

Chitosan/Alginate/Gellan Gum Hybrid Hydrogel as a Vehicle for Controlled Release of Drug

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Abstract: Hybrid hydrogel was fabricated by a classic sol-gel method using EDC/NHS as crosslink reagent grafting onto the thermoplastic polyurethane (TPU), nonwoven fabric, for controlled release of drug. In this study, precursor acetic acid (AA) was used to plasma deposit on the surface of TPU to form a hydrophilic thin film. Hybrid hydrogel was investigated through scanning electron microscopy (SEM), water contact angle (WCA) measurement, Fourier transform infra-red (FTIR) spectroscopy, UV/V is spectroscopy, equilibrium swelling ratio, MTT assay and drug delivery system studies. This polyelectrolyte complexes (PECs) formed hydrogel, pH-sensitive type, was evaluated at pH value of 1.2 and 7.4 of buffer solution and at temperature of 37°C to observe its rate of swelling and drug release features with caffeine. Moreover, the mechanism of caffeine release from membrane devices ($n=0.58$) are anomalous transport, non-Fickian diffusion, the value of n lies between 0.43 and 0.85. It has an excellent release ratio up to about 90% absorption cumulative amounts of caffeine at pH 7.4 after 8h and could be a beneficial carrier for fragile drugs.

Keywords: TPU, Acetic Acid Plasma, EDC/NHS Grafting, Hybrid Hydrogel

1. Introduction

In the 1950s, the first developed commercial thermoplastic polyurethane, TPU, was established in the U.S. by B. F. Goodrich and in the Germany by Bayer-Fabenfabriken. TPU commonly made by polyol, diisocyanate and a chain extender. It is a block copolymer composed of soft and hard blocks constituting a two-phase micro-structure. One is the soft block consists of polyester or polyether given their elastomeric and flexibility properties, another is the hard block built out of isocyanate and a chain extender offer TPU its high modulus, toughness and tensile strength. Therefore, this nonwoven has some advantages such as vast surface area [1], transparent, weathering resistance [2], heat and aging resistance [3], elasticity [4], water-proof [5].

Chitosan/ Alginate / Gellan gum hybrid hydrogel is a three-dimensional network hydrophilic heteropolymer,

polysaccharide. First of all, chitosan is a linear cationic polysaccharide [6] and primary aliphatic amine: β (1, 4)-linked 2-amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan [7, 8]. It is apt to soluble in acidic aqueous solution and to form positive charges (NH_3^+) at acidic pH ($\text{pH} < 6.5$) [9, 10]. Main sources of chitosan are crabs, shrimps, molluscs, and squids [11, 12]. Generally speaking, chitosan is usually described in detail by degree of deacetylation (DD) and average molecular weight (Mw). Chitosan to form polyelectrolyte complexes (PECs) [13, 14], frequently used opposite charge polysaccharide [15] such as pectin, alginate [16], gellan gum [17] and κ -carrageen [18] in order to avoid chitosan drawback in the strong acid environment, and to promote water-absorbing, swelling phenomena, and lower critical solution temperature (LCST).

Alginate is a linear anionic polysaccharide, consisting of (1,4) linked α -L-guluronic acid (G) and β -D-mannuronic acid

