A pH-mediated electronic wound dressing for controlled drug delivery

A thesis submitted by

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Abstract

Topical administration of drugs in a timely controlled manner according to the physiological needs can enhance the healing process in chronic wounds. Here a pH-mediated electronic wound patch is presented. This smart patch is capable of pH-based active drug release where the pH of the patch was itself controlled electronically. The wound patch is comprised of electrodes coated with a PEGDA/Laponite hydrogel layer containing chitosan nanoparticles (CHPs) as drug carriers. The engineered patch is pH responsive and enables the on-demand release of drug by electronically adjusting the pH. Applying electrical voltage between working and reference electrodes triggers charged ions and a consequent pH change. In basic pH, the CHPs were stimulated and drugs were released while in acidic pH the release profile was negligible. The pH shifting implemented by electrical volatge would not affect the pH of the wound site and its biocompatibility was confirmed in this study. This platform can provide precise control over administration of drug in order to improve the therapeutic effectiveness while reduce the side effects of the drugs.

Keywords: Controlled drug delivery, pH responsive material, smart wound dressing, stimuli responsive drug delivery, electrical stimulation

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Introduction

Chronic wounds do not heal in an orderly manner due to the lack of timely release of biological factors essential for healing (Bello & Phillips, 2017). Chronic wounds are a major medical challenges in the United Stated; and the treatment of these wounds are becoming more challenging every day (Fonder et al., 2008). Furthermore, chronic wounds are prone to severe complications, which not only affect the time to heal but also have a negative impact on the patients' quality of life (Siddiqui & Bernstein, 2010).

Smart wound dressings are typically made of stimuli-responsive particles and a controller (Mostafalu et al., 2017). There are two different types of stimuli-responsive particles used for drug delivery depending on the source of stimulation factor. If the system responds to local changes in environment, it is called self-regulated or a closed loop system and this normally occurs with enzyme or competitive substances. On the other hand, externally regulated mechanisms governed by outside stimulation forces such as ultrasound, temperature, electric and magnetic fields provide more user control (Meng, Zhong, & Feijen, 2009). Use of an externally regulated stimuli-responsive system enables the immediate treatment and precise release profile control by adjusting the rate and dosage, externally. Recently, we have used temperature as a stimulus for a smart dressing through use of the thermo-responsive particles (Mostafalu et al., 2017; Bagherifard et al., 2016). However, the wound's temperature varies from patient to patient and environmental thermal noise prevents precise release control. Recently, pH has been introduced as an alternative stimuli-factor for drug delivery applications; a slight pH difference between healthy and cancerous tissue was exploited to design anticancer drug delivery systems. Chitosan swelling due to the amino-group protonation (pKa ~6.3) leads to the release of encapsulated tumor necrosis factor alpha (TNFα) in the local acidic environment of tumor tissues. pH responsive materials have also

been widely used for oral drug delivery to protect the drugs from harsh environments in stomach and to improve the drugs absorbance in intestine (Mura, Nicolas, & Couvreur, 2013). Swelling or dehydration of pH-responsive materials allows for release profile control. However, application of these studies is limited as the pH was used for the passive delivery of the drugs and there is a lack of precise control over the release profiles.

Incorporation of pH responsive materials for active delivery in management of chronic wounds would be a superior alternative, as it is not significantly affected by external environmental factors (Alexander, Andreas, Grabbe, & Dissemond, 2007). Only severe infection of the chronic wound might inhibit the pH stimulus. However, it would lead to a positive consequence and enables self-regulation in treatment of such wounds. In this research, we engineered a pH responsive wound dressing capable of on-demand release of drugs by electronically controlling local pH. Use of an electronically regulated system enables on-demand delivery by temporal control of the release profile.

2. Motivation

Existing wound dressings such as Alginate, foam, hydrogel dressings are designed to cover the wound to maintain humidity and pressurize the wound (Kiaee & Kiaei, 2016; Ovington, 2007; Song, Rane, & Christman, 2012). These dressings all provide the passive release of one drug which alone is not sufficient to complete the healing process. However, Smart wound dressings can provide superior healing support by enabling the on-demand release of drugs if needed (Ganta, Devalapally, Shahiwala, & Amiji, 2008). The controlled release of drugs can provide more efficient therapy by reducing side effects and enhancing patient compliance (Takahashi, Yamada, Kataoka, & Nagasaki, 2005).

Controlled drug delivery to the wound makes this possible to deliver drugs in a consistent and sustained manner over long period of time without the need for frequent dressing changing (Alhusein, Blagbrough, & De Bank, 2012). Controlled release of drugs to a target tissue can prolong the action of the active drugs over time by allowing continual release from the polymeric dosage form which lead to the increase in therapeutic efficiency. Some drugs have an optimum range of concentration within which the maximum therapeutic effect can be seen and the drug concentration above and below this range can be toxic or produce no therapeutic effect. Sustained or continuous release of drug over an extended period of time can be achieved with polymers that release drugs at a constant rate due to diffusion or by degradation of the polymer over time. In some cases, sustained release is not the optimal way of drug delivery and pulsatile release of drug is preferred in order to mimic the natural way of body. For example, the continuous administration of some hormone can cause the desensitization of receptors and may suppress the release of that hormone. Insulin is the well-known example of compound produced by the body in a pulsatile manner.

We engineered a pH responsive wound dressing capable of on-demand release of drugs by controlling the local pH change using the electronic system. Incorporation of the electronic system enables on-demand delivery, and temporal control of the release profile. The proposed pH responsive wound dressing is comprised of electronic driver, microelectrode fabricated on flexible substrate, and PEGDA/Laponite hydrogel contained chitosan drug nano-carriers (Figure 1). A localized pH was changed electronically inside the hydrogel around the electrodes without significant alteration of the wound pH. It resulted in manipulation of the drug carriers and release profile (Figure 1). This platform enabled us to control the pH of the hydrogel dressing precisely.

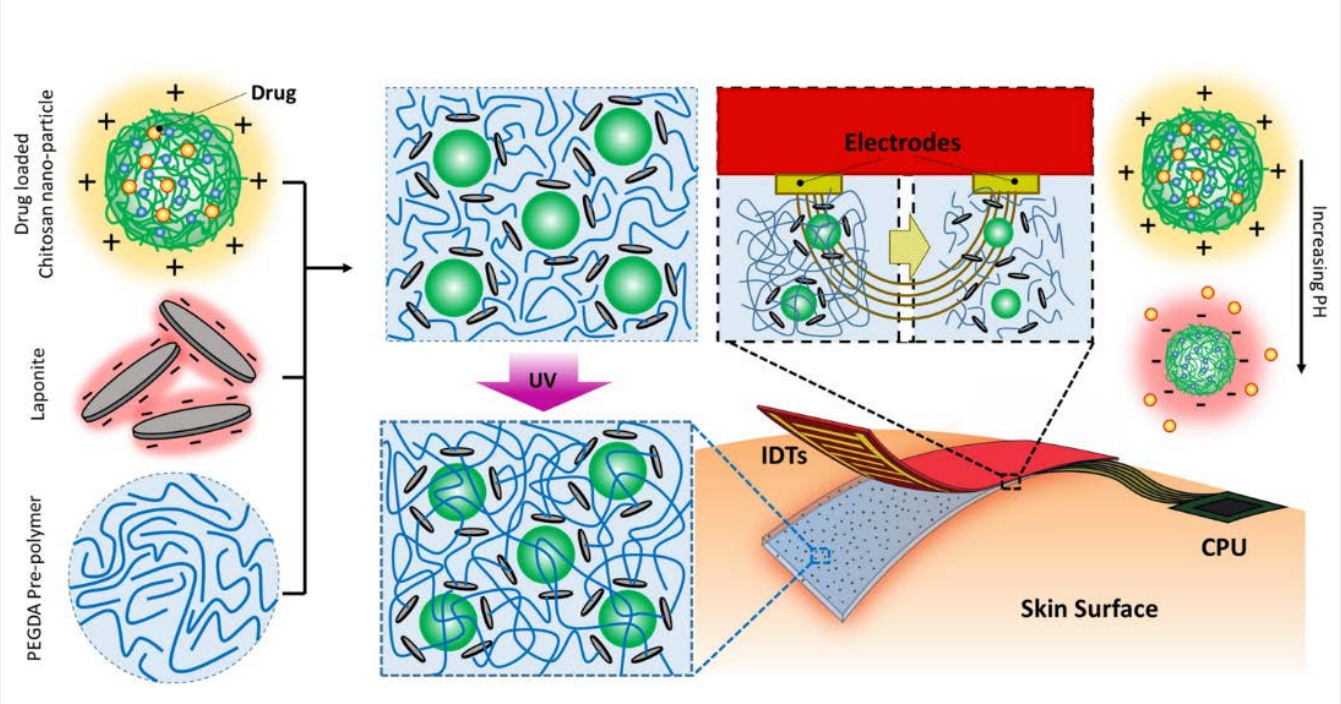

Figure 1. Schematics of an integrated electronic wound dressing for the transdermal drug delivery in which a pH responsive hydrogel layer carrying drug loaded chitosan nanoparticles was placed on a flexible electrode and connected to a microcontroller that will power them up. The inset shows the mechanism of the drug release with the application of electric field. The electrostatic interaction between negative face of Laponite and positively charged chitosan nanoparticle in acidic pH would be reduced in basic pH produce by electric field and lead to the drug release.

