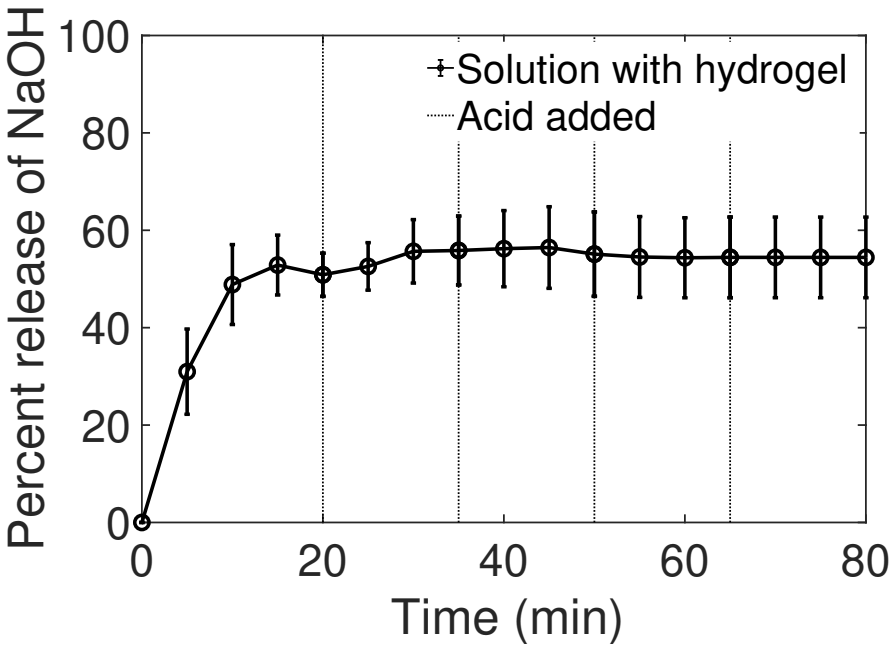

Figure 2: Aggregate release of NaOH from hydrogel over 80 minutes in percentage, normalized by the initial amount of NaOH in the hydrogel. Vertical dotted lines indicate the times when varying amount of HCl was added to the solution. Same dashed lines are used in subsequent figures for the same purpose unless otherwise specified.

4.3 Active pH Control

As described in section 3.3.1, once the hydrogels are introduced into a solution they diffuse the OH^- until an equilibrium is reached between the solution and the hydrogel. However from then on, increase in solution acidity is counteracted by additional OH^- diffusing out of the hydrogel. To demonstrate this effect and its versatility we have conducted series of experiments with varying amount of acid/base, these are split in three different categories: extreme acidic, weak acid and weak basic. These are discussed in detail below.

4.3.1 Highly Acidic

Figure 3 demonstrates the ability of these hydrogels to survive and perform in high basic environment. Initial pH inside the hydrogel was 13.0 and they were added to a solution with pH 10.5 and 10.7 respectively. Once the hydrogels were added to both the solutions, pH rose and reached a new equilibrium. Over next 60 minutes, acidic solution prepared using concentrated HCl (25% wt) with a pH of 1.7 was added in following amounts: 500, 200, 200, 500 mg for experiment Figure 3(A) and 200, 200, 200 mg for experiment Figure 3(B). pH was measured before adding the acid and immediately after, thus the corresponding time

indices have two data points. Over the next 80 minutes, equal amount of acid was added three more times. One can see that the data has extremely small error bars, which speaks to the quality of the data and its repeatability. One can see that Figure 3(A) shows release after first acid was added again however Figure 3(B) shows no more release after first 20 minutes, suggesting yet again that the release is dependent on the concentration difference between the hydrogel and solution. Both the experiments also show that while the pH of control solution becomes quite acidic whereas pH of solution with gel remains above 10.0.

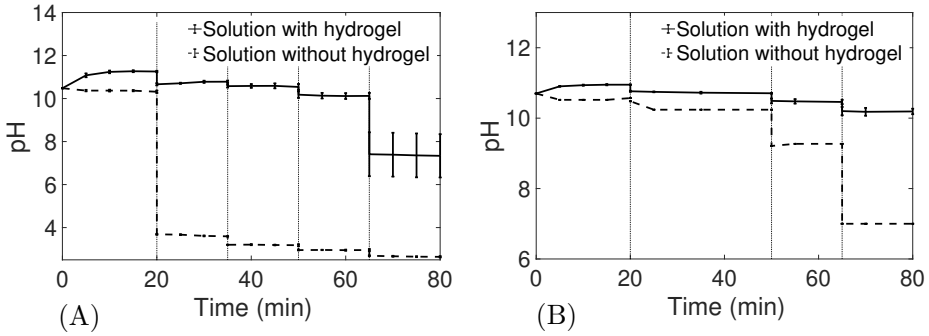

Figure 3: Active pH control of NaOH encapsulated hydrogels. (A) pH values of solutions containing hydrogel with 0.08mg NaOH (50% wt) and (B) pH values of solutions containing hydrogel with 3.56mg NaOH (50% wt) are shown with control solutions (without hydrogel) over 80 minutes during which varying amount of HCl was added multiple times to the solution.