(M) two units, were arranged in homopolymeric M- and G-blocks or alternating MG-blocks difference between the both proportion. Alginate is generally obtained from bacteria and seaweed [19]. It is a mono-valence, and water-soluble copolymer. Its salt transformed into water-insoluble salt due to the addition of divalent ions [20] such as calcium [21], barium, and strontium. Especially, calcium ion has not equal affinity between mannuric acid and guluronic acid, bound to the polyguluronate chain series to form “egg-box” shape. Therefore, alginate’s structure has two units demonstrated that the polyguluronate (GG) residues much tougher than polymannuronate (GG) residues and both tougher than altering (MG) residues. Its structure is unstable and easy to drawback under strong acid environment [22, 23]. Otherwise, gellan gum is added to hydrogel to avoid drawback, based on it is able to maintain stable in the lower acid medium. Gellan gum is a linear anionic polysaccharide, has some excellent advantages such as hydrophilic, biodegradable, biocompatible, non-toxic, and cheap. It produced by the microorganism *Sphingomonas elodea* [24] composed of repeating tetrasaccharide (1,3- β -D-glucose, 1,4- β -D-glucuronic acid, 1,4- β -D-glucose, 1,4- α -L-rhamnose) units, in the molar ratios 1:2:1 (α -L-rhamnose, β -D-glucose, and β -D-glucuronate) and possessed one carboxyl side group [25]. Native gellan gum has lower LCST (lower critical solution temperature), soft, and easy to reform gels [26, 27]. Contrary, the deacetylated one is rigid and brittle gel [28]. Gellan gum is efficiently able to form gel by the addition of cation (Na^+ , k^+ , Ca^{2+} , Cu^{2+}) and characteristics of changeful texture depend on the gelation conditions [29, 30]. This hydrogel has usually used in the food additive agent (used as a thicker, stabilizer, and emulsifier), cosmetic, and pharmaceutical used. Hence, this polyelectrolyte complexes has usually been used to control drug release [31-34]. In this study, caffeine was used as a release drug owing to its cheap, harmless, convenient and productive medicine for pharmacotherapy [35-38].

Lately, nonwoven fabric of TPU has been extensively used in various fields (bioreactor, biomedical material, biosensor), which has many advantages including mechanical strength, large surface area, resistant acid and base and bioavailability. In this study, it is used as a medicine carrier substrate, hydrophobic fabric. Hence, TPU surface is hard to bind with polar surface of material (collagen, enzyme, cell, chitosan), leading easy to peel off objective. For this reason, plasma technologies [39] (corona [40], glow [41], photo-grafting, UV induced and graft polymerization [42, 43]) have been used to modify the surface of TPU. It is a very effective way to promote hydrophilicity of TPU surface by applying free radicals, peroxides (OOR, OOH), and functional groups (SiO_2 , CH_3 , OH, COOH, C=O, and NH_2 [44]) introduced by plasma or grafting of gas or monomers [45] such as O_2 [46, 47], He, Ar [48, 49], N_2 , CH_4 , C_2H_2 , CO_2 , acetic acid, acrylic acid [50, 51], N-isopropylacrylamide (NIPAAm) [52], hexamethyldisilazane (HMDSZ), isopropanol. This hybrid hydrogel made from Chitosan, alginate, and gellan gum three element hydrogel and formed a three-dimensional network

hydrophilic polymer. TPU membrane surface was modified by PECVD [53] or lysine [54, 55]. Coming after, N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride as coupling agents [56- 59] are used to crosslink hydrogel (carboxyl side group) and TPU membrane, and then Caffeine loaded hydrogel-based polymeric film for controlled drug delivery. Finally, the surface characterizations of film and drug delivery system were made use of water contact angle measurement, scanning electron microscopy, Fourier transform infra-red spectroscopy, UV/Vis spectroscopy, swelling behavior of films and MTT assay were also tested

2. Materials and Methods

2.1. Material and Reagents

Deacetylated gellan gum, sodium alginate (MW=10,000~600,000), acetic acid, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide. HCl (EDC), polyvinylpyrrolidone (PVP) were provided by Wako Pure Chemical Company, USA. Chitosan (deacetylation degree > 93%, M.W. =190~310K) was purchased from Aldrich Company. Caffeine anhydrous and sodium tripolyphosphate (Na-TPP) were obtained from Sigma Company. Thermoplastic polyurethane fabric nonwoven (TPU) was supplied by Taiwan Textile Research Institute. Argon (purity>99%), oxygen (purity > 99%) and carbon dioxide were purchased from Sanfu chemical Company. N-hydroxysuccinimide (NHS, purity>97.0%) was obtained from Fluka. Trypan blue stain, Minimum essential medium Eagle, Dimethyl sulfoxide, Sodium bicarbonate and 3-(4, 5-Dimethylthiazo-2-yl)-2, 5-diphenyl tetrazolium bromide were purchased by Sigma. CCD-966SK cell line (Human skin fibroblast) was obtained from Bioresource Collection and Research Center, Hsinchu, Taiwan. MEM non-essential amino acids solution, Penicillin, Streptomycin, L-glutamine, and Dulbecco’s Modified eagle medium were purchased from GIBCO/BRL, USA. Fetal bovine serum, and Trypsin-EDTA were obtained from HyClone, USA. All of the other chemicals and reagents were of analytical grade and used without further purifications.