3. Literature Review

3.1 Chronic wound

Wound is described as break in the skin as a result of physical or thermal damage or presence of an underlying medical or physiological condition (Siddiqui & Bernstein, 2010). Wound can be classified as acute or chronic wounds based on the nature of the repair process (Fonder et al., 2008). Acute wounds are usually healed within 8-12 weeks; however it takes more than 12 weeks for the chronic wound to heal completely. Normal wound healing has three phases: inflammation, proliferation and remodeling with wound contraction. Chronic wounds have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation (Bello & Phillips, 2017). Different factors can cause chronic wounds such as diabetes, vascular insufficiency, neurological defects, and advanced age. Chronic wounds have long been the major concern of health care provider. The burden of treating chronic wounds is growing rapidly due to increasing health care costs, and an aging population in the United States and beyond (Fonder et al., 2008). These wounds affect the life of more than 7 million in the US and require regular screening by medical professional. Non-healing wounds cause enormous health care expenditure with the total cost of more than 3 billion dollars per year (Fonder et al., 2008). Chronic wounds are prone to a severe complication, which not only affect the time to healing completion but also have a negative impact on the patients themselves (Siddiqui & Bernstein, 2010).

3.2 Wound treatment

Wound dressing have developed from the natural products such as animal fat and honey to tissue engineering scaffold. The current wound dressing can retain and create moist environment around wound to facilitate wound healing. The engineering scaffold can also deliver bioactive material

such as growth factor and antibiotic to the wound. The local administration of antibiotic may be a preferred option to systemic administration due to the lower antibiotic doses required in topical administration in compare to the systemic administration which reduced the systemic toxicity. In addition, the local delivery from dressing can overcome the problem of ineffective systemic antibiotic therapy resulting from poor circulation especially at the extremities in diabetic ulcers. While the antibiotic administration can treat and prevent infection, the growth factor administration can affect the inflammatory, proliferation and migratory phases of wound healing.

3.3 Stimuli responsive drug delivery

Stimuli responsive polymer can be a great option to deliver drugs in a controlled way. There are two different types of stimuli responsive drug delivery depending on the source of stimulation factor. If the system responds to local changes in environment, it is called self-regulated or a closed loop system and this normally occurs with enzyme or competitive substances. On the other hand, externally regulated mechanism governed by outside stimulation forces such as ultrasound, temperature, electric and magnetic fields.

The application of external regulation stimuli-responsive systems enables the immediate treatment and precise control of release profile by adjusting the rate and dosage externally. Recently temperature was used as an stimuli-factor for the activation of the smart dressing by incorporation of the thermo-responsive particles (Bagherifard et al., 2016). However, the wound's temperature varies from patient to patient and environmental thermal noise disturb the precise control of the release. On the other hand, the pH was introduced as another stimuli-factor for the drug delivery applications. For example, the slight difference of pH between healthy tissue and tumor was exploited to design anticancer drug delivery systems. Chitosan swelling due to the amino-group protonation (pKa ~6.3) leads to the release of encapsulated tumor necrosis factor alpha (TNFα) in

the local acidic environment of tumor tissues. The pH responsive material also have been widely used for oral drug delivery to protect the drugs from harsh environment in stomach and to improve the drugs absorbance in intestine (Mura et al., 2013). Swelling or de-swelling of pH-responsive materials allows the control of release profile. In these studies, the pH was used for the passive delivery of the drugs and there is a lack of precise control on the release profiles.

Incorporation of pH responsive materials for the active delivery in management of chronic wound would be a superior alternative, as it is not affected significantly by other environmental factors. Only severe infection of the chronic wound might interpolate the pH stimuli materials. However, it would lead to a positive consequence and enable self-regulation in treatment of the wound.

3.4 pH responsive material

3.4.1 Polyelectrolyte

Polyelectrolytes are polymers with relatively high Ionizable groups along the backbone chain. In polar solvent such as water, Ionizable groups can be dissociate completely or partially depending on the polymer dissociation constant (pK_a or pK_b), and moreover their charge can be modified by changing the solution pH, counter ion concentration, or ionic strength. The physical properties of polyelectrolyte solutions are strongly affected by this degree of charging. The polyelectrolyte can affect the solution's ionic strength, since their dissociation is released counter-ions. This in turn affects other properties, such as electrical conductivity. Some example for polyanion are polymer with anionic groups such as, sodium polystyrene sulfonate, sodium polyvinyl toluene sulfonate, sodium polyacrylate, sodium salts of the hydrolyzed copolymers of styrene with maleic anhydride, sodium polyvinyl sulfonate, and the corresponding free acids (when sufficiently water-soluble) as well as corresponding salts of other alkali metals. Polymers containing cationic groups include

polyvinyl benzyl trimethyl ammonium chloride, polyethyleneimine, polyvinyl pyridine, poly(dimethylaminoethyl methacrylate), quaternized polyethylene imine, quaternized poly(dimethylaminoethyl) methacrylate, polyvinyl methyl pyridinium chloride. The preferred polymers are those containing sulfonate groups and those containing quaternary ammonium groups.

Layer by layer assembly of polycation and polyanion in the form of thin film layer or microcapsule, have attracted much attentions for the development of drug delivery system (Sato, Yoshida, Takahashi, & Anzai, 2011). The electrostatic interaction between charged chain due to osmotic and Coulombic forces can be modulated by charge screening with the use of solutions of varying ionic strength or pH. When the pH becomes higher (in the case of a polybase) or lower (in the case of a polyacid) than the pKa, the polyelectrolytes become uncharged, which in turn, disassembles the capsules or film (Petrov, Antipov, & Sukhorukov, 2003). It happens by the application of an electrode potential to induce local pH changes by the electrolysis of water or hydrogen peroxide on the electrode surface. The pH changing can accelerate the diffusion of the drug encapsulated in the film layer or the microcapsule by enhancing the permeability (Sato et al., 2011).

3.4.2 Chitosan

Chitosan, a (1–4) 2-amino-2-deoxy-b-D glucan, has structural characteristics similar to glycosaminoglycans. This polycationic biopolymer is generally obtained by alkaline deacetylation of chitinwhich is the main component of the exoskeleton of crustaceans, such as shrimps (Khor & Lim, 2003). Chitosan has interesting biopharmaceutical characteristics such as pH sensitivity, biocompatibility and low toxicity (Yao et al., 2014; Kim et al., 2007). Moreover, chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered as biodegradable (Peter et al., 2010). Due to these favorable properties, the interest in chitosan and its derivatives as

excipients in drug delivery has been increased in recent years (Ueno, Mori, & Fujinaga, 2001; Govender et al., 2005). The interest in chitosan arises mainly from the fact that this natural polysaccharide allows the production of biocompatible and biodegradable drug delivery systems. It is extremely important that chitosan be aquosoluble and positively charged. These properties enable it to interact with negatively charged polymers, macromolecules and polyanions on contact in an aqueous environment. Chitosan has the special feature of adhering to mucosal surfaces, a fact that makes it a useful polymer for mucosal drug delivery (Govender et al., 2005; Mi et al., 2002). Many studies have reported the use of chitosan in the formation of gels, particles, and microspheres. Chitosan also has pH-sensitive properties due to the protonation–deprotonation equilibrium of the amino groups, which allowing the fabrication of pH controlled release carriers that are based on chitosan (Pasparakis & Bouropoulos, 2006). Natural polymers, such as collagen, alginate, and chitosan carry a number of advantages over the synthetic polymers. One such example is the reduced need for harsh processing conditions (Venkatesan & Kim, 2010). The natural polysaccharides are also very abundant and their production is both relatively environmentally safe and of low cost (Peter et al., 2010).

3.4.3 Polyethylen diacrylate

Poly (ethylene glycol) (PEG) is a hydrophilic, water soluble, biocompatible polymer that has been suggested for use in a variety of biomedical applications. Substituting terminal hydroxyl groups with acrylates, forming poly (ethylene glycol) diacrylate (PEGDA), allows the polymer to be crosslinked to form a three-dimensional polymer network. While PEGDA may be crosslinked by a variety of methods, the use of photopolymerization is particularly versatile for use in tissue-engineering applications. The polymer is poorly immunogenic, non-toxic at molecular weights above 400 Da, is readily cleared by the kidneys, and is approved by the FDA for internal

consumption. The polyether backbone cannot be degraded by mammalian enzymes. Enzymatic degradation of a cross-linked polymer can therefore be initiated only by the controlled inclusion of degradable sequences at crosslinking sites (Browning, Cereceres, & Luong, 2014). The PEG molecule is neutral, highly mobile, and heavily hydrated in aqueous solution, with a large exclusion volume (Takahashi et al., 2005). These properties have been used to explain PEG's inherent resistance to protein adsorption when covalently crosslinked: the molecule has few sites for protein binding, its high mobility allows little time for proteins to form positive attachments, and the surrounding water molecules exclude other molecules from nearing the polymer surface (Takahashi et al., 2005) . The resistance to protein adsorption allows PEG-based hydrogels to act as blank slates for cell adhesion, as they can be rendered selectively cell adhesive by the addition of specific ligands but will otherwise not support the adsorbed protein layer that mediates cell attachment to most materials used in biological applications (Yinghong Xiao, 2012; Browning et al., 2014). Photopolymerization allows for spatial and temporal control of polymerization as well as formation of complex shapes (Yinghong Xiao, 2012). With careful selection of an appropriate photoinitiator, crosslinking can occur under sufficiently mild conditions to permit encapsulation of living cells within the polymer matrix (J. Liu, Nguyen, Andya, & Shire, 2005). PEGDA hydrogels are highly tunable. The mechanical properties of the hydrogels can be controlled by varying the molecular weight or concentration of the polymer, with an increase in elastic modulus with increasing polymer concentration or decreasing polymer molecular weight (Wang, 2011; Ghadiri et al., 2013; Shi, Li, Maciel, & Tom, 2011). The mesh size and swelling ratio can be similarly controlled (Pasparakis & Bouropoulos, 2006), and the mechanical and biochemical properties can be varied independent of one another (P. Li, Hoon, Hui, Yop, & Hee, 2009).