4.3.2 Weak Basic

Another set of experiments we did were with hydrogels encapsulated with acid instead of base. The pH of the gel was initially at 2.85 and the pH of solution was at 7.5. After reaching initial equilibrium, concentrated NaOH solution was added twice to ensure that the pH was increased to 9. As shown in figure4, hydrogels brought the equilibrium pH down everytime it was artificially increased. Amount of acid released from the gel is a lot less compared to the previous case, initial 20 min show release of 1 % , followed by 0.5% for the two times after acid was added. Concentration difference of hydrogen ions between this case and previous is about 3 orders of magnitude smaller and since concentration difference governs release, it explains the extermely small release observed. Conclusions drawn from both these experiments are quite significant, they suggest a novel method to selectively alter pH of any solution. An ability to actively counteract any external perturbations in a way to maintain equilibrium pH.

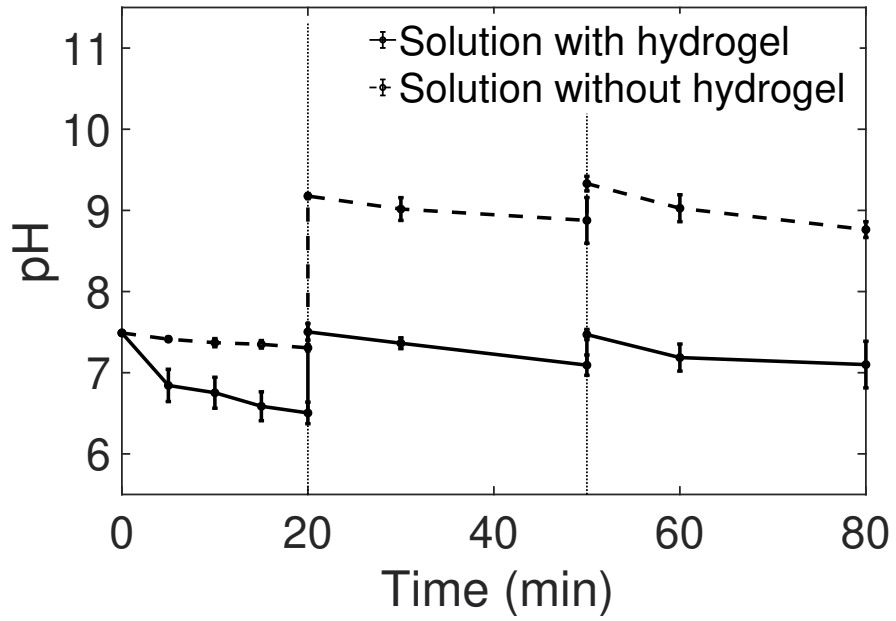

Figure 4: Active pH control of HCl embedded hydrogels. pH values of solutions containing hydrogel with 5mg of HCl(25 % wt) are shown with controlled solutions (without hydrogel) over 80 minutes during which varying amount of NaOH was added multiple times to the solution. Vertical dotted lines indicate the times when NaOH was added to the solution.

4.3.3 Weak Acidic

As demonstrated by the cases above, these hydrogels can be tailored for a variety of applications. Previous section demonstrated the ability to control pH in highly acidic and weak basic conditions, next we look at a specific case of controlling pH around a cancerous tumor. As described in introduction there is a small but significant pH difference between extracellular matrix around cancer tumor vs healthy tissue. Previous work have demonstrated that if the pHe is brought above 7, it can reduce metastasis rate and potentially also reduce growth rate of the tumor[7]. We believe these hydrogels can achieve both reduction in metastasis rate and reduce tumor growth rate. We ran another experiment to demonstrate this effect, the hydrogels would be required to control pH within a highly sensitive scale, namely 6.5-7.5.