2.2. Cold Plasma of Surface Treatment

Samples (TPU, thickness:0.2cm) were cut into the pieces ($1\text{cm} \times 1\text{cm}$) as substrates, ultrasonically cleaned in alcohol and deionized water to remove film surface impurities and dried at room temperature. Advanced plasma system (Model PD-2S plasma deposition system manufactured by SAMCO Company) with 13.6 MHz radio frequency generator and a bell jar type reactor were used to activate the surfaces of the films. All process of the cold plasma treatment was described in detail in previous study [42]. Foremost, samples were treated by placed over the electrode, a horizontal holder, and the chamber was pumped to a base pressure of 30 mtorr before the cold plasma treatment which was stabilized. Next, the acetic acid (AA) monomers were introduced into the

chamber (pressure: 50mtorr, and at a discharge power of 40W) for treatment times of 1, 5, 10, 15, 30 minutes, respectively, for sample surfaces to produce $\cdot\text{COOH}$ group.

2.3. Hybrid Hydrogel Coupling to Surface of Treated TPU by EDC/NHS

The AA-treated film was immersed in 2mM EDC/NHS aqueous solution (0.1M MES buffer, pH 5-6) at room temperature for 2 h, and then washed the pH 7.2-7.5 with 0.05M phosphate buffer immediately before reaction to the hybrid hydrogel. Allowing EDC/NHS-treated films reacted with the hybrid hydrogel for 2h at room temperature. After grafting treatment, and washed with deionized water to remove un-reacted reagent and dried them at room temperature for overnight. For the preparation of the alginate/gellan gum/chitosan hybrid hydrogel, chitosan power was dissolved at 1% (w/v) concentration in aqueous acetic acid (1%v/v) with constant stirring at a room temperature. Polyvinylpyrrolidone (PVP) was dissolved in deionized water (0.5%). Both chitosan and PVP solutions were mixed and stirred at room temperature for 2 h. Alginate was dissolved at 1% (w/v) concentration in the deionized water with constant stirring at a room temperature. Gellan gum was dissolved at 1% (w/v) concentration in the deionized water with constant stirring at a temperature of 80°C for 2h. The AA-treated films were soaked in an aqueous solution of mixed chitosan/PVP, alginate and gellan gum solution in the ratio (w/v) 1:1: 0, 1:1:1, 1:1:2, 0:1:0, and 1: 0: 4 at a temperature of 50°C for 2 h, and put them into 1% Na-TPP aqueous solution for overnight. Next, the grafted films were washed with distilled water overnight to remove the unbinding reactant.

2.4. Surface Characterization

The surface morphology of the films was observed using scanning electron microscope (SEM, JSM 5600). The water contact angle (WCA) of original and modified films were measured by CCD camera (Goni-meter type G-1 made by

ERMA Optical Works Company). The Fourier transform infrared spectrometer (Jasco FT/IR-6200) was used to analyze the surface functional groups after the plasma modification, graft polymerization, and immobilization of hybrid hydrogel (chitosan, alginate, and gellan gum). The grafting density was also measured.

2.5. Swelling Characteristics

The swelling behaviors of films were examined in simulated gastric fluid (pH 1.2, 0.1M HCl / NaCl buffer solution, SGF), and simulated colonic fluid (pH 7.4, 0.05M phosphate-buffered SCF). Films of known weight were successively immersed in 10 ml of SGF, and SCF at a temperature of 37°C. The swollen films were removed to a fresh 10 ml buffer solution and weight at fitting interval, sucking them with filter paper to remove the surface film of liquid adherence at once. The swelling ratio (SR) of film was calculated, using the following equation:

$$\text{SR (\%)} = [(W_1 - W_0) / W_0] \times 100 \%$$

Where W_1 is the weight of the swollen test film at fitting intervals and W_0 is the dried weight of film.