3.4.4 Laponite

Natural clays have been used by humanity for purposes ranging from filtration, cosmetics, architecture, and even healthcare for millennia. Wound treatment involved clays as early as 2500 BC. Other examples include clays as absorbents for topical or gastrointestinal ailments. However, exploration of clays as a scientific field began in the 1930s and pharmaceutical interest in clays has increased over the past two decades (Dawson, Kanczler, Yang, Attard, & Oreffo, 2011). A variety of clays have found utility in pharmaceutical applications. Laponite is a synthetic example of a hectorite clay mineral, belonging to the smectite clay group (Mongondry, Tassin, & Nicolai, 2005). These clays are part of a larger group of silicate-based materials known as phyllosilicates, which categorize minerals composited of tetrahedrally coordinated silicates. Clays within the smectite group have a 2:1 layered structure, with an octahedral sheet sandwiched between two tetrahedral sheets (TOT) (Ghadiri et al., 2013). This structure generates sheets that are commonly 1 nm thick while the width of the sheets can vary. Synthetic techniques have been able to achieve a well-controlled diameter of 25-30 nm for Laponite materials. The smectite group is relatively charged (~0.2-0.6 charge/formula unit), due to hydroxyls within the tetrahedral structure and are countered by cations. Lastly, the species of Laponite clays, hectorite, a trioctahedral subtype, all have the following formula:

$$(Si_4)^{IV}(Mg_{3-y}Li_y)^{VI}O_{10}(OH)_2 \cdot yM^+ \cdot nH_2O$$

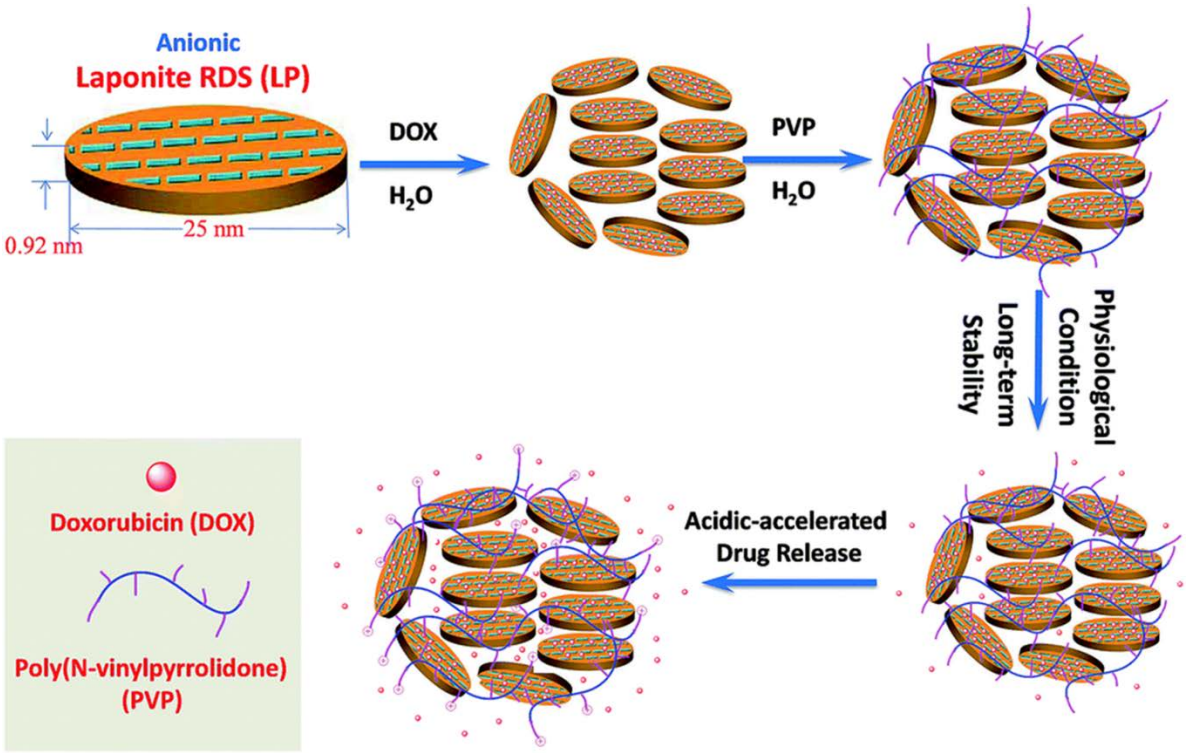

Figure 2. Basic outline for designing a functional drug carrier from ref (Mongondry et al., 2005).

Of importance to their use as therapeutic drug carriers is the presence of water in the equation. These clays are hydratable and capable of ion exchange between the cations intercalated between layers (Mg^{2+} or Li^{+}) and other molecules (Figure 2).

As is characteristic of all hectorite clays, the planar silicate sheets are hydratable, resulting in the ability to swell in aqueous solution and difficulty when attempting to dissolve in organic or high ionic strength solutions (Dawson et al., 2011). The swelling process of Laponite is dependent upon the chemical potential of the aqueous solution it is in as well as the cation present in the interlayer space (P. Li et al., 2009). When dry, Laponite and other hydratable clays form stacks of TOT sheets, between 10 and 100 layers thick. When mixed in aqueous solutions, dependent on salt concentrations, individual sheets can be completely exfoliated, characterized by XRD or SAXS,

or generate 2-4 layer hydrates at higher salt concentrations (Shi et al., 2011). This cation-dependent exchange can be described by the following reaction:

$$z_j X_{z_i} C_i + z_i C_j^{z_j+} \leftrightarrow z_i X_{z_j} C_j + z_j C_i^{z_i+}$$

Where z is the valence of the ion, X is the negatively charged exchange site, and C is the cation, either in solution or on the clay surface. The exchange of selected ions is dependent on the species of clay and must be determined experimentally. This exchange is what allows uptake of other charged species in solution, such as drug molecules, into the interlayer spaces of the particles.

The water that is finally present in the interlayer spaces is unique compared to bulk water and strongly associates with the cation. When hydrated in solutions with well-characterized dyes, the absorbed dyes are noted to have a red shift in UV spectra based on the new, more polar environment encountered within the interlayer space. Additionally, with higher concentrations of dye, the clays become more hydrophobic and eventually precipitate, making knowledge of the cation exchange capacity (CEC) crucial when loading with small molecules (Table 1).

Table 1. Characteristics of Laponite Pertinent for

Applications

Property	**Common Values**	**Comment**	**Ref.**
Charge	~0.2-0.6 charge/formula unit		(Dawson et al., 2011)
pH	Coagulation by salts pH>6	pH can serve as a signal to release bound drug molecules	(Shi et al., 2011)

	Spontaneous coagulation pH<4		
Cations	Mg^{2+}, Li^{+}, Na^{+}	Hydratable clays can swell as cations are exchanged for small molecules	
Cation Exchange Capacity	115 mEq/ 100 g for hectorite	Total specific amount of charge that is balanced by exchangeable cations; determines amount of drug able to be loaded	(Nair & Sharma, 2012)

The charge on Laponite dictates much of its mechanical properties, in addition to its size. Edges and surfaces (basal planes) are differently charged and can therefore adsorb charged species and water differently. Laponite has a permanently positive charge around the edge of the particle. However, it is pH-dependent, capable of being protonated or deprotonated based on the acidity or basicity of the solution (Shi et al., 2011). Altering the pH is a mechanism by which to release loaded molecules and must be considered as a factor when designing drug carrier systems.

As concentrations of clays are increased in aqueous solutions or as ionic strength is increased, changes in dispersion, aggregation or coagulation are observed. Critical to clays dispersion in solution is their critical coagulation concentration (c_k) which is dependent on the type of counterion used. Laponites have c_k~10 mmol/L in sodium chloride aqueous solutions. Phosphates, common in many biological buffers also impact c_k, likely due to their additional capability to strongly buffer solution pH and impact the ionic layer thickness surrounding clays (Table 1).

With this sensitivity to ions, various structures can form depending on the pH, ionic strength, and concentration of the clay particles, which will impact the gel properties. A common cartoon used in Laponite research is the house of cards structure, generated by preferential interactions between the positively-charged edges and negatively charged basal plane of the clay particles. However, this is only true at lower salt concentrations ($< c_k$) or lower pH values (less than pH at the point of zero charge (p.z.c.)), where the positive charge on the edge is able to significantly factor into the system. At higher salt concentrations, face-face van der Waals attractions are able to dominate and generate band-type aggregates which can still transition into the card-house structure. However, salt concentrations that are too high will cause excessive aggregation and sedimentation of the clay solution. Laponite, possibly due to its smaller uniform size is capable of forming stable gels at lower concentrations and higher salt concentrations than other smectites (Figure 3) (Mongondry et al., 2005).