The experimental setup varied slightly from the previously presented ones. We started with an initial solution pH of 6.5. In order to get stable readings and minimize ambient noise we took the following measures: we increased the sensitivity of the probe by adding sodium chloride to the solution and autoclaved the apparatus before starting the experiment. In order to better simulate the application and prove that the results observed are time

independent we performed this experiment over 5 days, significantly longer than the previous data. Acid was added to the solution everyday and data measured multiple times during the day. The quantity of acid added each time was varied (although same amount was added in all 6 beakers every turn), amount to be added was precalculated to ensure that the beakers with hydrogel would result in a pH of 6.5. As seen in plot 5 the results meet design predictions, after 5 days and adding acid 4 different times the control solutions have fallen to very acidic pH 3.3 whereas the solutions with hydrogels have successfully able to keep the pH at 6.7. The error bars appear to be quite consistent as well, to have this tight of a variance specifically in this pH range is quite challenging to achieve. This work

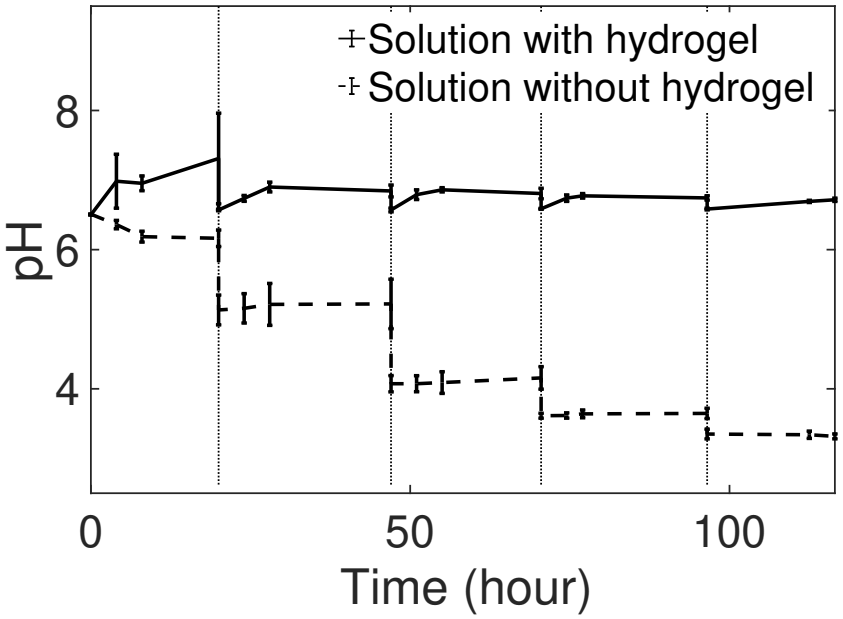

Figure 5: Active long-term pH control of NaOH encapsulated hydrogels at near-neutral pH region. pH values of solutions containing hydrogel with 0.04mg of NaOH(50 % wt) NaOH are shown with controlled solutions (without hydrogel) over 120 hours during which varying amount of HCl was added multiple times to the solution.

demonstrates that these hydrogels can be tuned to suite applications as needed. That they can be used in highly acidic and basic conditions and also in sensitive neutral conditions. In all the cases they can successfully counteract external perturbations to affect the pH of the solution. This versatility and combined with the gels ability to turn on and off based on need make them a long-lasting solution in a wide array of applications.

5 Conclusion

Our experimental and simulation work demonstrates results for a pH system in equilibrium despite external effects, which was achieved through gellan based hydrogel encompassed with acid or base.

We were also able to demonstrate by correlating our experimental data with computer modeling that the release seen is dominantly Fickian based. This indicates that the overall release amount is based on hydroxide ion concentration difference between hydrogel and external solution. It also provides us with an ability to tailor these gels for sensitive applications like impacting metastasis rate to extreme cases like increasing efficiency of hydrogen production. Of which we have demonstrated 3 cases: 1) controlling pH of a system which is attempting to change from pH of 10.5 to 3.5 2) controlling pH of system that is trying to transition from 6.5 to 7.5 3) lastly, controlling pH of system trying to transition from 7.5 to 6.5. Based on the maximum allowable release, release rate from our simulation work and knowing the concentration difference between hydrogel and solution we are able to create a relationship that allows us to engineer hydrogel design to control pH to a level that is required.

The above mentioned experimental data proves that our hydrogels can actively keep the environmental pH stable. Even though overall release is limited to 50% , it can control pH in extreme conditions. It can maintain the pH of a solution to an equilibrium value over varying conditions. This is an important trait that is unique to this product and required in numerous applications, some of which are described above. Although the focus of this paper is more for cancer research, it is equally applicable in energy production. Existing methods to regulate pH are specific to lab applications as described in introduction.

These experiments suggest using hydrogels as pH regulators could be very beneficial. Ongoing work includes to tailor the gels specific to application requirements. With a focus towards cancer application, we are pursuing : 1) to reduce the size of the gel to nanometer length scale, 2) to design and conduct an *in-vitro* experiment.Shrinking the gels to a micro size is the next progression of this research [41][42].

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