2.6. Drug Release Test

The Caffeine profile of film was measured for the purpose of impersonation gastrointestinal tube (GIT, included: pH 1.2, in SGF, and pH 7.4, in SCF) from stomach to colon. The caffeine-loaded film was equilibrated in a solution of 30 mg drug and 1L deionized water (30ppm) [60] and incubated at 37°C for 24h. The drug release test was carried through by transferring previously incubated drug hydrogel into a 10 ml buffer solution at 37°C and at fitting interval. The film was constantly removed and transferred into a fresh 10 ml buffer solution at different time interval. Capacity of the caffeine in film was determined at 273 nm by making use of a UV-Vis spectrophotometer (JASCO 1700).

$$\text{Loading efficiency (\%)} = \frac{\text{Actual amount caffeine in hydrogel}}{\text{Theoretical yield of caffeine loaded hydrogel}}$$

2.7. Cell Culture Protocol

CCD966SK cell line (BCRC 60153) was obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan). Cells were seeded at a density 3.0×10^4 cells per well into 24well plates and grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 100Mm non-essential amino acid, 100U/ml penicillin and 100U/ml streptomycin mixed antibiotics, NaHCO_3 and 2mM L-glutamine. All cell cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C and exposed to hydrogel films for 1 to 3 days. Assessment of cell viability was determined by MTT assay based on live cell's mitochondrial (succinate dehydrogenase) reduction of yellow MTT tetrazolium salt to blue purple formazan crystal. The medium was regularly changed and incubated with MTT reagent

(5mg/ml) at 37°C for 4h. The reaction was stopped by adding dimethyl sulfoxide (DMSO) and measured optical density (Multiskan Spectrum Microplate Spectrophotometer) at 540nm (OD_{540}).

3. Results and Discussions

3.1. Surface Morphology Survey

Figure 1 SEM images of the surface morphologies of (a) un-modified and (b) hybrid hydrogel-treated TPU. The surface of un-modified TPU film revealed a lot of rough squamae. After acetic acid plasma treatment and grafting alginate/chitosan/gellan gum hybrid hydrogel, the treated surface of TPU was apparently changed as Figure 1(b) shown. Both of SEM images created diversely a

transformative surface of TPU from coarse to flat structure. Therefore, surface of TPU film could be modified by plasma treatment and gelatin, has some alterations.

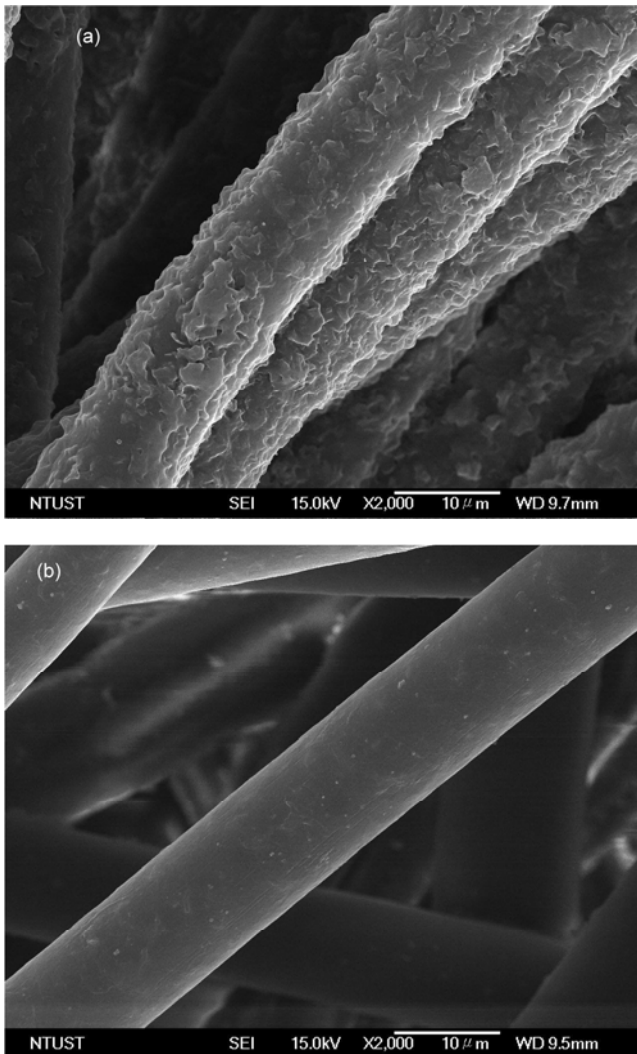


Figure 1. SEM of (a) un-modified and (b) hybrid hydrogel-treated TPU film, hybrid hydrogel in the ratio, Chitosan: Alginate: Gellan gum = 1:1:2 (w/v %)