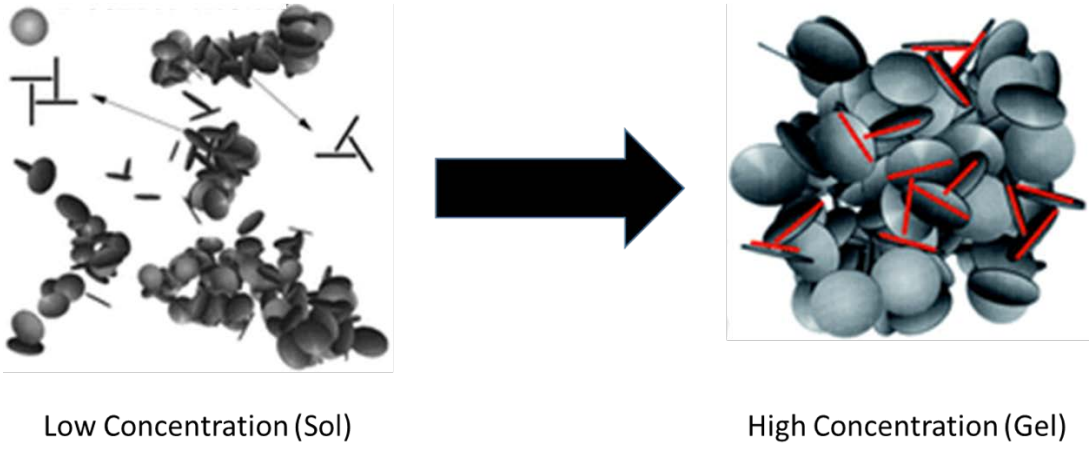

Figure 3. Image highlighting the structure of Laponite networks at lower and higher concentrations (Ghadiri et al., 2013).

This dependence on salt concentration is not so straightforward, as systems can have a narrow window of ion concentrations at which increased mechanical properties (i.e. viscosity, strength)

are observed (Ghadiri et al., 2013). Weak sols are present on both extremes of some smectite clays while others see a reemergence of a gel region at higher salt concentrations. Laponite also can have the property of thixotropy, where there are time dependent changes in the mechanical properties. Laponite experiences a monotonic increase in mechanical properties due to the constant reorganization of micro domains within the network. The network continues to become stiffer, causing slower changes to the mechanical properties (Wang, 2011).

When polymers are included in the system, flocculation or stabilization can occur, depending on the concentration of polymer and their interactions with the clay (Wang, 2011). If the solvent is good for the polymer, steric stabilization can occur, where there are bridges between clay particles of polymer. Destabilization can occur with the addition of a lower quality solvent for the polymer. At higher concentrations of polymer, steric stabilization can occur where the space between particles has a high density of chains, causing solvent to penetrate and prevent precipitation. Dependent on the type of polymer, flocculation, stabilization, or nanocomposites can be formed, as summarized in Figure 4.

Introducing pH sensitive polymers (Maliheh Ghadiri, Herman Hau, Wojciech Chrzanowski, 2013) or stabilizing polymers (Guangxiang Chen, Du Li, b Jingchao Li, a Xueyan Cao, a Jianhua Wang & Guo, 2015) can impact the release and stability of drug loaded systems after delivery. Natural polymers like chitosan and alginate have been used to generate films to coat implants for antibiotic delivery or to improve the loading capacity and sustained release of drug loaded Laponite (Xiao et al., 2016). The charges on the Laponite surfaces are negative and the edge charges are pH dependent which makes Laponite pH responsive. The positive charge of Laponite edge is reduced by increasing the pH. In the same way the negative charge of drug compounds will also increase by rising the pH.

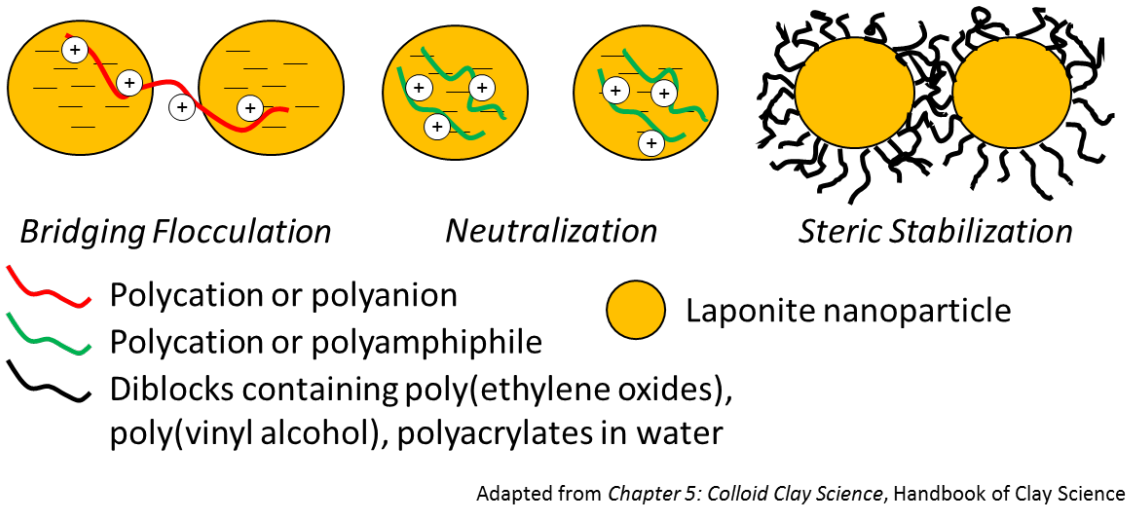

Figure 4. Table of flocculation and stabilization mechanisms possible with clay-polymer systems with accompanying schematics highlight the role of the clay (circles) and polymers (bold lines) in impacting solution state (Shige Wang, et al, 2013).

Because these clay materials have high swelling ability, diffusion of small molecules and permeability of water through colloidal suspensions or gels of them is of interest. However, this behavior is dependent on the age of the solution, as the viscosity increases over time due to the dynamics of clay particle diffusion. Depending on the orientation of the clay particles within the gel or nanocomposite, the permeability of water and other small molecules through the system can be impacted. Well-oriented structures of clay particles can be utilized as barriers to gases and liquids (Viseras, Cerezo, Sanchez, Salcedo, & Aguzzi, 2010). Additionally, randomly oriented particles can present a sufficiently tortuous path to decrease permeability. The size and charge of the molecules also will impact its passage through clay systems. Water can be tightly associated with the clay surface and result in slow dynamics (Duval, Porion, & Faug, 2001). Larger molecules have diffusions dependent on the ratio between their size and the spacing of the clay particles (Petit et al., 2009). As noted above, positively charged molecules can exchange and intercalate between clay particles.

3.5 Local pH changing with DC electrical field

The main mechanism behind pH changing with electrical stimulation is electrolysis of water and the movement of ion toward electrode (electrosmosis). By applying electrical voltage, we are going to have a difference in electrical potential energy between two points (cathode and anode) and consequently the movement of charged particles toward their oppositely charged electrodes (Y. Li et al., n.d.). This movement can directly affect drug release or indirectly change the balance of ionized molecules and pH variance. In addition, Electrical current induces water hydrolysis and produces H^+ and OH^-. The hydrolysis reaction needs a potential difference of 1.23 V to happen (Richards, n.d.). Hydrogen will appear at the cathode and oxygen will appear at the anode. The amount of hydrogen produced at the cathode is twice the amount of oxygen and both are dependent on the applied voltage (Vanags, Kleperis, & Bajars, n.d.). The electrolysis of pure water happens slowly due to the self-ionization of water (Carmo & Fritz, 2013). The addition of electrolyte (such as salt, acid or base) increased the efficiency of water electrolysis. By using an electric field, one can create a pH gradient in the solution. The discharge of H^+ at the cathode in the form of hydrogen gas and leaving behind the hydroxide ion in the solution lead to the increase in the pH of solution (Y. Li et al., n.d.).

The hydrogel response in electrical field (bending, swelling or deswelling) depends on the the shape of the hydrogel and its position relative to electrode determine. For example, the parallel position of the hydrogel to electrode lead to bending whereas perpendicular position of hydrogel to electrode cause the deswelling of hydrogel to happen. Polyanions shrink at the anode while polycationics shrink at the cathode. The hydrogel response to electrical stimulation is happened slowly since volume changes of responsive hydrogels are usually diffusion-controlled (unless gel microparticles are used, where the response is faster). The anisotropic deswelling of gels can often

be observed visually. The gel deswelling may also be accompanied by an increase in gel opacity. The extent of gel deswelling increases with the magnitude of the electric field but is not linearly proportional to it. At higher voltages, the magnitude of the gel response decreased. It is possible that at higher voltages, when gels have deswelled to a certain extent their resistivity to the passage of charges increase as the content of free water decreases. Subsequently, a smaller amount of charge passes through the gel whose response is proportionally smaller.