3.2. Wettability of Modified Surface

TPU thin film, nonwoven fabric, has specifically a hydrophobic property. Figure 2 (b) shown water contact angle of TPU surface has been obviously changed owing to plasma treated. Water contact angle (WCA) of TPU film was measured by the sessile drop method with distilled water at room temperature. The data were recorded by a CCD camera (Goni-meter type G-1, ERMA Optical Works), which of un-modified TPU is 116.70° . After plasma deposition of acetic acid, at power of 40 W and 50mtorr for 30 minutes, WAC is 106.4° , demonstrated hydrophilic leaning was due to cover with acetic acid. Of TPU has dramatically changed by acetic acid plasma treatment to form hydrophilic film.

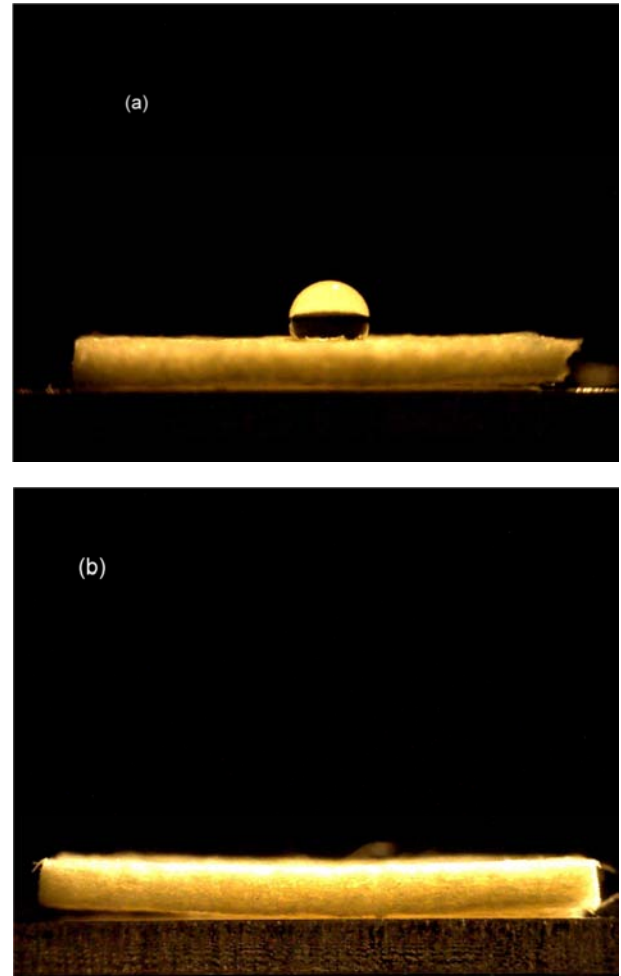


Figure 2. Water Contact Angle of TPU due to acetic acid Plasma treatment (a) un-modified (116.7°) (b) AA-treated acetic acid TPU (0°). plasma treatment condition: 40W, 50 mtorr acetic acid, and treatment time = 30minutes.

3.3. FTIR Spectral Analysis

Figure 3 illustrated the FTIR spectra of TPU of acetic acid plasma and hybrid hydrogel treatment were composed of chitosan, alginate and gellan gum. Alginate and gellan gum represented one carboxyl ($\text{C}=\text{O}$) peak at 1640cm^{-1} slightly shafted to 1653.1cm^{-1} , the $-\text{C}-\text{O}-\text{C}-$ peak at 1221.21cm^{-1} stretching due to carboxylic acid. TPU has characteristic sharp peak at 1157.5cm^{-1} , 1538.4cm^{-1} , 1722.8cm^{-1} owing to $-\text{C}-\text{N}$ stretching vibration and $-\text{N}-\text{H}$ bending vibration overlapped, and $-\text{H}-\text{N}-\text{C}-\text{O}-$ stretching vibration, respectively. The peak at 2848.8cm^{-1} and 2910.8cm^{-1} exhibited asymmetric and symmetric stretching vibration of $-\text{CH}_2$. The characteristic peak of $-\text{CO}$ stretching vibration of acetic acid was observed at 1061.6cm^{-1} in Figure 3 and at 932.2 , 1451.9cm^{-1} assigned to $-\text{C}-\text{H}$ bending and scissoring vibration. Hence, FTIR spectra of hybrid hydrogels for chitosan demonstrated in Figure 3, shown sharp peak in at 3210.4cm^{-1} , 1653.1 , 1577cm^{-1} are due to the $\text{O}-\text{H}$ bending vibration overlapped with the $\text{N}-\text{H}$ stretch vibration, the amide I ($\text{C}=\text{O}$ stretching vibration of acetyl group), amide II ($\text{N}-\text{H}$ shift stretching vibration), respectively.

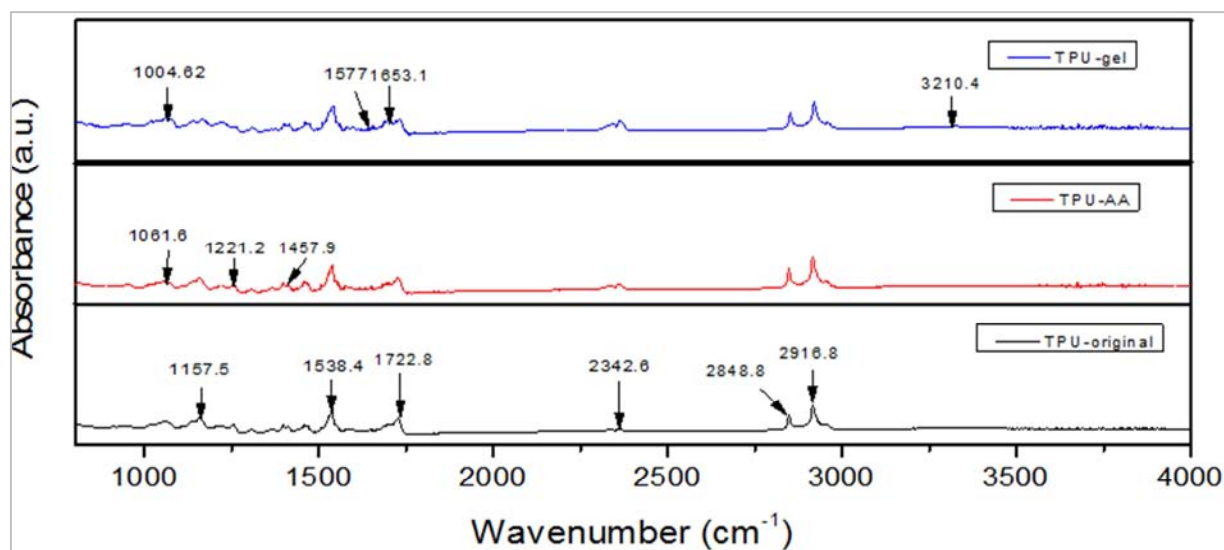


Figure 3. FTIR spectra of TPU of acetic acid plasma and hybrid hydrogel treatment, ratio of Chitosan: Alginate: Gellan gum is 1: 1: 2 (w/v %).

3.4. Equilibrium Swelling Study

Effect of hydrogel variation compositions (w/v %) of TPU film are due to equilibrium swelling in alkaline and acid conditions shown in Table 1. Swelling behavior of hybrid hydrogel was measured with water uptake at a fitting interval. Next, the dry copolymer hydrogel was placed in buffer solutions with pH 1.2 and pH 7.4 at 37°C for 8 h to be allowed hybrid hydrogel to achieve equilibrium swelling state. The weight of hydrogel film is $0.167 \pm 4.71 \times 10^{-5}$ mg/cm² (mean \pm SD, N=3). Table 1 showed a variety of SR (%) for hybrid hydrogel, investigated that contained alginate (-COOH), chitosan (-NH₂) and gellan gum (-COOH) of different ratio at 37°C. In acidic environment, pH 1.2, swelling behavior of the hydrogel film was lower, the performance exceedingly increased in alkaline environment,