The ionizable polymer hydrogel gives rise to important electrochemically modulated electroosmotic swelling and deswelling characteristics and this has been explained in terms of electrokinetic processes. Hence, an ionized hydrogel changes volume discontinuously as the solvent composition is continuously varied (i.e. a phase transition occurs). This phase transition is induced not only by a change in the solvent composition but may also be induced by a change in pH, ionic strength (salt concentration), temperature and the application of an electric field. For example, a polyelectrolyte hydrogel when placed between a pair of electrodes deswells under an applied DC voltage with concomitant exudation of water. This property is associated with the electrophoretic and electroosmotic transport of highly hydrated macromolecules and of its counterions. The shape change of hydrogels has also engendered applications as hydrogel actuators in bioMEMS devices due to the hydrogel's dramatic changes in physical dimensions caused by changes in the polarity of the electric field. These chemo-mechanical devices can allow dynamic control of delivery of drug and other solute molecules across and from within hydrogel membranes (S. Lankalapalli and V.R.M Kolapalli, n.d.).

4. Engineering electronic wound dressing for in vitro drug release

4.1 Chitosan nanoparticle fabrication and characterization in different pH

To develop pH responsive drug delivery platform, CHPs were fabricated as pH sensitive materials. The nanoparticles with the drug encapsulation efficiency of 18.5 % were fabricated using ionically cross-linked approach. We prepared 0.2 % chitosan aqueous solution in citric acid 5% containing drug (FITC), then we ultrasonicated it for one minute, and added tripolyphosphate solution in a ratio of 1:5 chitosan solution/TPP and continued the ultrasonication process for 4 minutes. We centrifuged the solution to separate the chitosan Nanoparticle at 14000 rpm for 20 minutes. We removed supernatant and washed chitosan nanoparticle with four different pH buffer, pH 2, pH 5.5, pH 7.4 (PBS) and pH 14 and then dispersed in PBS. The size and zeta potential characterization was done with Zetasizer Nano-ZS90 (Malvern Instruments), the measurement was performed at a scattering angle of 90° at a temperature of 25°C. In summary, the electrostatic interaction between positively charged chitosan (0.2% in citric acid) and negatively charged tripolyphosphate (TPP) (1 mg/ml in water) lead to the formation of chitosan nanoparticle under ultrasonication. The concentration of chitosan solution and ultrasonication speed and duration control the size of the particles. Chitosan is a very established polycation polymer which respond in pH above the 6.5 (H. Liu, Wang, Zou, Wei, & Tong, 2012), and provide a suitable deformation in non-healing chronic wound. Chitosan nanoparticles deprotonated in higher pH and charge acclamations changes. The Zeta potential of these nanoparticles were +40, +25, +1.5, -10 mv in pH 2, 5.5, 7.4 and 14 respectively that confirmed the pH responsive functionality (Figure 5A).

A hybrid hydrogel of PEGDA and Laponite was employed as a hydrogel dressing and chitosan nanoparticles were embedded into it. Poly (ethylene glycol)-diacrylate (PEGDA) is an inert and biocompatible hydrogel, which is not cell adhesive and is a promising material for the wound

dressing. Laponite as a synthetic silicate nanoparticle, consists of disks of nanometric dimensions (25 nm in diameter and 0.92 nm in thickness) with non-toxic degradation products (Na^+, $Si(OH)_4$, Mg^{2+}, Li^+). Laponite particles have negatively charged faces and positively charged edges. Laponite can be added to PEGDA in order to control the drug release more effectively. The self-association of the positively charged chitosan nanoparticle and negatively charged Laponite faces can prolong the release of nanoparticle (Wang, 2011). The competition between the positively charged chitosan nanoparticle and other cations in solution (Ca^{2+}, Na^+, and K^+) determine the amount of nanoparticle that can be encapsulated. The stronger electrostatic interaction between particles and Laponite, the higher the encapsulation efficacy would be. The strength of this interaction calculated by the charge of chitosan nanoparticles which depends on the pKa of chitosan and the pH of the solution. The dissociation of nanoparticles from Laponite happened when other cations take the place of chitosan nanoparticle due to the decrease in particle charge in pH above 6.5 (pKa). Following the particle dissociation in basic pH, the de-swelling of chitosan nanoparticle lead to the drug release. The pH changing from acidic to basic caused the reduction of the intramolecular electric repulsion inside the particle due to the deprotonation of Amin group in Chitosan. The size of nanoparticle diminished from 458 ± 8 nm in pH 2, to 141.77 ± 20 nm in pH 14 which represents this de-sewelling process. The nanoparticles have the size of 255 ± 12 nm and 341± 10 nm in the pH of 7.4 and 5.5 respectively (Figure 5B).

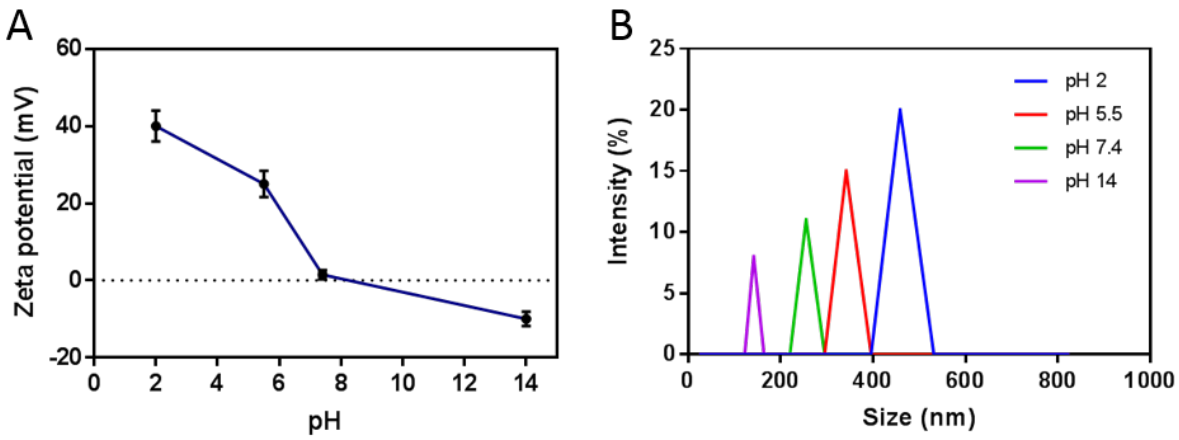

Figure 5. The characterization of chitosan nanoparticle in different pH A) the reduction in CHP size by shifting the pH from 2 to 14 (particle size) confirmed the dehydration process of CHP which was due to the decrease in positive charge of CHP. B) Zeta potential results in different pH.

4.2 Measurement of pH changes around electrode by electrical stimulation

Zinc and Copper was used as an anode (positive terminal) and cathode (negative terminal) respectively. We placed electrodes with spacing of 5 cm in 20 ml release media (PBS) with pH of 5.5. We measured pH changes around anode after applying DC voltage with pH meter. First, we applied different voltage from 0.5 to 5 V and measured the pH changes around anode during electrical stimulation. The maximum pH of 14 was achieved with 5 V after 2 seconds. In another experiment, we applied 2.5 and 4 V in an on/off pattern for 19 seconds to demonstrate the pH changing reversibility around anode. Then in a cyclic pattern with the interval of one minute, we applied 2.5 and 4 V for 9 cycles. We also calculated the rate of pH changing in the distance of 2.5 cm from anode and cathode with the application of 2.5 V and 4 V for 5 minutes. The application of an electrical field, lead to local pH changing due to the electro-osmosis and slight electrolysis of water. The motion of charged ion induced by applied potential can make the gradient of pH. The move of positive ions such as H^+ toward the cathode lead to acidic pH around cathode. At the

same time, negative ions move toward the anode and render basic pH. The local pH changes is temporary and after stopping the electrical stimulation the pH will go back to normal due to the diffusion of hydrogen and hydroxyl ions (Y. Li et al., n.d.). The kinetic of ion movement is voltage dependent, which can affect the rate of pH changing around electrodes, as with 4 V, the rate of pH changing was faster than with 2.5 V (Figure 6B, C). The maximum pH of 14 was achieved in 2 seconds by applying 5 V (Figure 6A). The rate of pH changing also has direct relation with the distance from electrode (Figure 6D). A value of pH=12 was achieved in 2 seconds around anode with the application of 4 V (Figure 6C), however it took 5 minutes to reach that pH in distance of the 2.5 cm from anode (Figure 6D). In addition, the pH buffering capacity of phosphate buffer solutions (PBS) keeps pH at constant range in the region far from electrodes and prevents abrupt pH changing in the solution. We demonstrated the reversibility and reproducibility of pH changing around anode by the application of electrical field in an on/off pattern (Figure 6C). The cyclic electrical stimulation with one-minute interval of applying 4 V and 2.5 V also confirmed the transient pH changing around anode for longer duration of time (10 minutes) (Figure 6B).