pH 7.4. Under the lower PH environment, pH 1.2, the -NH₂ group kept to proton, owing to H⁺ ion promoted and raised electrostatic repulsive force. Consequently, hybrid hydrogel (-NH₃⁺, -COOH) could be to swell up, -COOH part of hydrogel was obviously inhibited. At higher pH value of alkaline environment, -COOH part of hydrogel ionized to -COO⁻ that exerted electrostatic repulsive force, and -NH₂ part of hydrogel was inhibited. Accordingly, it resulted in volume of alteration at different PH, this swelling behavior of the hydrogel network depended on ratio of alginate, chitsan and gellan gum. The pH-sensitive experiment of hybrid hydroge of swelling ratios found them up to maximum after 8h. When hybrid hydrogel, alginate: chitosan: gellan gum = 1: 1: 2 (w/v %), operated at 37 °C for about 8h, the SR % ratio was able to gain a maximum divergence.

Table 1. Effect of hydrogel variation compositions (w/v %) of TPU film is due to the equilibrium swelling in alkaline and acid conditions. (mean \pm SD, N=3).

Hydrogel Types					Equilibrium swelling (%)	
Chitosan	:	Alginate	:	Gellan gum	pH1.2	pH7.4
1	:	1	:	0	185.47±25.19	332.93±35.60
1	:	1	:	1	166.40±10.74	276.80±16.25
1	:	1	:	2	166.00±14.06	355.45±20.11
1	:	0	:	0	139.20±23.83	320.13±19.82
1	:	0	:	4	200.93±74.95	276.67±30.14
0	:	0	:	0*	0	0

*un-treated TPU

3.5. Caffeine Release and Cytotoxicity Studies

Figure 4 showed that the caffeine release curves of various pH investigated at 37°C. Cumulative release was higher amount of caffeine due to suitable ratio of hybrid hydrogel, Chitosan: Alginate: Gellan gum = 1: 1: 2 (w/v %). These films loaded drug amount are $6.24 \times 10^{-4} \pm 2.24 \times 10^{-5}$ mg/cm² (mean \pm SD, N=3). Loading efficiency (%) is $92.45 \pm 1.05\%$ (mean \pm SD, N=3). Table 2 exhibited drug release from caffeine-loaded hybrid hydrogel followed non-Fickian model transport mechanism in view of diffusion exponent at PH1.2

and PH 7.4 were 0.57 and 0.58, respectively. The drug released behavior was due to volume alteration to form stress effect rather than non-Fickian diffusion mechanism. Cytotoxicity assay of hybrid hydrogel was done by MTT testing. Figure 5 demonstrated that plot of viability of % CCD966SK cells line for 24~72h. The hybrid hydrogel is a polyelectrolyte complexes (PECs) formed hydrogel. It has some positive charges and characteristics of smooth surface texture could be apt to adhered cell. Therefore, the cell viability values were found out to be $126.39 \pm 0.04\%$ and

192.36 \pm 0.17% (mean \pm SD, N=3) for hybrid hydrogel compared with control (3×10^4 cell/well).

Table 2. Release kinetic data of Caffeine loaded hydrogel (w/v %) of TPU film.

Hydrogel Types	Korsmeyer-Peppas Model		Caffeine cumulative release rate (%)
Chitosan:Alginate:Gellan gum 1:1:2	k	N	(mean \pm SD, N=3)
pH 1.2	0.069	0.57	42.30 \pm 2.54
pH 7.4	0.173	0.58	88.77 \pm 2.65

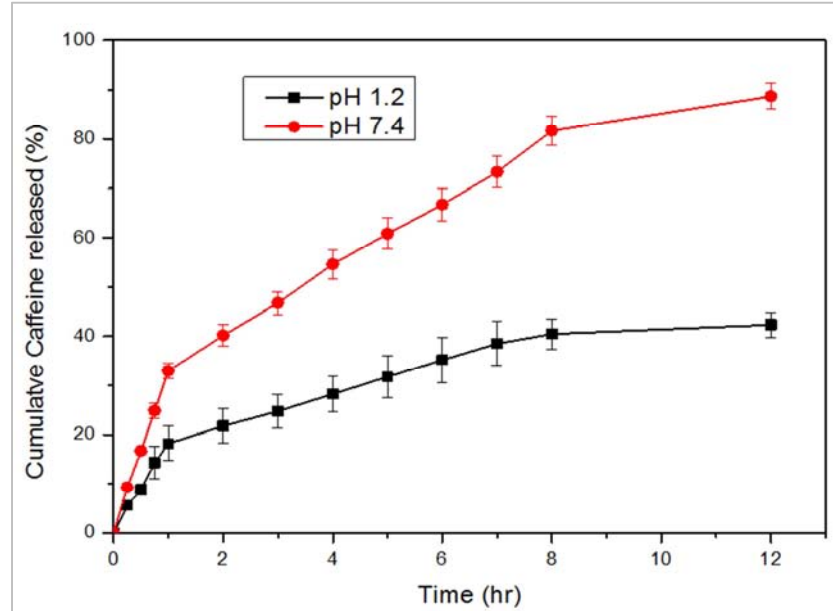


Figure 4. The caffeine release curves of various pH investigated at temperature of 37°C, ratio of Chitosan: Alginate: Gellan gum is 1: 1: 2 (w/v %).

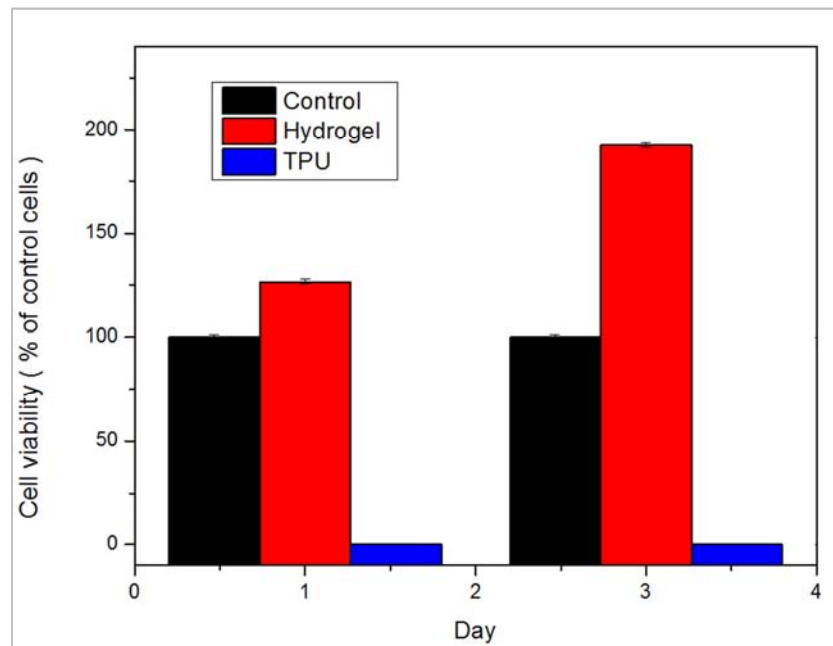


Figure 5. In vitro cytotoxicity analysis of the CCD-966SK cells on the hydrogel, Chitosan: Alginate: Gellan gum = 1: 1: 2 (w/v %). CCD966SK cells (3×10^4 cells/well) were incubated on the hydrogel for 1 and 3 days, respectively. (mean \pm SD, N=3).

4. Conclusions

Caffeine is widely used to consume as psychoactive drug. In this study, pH-sensitive hybrid hydrogel of chitosan, alginate and gellan gum were prepared by ionotropic

gelatification to load caffeine, which concentration ratio of hybrid hydrogel has a crucial influence on the swelling rate and release performances. Surface of TPU nonwoven film was hydrophobic which binding polar material is difficult, so utilized acetic acid plasma treatment improved hydrophilic of

surface. Also, hybrid hydrogel was settled on the surface of TPU nonwoven film by EDC/NHS crosslinking reagent. This pH-sensitive hybrid hydrogel is a three-dimensional network and hydrophilic polyelectrolyte, polysaccharide, could tolerate lower pH to release caffeine which avoided being destroyed. It is based on gellan gum could not be split up in strong acid environment. Therefore, drug could be almost performed to target, released cumulative ratio up to about 90% absorption amounts of caffeine after 8h. This drug release system followed non-Fickian type transport mechanism and electrostatic repulsive force. This results exposed that was able to attenuate the release of caffeine at the acid medium, pH 1.2, to be suitable for drug release at the alkaline environment, pH 7.4, minimized the loss of caffeine at the acid medium to the first-pass metabolism and improved on bioavailability.

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