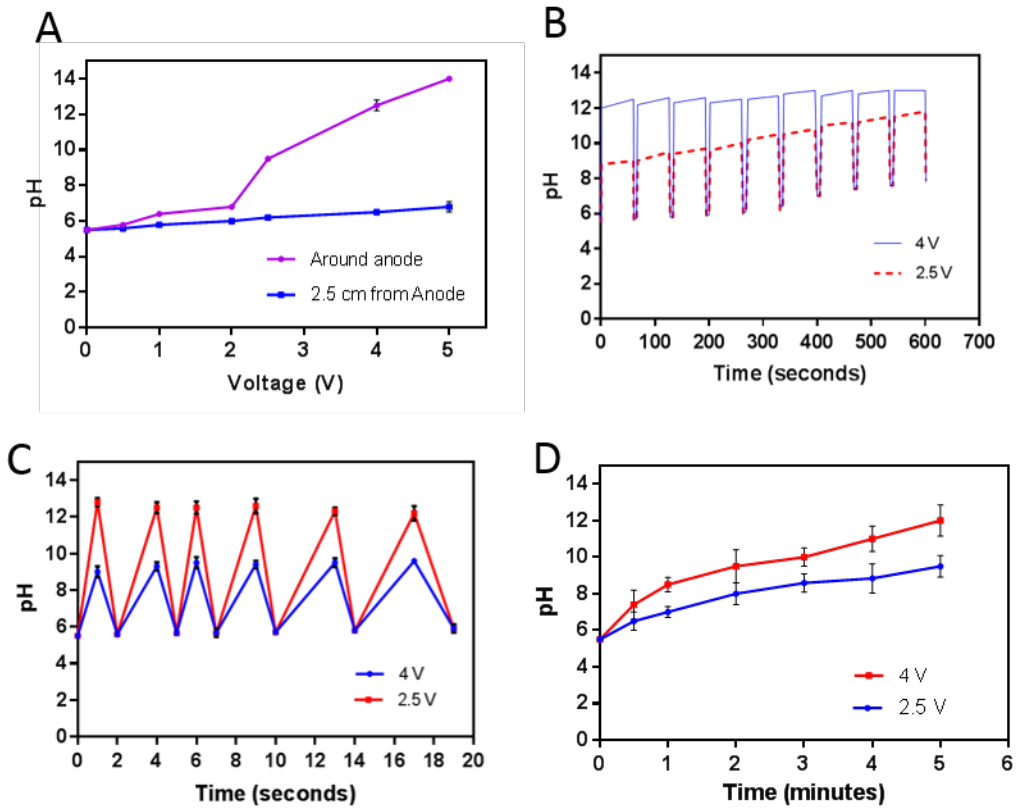

Figure 6. A) The rate of pH changing depends on the applied voltage and the distance from electrode, the pH of 14 was achieved in 2 seconds with 5 V however, in distance of 2.5 cm from anode, the pH changing was slow. B) The control of pH around anode after cyclic electrical stimulation with one minute intervals. C) The dramatic pH changes around electrode was transient and controllable by the application of electrical field. D) The rate of pH changing, 2.5 cm distance from electrode with constant application of electrical field for 5 minutes

4.3 In vitro drug release in different pH

We conducted the *in vitro* drug released test at different pH. FITC-dextran was used as a drug model to study the release profile. We resuspended Chitosan nanoparticles in PEGDA 10%, PI 0.5%, Laponite 1% aqueous solution and UV cross-linked it for 3 minutes, and then the known mass of crosslinked sample with approximate dimension (1 cm ×1 cm) placed into 2ml of release media under constant stirring at 36.5°C. Four different release media with pH 2, pH 5.5, pH 7.4 (PBS) and pH 14 were used to study the effect of pH, on drug release. At each time interval, we

replaced the whole 2 ml of released media with fresh media to guarantee the continuation of the release. We measured the concentration of released drug with ultraviolet-visible spectrometer (UNICAM Series 8700 model, Philips Co., Amsterdam, the Netherlands) using the calibration curve prepared by measuring the intensities of known concentrations in different pH in order to determine the concentration of the released drug models (FITC-dextran: λex 485 nm and λem 530 nm). Then we normalized the results and converted it to percentage cumulative.

The drug release followed distinctive pattern in a different pH. In pH above 6.5, the some of the positive-charged chitosan particles were balanced, thus the ionic interaction between chitosan and TPP which made the particle was diminished and caused the particles to dissemble. In addition, the electrostatic attraction of chitosan particle and Laponite faded away due to the decrease in particle zeta potential. However, in acidic pH, the protonated chitosan formed a strong ionic bond with TPP and retained drug inside the particle. The Figure 7A shows that 3.54% and 7.46% of drug was released after 6 hours in pH of 2 and 5.5 respectively. However, 62% and 100% of drug was released in pH of 7.4 and 14 for the same amount of time. The release rate was also controlled by switching the electrical voltage on and off resulting the basic and acidic hydrogel (Figure 7B). The dissociation of chitosan particle from Laponite happened at pH above 6.5, and the chitosan particle were substitute with another cation like Na^+ due to the reduction in its positive charge. The replacement of chitosan particle by simple cation like Na^+ or Ca^{2+} in acidic pH is difficult to achieve due to the stronger interaction that positively charged chitosan nanoparticles could make with negative face of Laponite (Ghadiri et al., 2013).

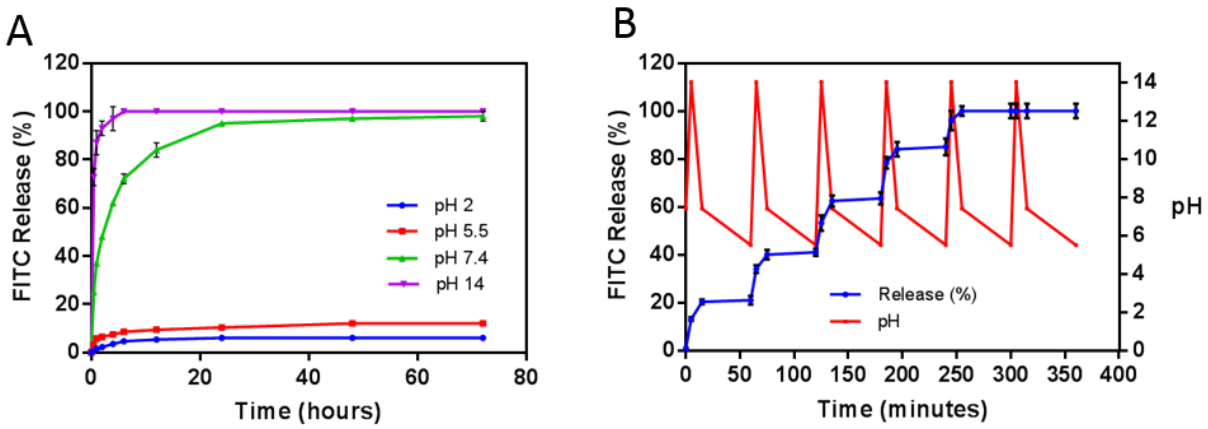

Figure 7, A) Release profile of FITC from chitosan nanoparticle embedded in PEGDA-Laponite hydrogel in different pH showed the abrupt release of drug in pH 14 and slow release in acidic pH B) B) Graph showing the control of release rate with pH change. Large release slope in pH 14 decreased when pH became 7.4 and then 5.5.

5. Hydrogel Characterization

Physicochemical properties of PEGDA/Lap/ChP in different pH, degradation of hydrogel and diffusion of drug play a critical role on drug release profile and require precise characterization.

5.1 Swelling and degradation test in different pH

We immersed the known weight of Crosslinked samples in 4 different released media at 36.5°C for 1, 6, 12, 24 and 48 hours. At each time point, we took the samples out of the solution, removed the excess solution on their surface and weighed the samples using a high precision scale, and then we put samples in oven 45 °C to reach a constant weight in order to measure the degradation ratio.

Swelling degree: $(M\text{-}M_i /M_i) \times 100$

Degradation percentage: $(M_d\text{-}M_i/M_i) \times 100$

M is the swollen weight of sample, M_i is the initial dry mass of sample and M_d is the dried mass of immersed sample in release media.

By comparing the swelling ratio of LAP/PEGDA with and without chitosan nanoparticle, it can be inferred that the addition of chitosan nanoparticle increased the swelling ratio from 290 % to 390% after one-hour incubation in pH 5.5 due to the presence of hydrophilic chemical groups such as NH_4^+ in structure of chitosan particles (Figure 8 A, B). The amine groups of chitosan will be neutralized in basic pH, however; the negative charged of laponite remains causing the electrostatic repulsion of laponite layer (Gaharwar, Dammu, Canter, Wu, & Schmidt, 2011). Therefore, more space would be provided to absorb water, which lead to burst swelling around 800% in pH 14 after one hour. In pH 14, OH^- attack the carbonyl group in acrylate ester of PEGDA and accelerate the degradation which cause the decrease in swelling degree after 6 hours (Kuen et

al., 2008). Taken all these together, the faster drug release in basic pH in compare to acidic pH can also be explained through the rapid degradation and burst swelling ratio of hydrogel. After 6 hours' incubation in pH 14 the hydrogel was degraded completely (Figure 8 C, D).

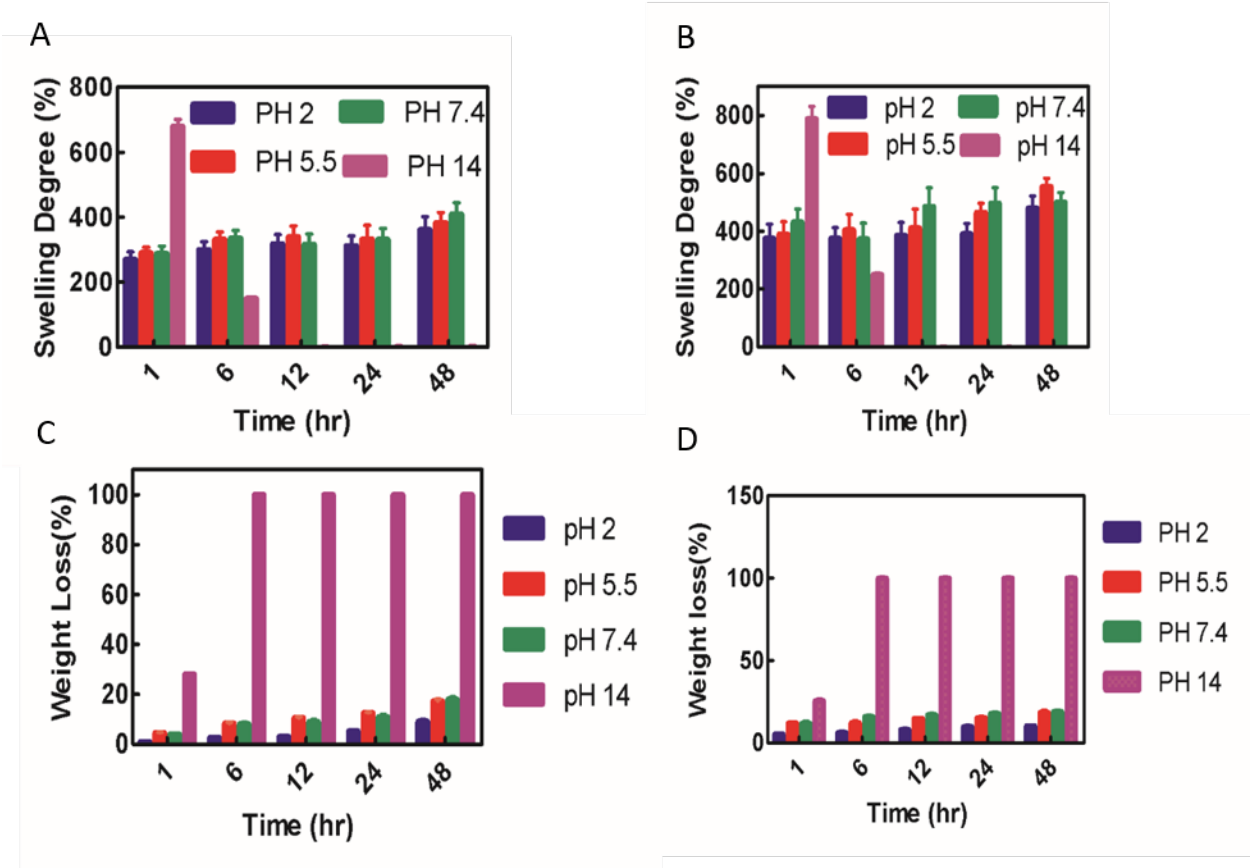

Figure 8. Swelling ratio of A) PEGDA/LAP B) PEGDA/LAP/CHP, in different pH and degradation rate of C) PEGDA/LAP D) PEGDA/LAP/CHP

5.2 Chemical analysis of Hydrogel with Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is used to evaluate the molecular and chemical interaction of compounds. To confirm the degradation of hydrogel in extreme alkaline environment, we need to know about the mechanism of degradation. The FTIR is one way of showing the mechanism of chemical reaction in alkaline environment.

we freeze dried the samples after one-hour soaking them in different pH, and studied their chemical structures with Fourier transform infrared (FTIR) spectroscopy using a Spectrum 100 FTIR spectrometer (PerkinElmer, Massachusetts, USA) fitted with an ATR attachment with the range of 4000–600 cm^{-1} for scanning, and the resolution set of 1 $cm^{-1.}$.

The similarity between LAP/PEGDA and LAP/PEGDA/ChP FTIR confirmed the complete intercalation of chitosan nanoparticle in laponite, the chitosan amine groups fully covered by laponite hydroxyl group (Figure 9C). However, after one-hour incubation in pH 14, a wide band around 3300 to 3450 cm^{-1} (the stretching vibration of N–H and hydroxyl group) was appeared related to the chitosan de-intercalation from laponite. The band at 1560 and 1080 cm^{-1} belonged to the chitosan amide II (N–H bending vibrations coupled to C–N stretching vibrations) groups, and glycosidic linkage (ether bond), respectively(Ting et al., 2010). Other peaks at 1000 cm^{-1} corresponded to Si–O stretching in laponite (Figure 9B)and the peak around 2882 cm^{-1} corresponded to alkyl-CH stretch in PEGDA and the peak at 1732 cm^{-1} is assigned to the –C=O stretching from ester bonds in PEGDA which in pH 14, it was disappeared (Figure 9A) (Yinghong Xiao, 2012).

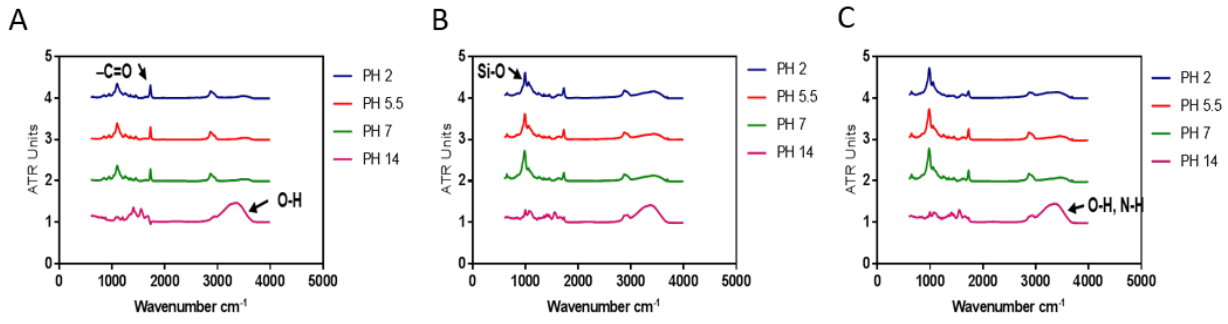

Figure 9, FTIR result of A) PEGDA B) PEGDA/Lap C) PEGDA/LAP/CHP in different pH

5.3 Morphology analysis of hydrogel with scanning electron microscopy (SEM)

To better understand the physicochemical property of hydrogel after the addition of chitosan nanoparticle to PEGDA/LAP, we took the SEM image of the hydrogel before and after the addition of CHP. We Freeze-dried PEGDA/Laponite and PEGDA/Laponite/ChP and then coated them with a thin layer of gold by a Bio-Rad E5200 auto sputter coater (England). We used Scanning electron microscope (CamScan MV2300 model, Oxford) with 5000X magnification to study the effect of addition chitosan particle on hydrogel pore size.

The SEM image of hydrogel showed that the addition of chitosan particle to LAP/PEGDA increased the pore size of the hydrogel (Figure 10). The increase in porosity can facilitate the water penetration to the hydrogel and subsequently the increase in swelling degree of hydrogel which has high in impact in drug release.

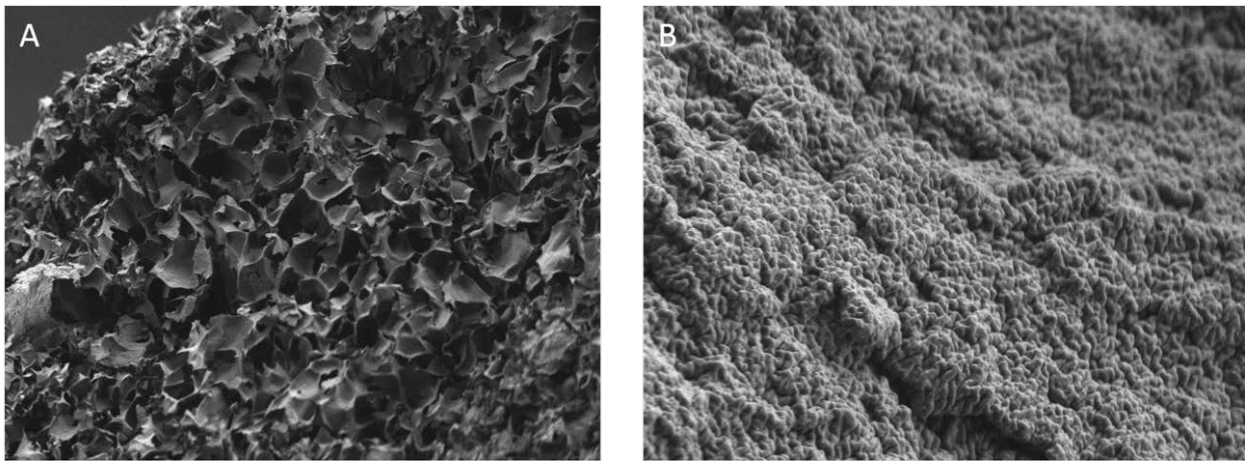

Figure 10, SEM image of A) PEGDA/LAP/CHP, B) PEGDA/LAP

6. Biocompatibility

We performed *in vitro* biocompatibility test to confirm the ability of our system to support the fibroblast proliferation after exposing to electrical field. The biocompatibility of electrical field and its effect on cell proliferation was investigated in several studies(Zhao, 2009; Kloth, 2005; Weiss, Kirsner, & Eaglstein, 2017; Hronik-tupaj & Kaplan, 2012). Exposing Fibroblast to Continuous electrostatic field of 1000 V/cm caused the increase in DNA and collagen synthesis after 14 days, and high voltage pulsed current induce the activity of fibroblast (Weiss et al., 2017; Zhao, 2009). The suggested mechanism for the fibroblast increased cell activity was attributed to the opening of voltage sensitive calcium channel in cell membrane and upregulating of insulin receptor on the cell surface, which triggered the DNA and collagen synthesis (Bourguignon, Jy, & Bourguignon, 1989; Gerard J. Bourguignon and and Lilly Y. W. Bourguignon, 2017).

The localized pH change around the electrode during electroporation decreased cell viability. It was shown that pH greater than 12.5 caused cell lysis due to cell membrane damage by hydroxyl ions. However, no abnormal cell morphology was seen after the alkaline stimulation with pH value 12 for 10 seconds (Y. Li et al., n.d.). They showed that there is a critical pH value of cell viability. Above pH 13, almost no cells survive. Additionally, longer pH shock lead to the less cell viability. The alkaline environment causes more severe damage to cells than acidic environment.

We cultured Fibroblast in a DMEM containing 10% FBS and 1% penicillin/streptomycin at 37°C and 5% CO2. We passaged Cells at 80% confluency. We added 100 000 cells into six well plates and incubated at 37 ◦.

6.1 Effect of electrical stimulation on cell viability in in vitro Wound closure experiment

Scratch assay was employed to evaluate the effect of the pH changes around the electrode on cells viability and migration. Three group were selected: group 1 as a control sample without application of the electrical voltage, group 2 and three were the samples exposed to 2.5 V and 4 V for 5 minutes. In Wound Scratch assay, Cells were seeded into six-well plates at 37 ◦C and 5% CO2. Once a confluent monolayer of cells was achieved, with pipette tip, 1 mm wound width was created, then suspended cells were aspirated and fresh media was added to each well plate. Cell cultures were divided into three groups: group one and two were exposed to 2.5 V and 4 V for 5 minutes respectively and group three stayed as control group. We took images at time 0, 16, 24, 48 hours after scratch with an inverted microscope, and the wound width percentage calculated at each time point as following: (W_t: the width of wound at time t, W_0 is the width of wound at time zero)

Wound width percentage: $W_t/W_0 \times 100$

The growth of cells was watched under microscope for 48 hours to measure the rate of wound closure. Although, there was significant difference between group 2 and 3 and control group (*P<0.05) at each time point, however after 48 hours, the wound width percentage reached to more than 5 % in all groups of studies (Figure 11B). Here, the most important point was to assure the normal growth of cells after exposure to electric field since the temperature and pH changing induced by electric current may affect cell growth (Weiss et al., 2017). The viability of cells was also assessed in an independent experiment by live/dead assay for day one, three and seven. We performed live/dead assay to determine the viability of cells after exposure to 2.5 V and 4 V for 5 minutes. We used the Live/Dead kit (Invitrogen, USA) according to the manufacturer's instructions, ethidium homodimer-1 (2 µl/ml) and calcein AM (0.5 µL mL−1) were mixed in PBS.

Cultures were incubated with the prepared solution at 37 ◦C for 15 min and were washed with PBS. Stained samples were imaged with an inverted fluorescence microscope (Nikon TE 2000-U, Nikon instruments Inc., USA).

Wound-edge Cells extended in to wound site and aligned perpendicular to wound direction in all groups. The pH changing around electrode did not show to have any toxic effect on cell growth at in vitro wound closure test 9 (Figure 11A).

6.2 Cell viability assay

We put fibroblast in contact with our hydrogel wound dressing and conducted proliferation test to assess the cytocompatibility of the PEGDA/LAP/ChP hydrogel. PrestoBlue cell viability assay was used to measure cellular metabolic activity after treating cells with PEGDA, PEGDA/Laponite and PEGDA/Laponite/Chitosan particle. Each well was incubated for 1 h at 37 ◦C with 400 µL of a solution containing PrestoBlue reagent and cell culture media in a ratio of 1:9. By subtracting the samples fluorescence values obtained from reader (Synergy HT-Reader, BioTek, Winooski, VT) from the background, the fluorescence values were determined for each day. Higher fluorescence values were associated with greater total metabolic activity. The test was repeated three times for each sample. No significant difference in proliferation activity of fibroblast was seen between PEGDA/Lap, and PEGDA/LAP/ChP, PEGDA and control groups (*P> 0.05) (Figure 11D).

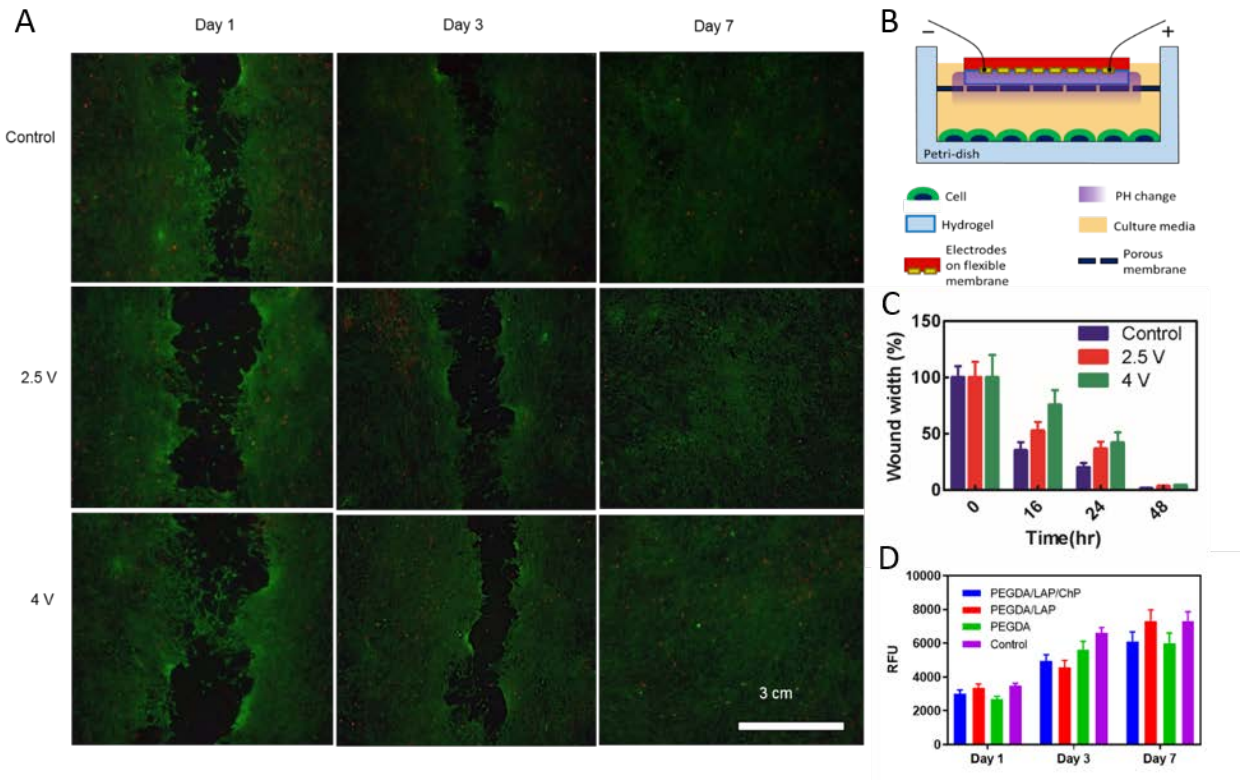

Figure 11, In vitro experiments to test the biocompatibility of the electronic wound dressing: A) In vitro scratch wound assay, application of 4 V, 2.5 V in contact with 3t3 for 5 minutes prior to the scratch test and control group without exposing to electric field, the live/dead images showed the wound closure after 7 days with high cell viability as green color present live cells and red dots present dead cells B) The schematic of the application of DC voltage in contact with cells C) There was significant difference between group 2 and 3 and control group (*p-value<0.05) at each time point, however after 48 hours, the wound width percentage reached to more than 5 % in all groups of studies D) PrestoBlue assay after 7 days of 3t3 plating on to hydrogels showed the biocompatibility of the proposed system (p-value>0.05).

7. Conclusion

In this work, we developed an electronic wound dressing made of pH responsive material for treatment of chronic wounds. The proposed wound dressing is consisted of electronic driver, microelectrode fabricated on flexible substrate, and PEGDA/Laponite hydrogel embedded with chitosan drug nano-carriers. Here, we have illustrated the on-demand delivery of drug due to the de-swelling of chitosan nanoparticle in response to alkaline environment. The localized pH changes around electrode during electrical stimulation was transient due to the diffusion of ions and PBS pH buffering capacity. The wound closure and cell viability studies confirmed the biocompatibility of the electronic wound dressing. In summary, controlled release of drug was confirmed due to the alkaline environment around anode.

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9. Appendix

Material

Chitosan (molecular weight), Laponite, poly (ethylene glycol) diacrylate, DAPI were obtained from Sigma-Aldrich (St. Louis, MO, USA). 2-hydroxy-1- (4-(hydroxyethoxy) phenyl)-2-methyl-1- propanone (Irgacure 2959, CIBA Chemicals) was used as a photoinitiator (PI). Dulbecco's modified Eagle medium (DMEM), 0.05% trypsin-EDTA (1X), fetal bovine serum (FBS), Live/Dead kit and antibiotics (Penicillin/Streptomycin) were acquired from Invitrogen (Carlsbad, CA, USA). PrestoBlue® Cell Viability reagent was received from Life Technologies.