Amphiphilic Interpenetrating Polymer Networks for the Oral Delivery of Chemotherapeutics

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New interpenetrating polymer networks (IPNs) composed of hydrophilic, pH-responsive poly(methacrylic-grafted-ethylene glycol), and hydrophobic poly(n-butyl acrylate) were formed for the oral delivery of chemotherapeutics. These amphiphilic IPNs were synthesized to express variations in swelling and hydrophobic properties to try and develop an optimized material for the loading and release of doxorubicin, a hydrophobic chemotherapeutic. Release profiles modeling the gastrointestinal (GI) transit from the stomach to the small intestine were investigated. Mucoadhesion was determined using fresh porcine small intestine, polymer samples, and a tensile tester. The biocompatibility of the materials was assessed against Caco-2 and HT29-MTX cell models representing the GI tract and SW620 cells serving as a colon cancer cell model. © 2013 American Institute of Chemical Engineers *AIChE J*, 59: 1472–1478, 2013
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Introduction

Chemotherapy can be used as adjuvant treatment for post-surgical cancer patients, because reduction in tumor burden, stimulation of cell replication, and increase in blood perfusion increase its effectiveness.[1–3] Chemotherapy is traditionally administered intravenously, which provides systemic circulation and improved results for metastatic cancers. However, systemic circulation is unbiased against healthy and diseased cells resulting in severe patient discomfort or toxic levels too high for continued treatment. Therefore, there is a need for new delivery strategies and targeting mechanisms to get toxic chemotherapeutics to the proper location at appropriate therapeutic concentration levels while reducing cytotoxicity to healthy tissues.

Novel polymer synthetic techniques have created a new class of materials, which can be used in biomedical and clinical applications and may be modified for the oral drug delivery of chemotherapeutics. The oral delivery of chemotherapeutics could dramatically improve cancer care by providing new methods of delivery that lower side effects and allows the patient more comfort during the treatment regime. Patient preferences include flexibility and convenience in dosing, reduction in hospital or clinical visits, and elimination of peripherally inserted center catheters (PICC-line) for easy connection and disconnection during intravenous administration.[4–6]

Oral delivery of chemotherapy must overcome a variety of challenges. First, chemotherapeutic agents are traditionally hydrophobic making them difficult to be distributed in highly aqueous environments such as the human body and hydrophilic materials. Second, the chemotherapeutics can be toxic to the stomach and the stomach's low pH environment harmful to the chemotherapeutic. Third, to determine if the chemotherapeutic should transport across the epithelium of the gastrointestinal (GI) tract into the bloodstream for systemic cancer treatment (breast or liver cancer) or remain in the GI tract for local cancer treatment (colorectal cancer). Lastly, to deliver enough chemotherapeutics to the appropriate site for tumor reduction or tumor death.

Biomaterials based on pH-responsive hydrogels may be appropriate for the oral delivery of chemotherapeutics. Hydrogels are three-dimensional networks that are insoluble in aqueous environments due to physical and/or chemical cross-links, but still imbibe large amounts of water or biological fluids.[7] These networks can be composed of biocompatible polymers and use the pH shift from the stomach to the small intestine as a stimulus for the release of encapsulated chemotherapeutics. However, these materials are dominantly hydrophilic and most be modified with

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hydrophobic properties to preferentially load and release hydrophobic chemotherapeutics.

Doxorubicin, an important and successful anthracycline antibiotic, intercalates between DNA resulting in cell death for a variety of cancer types including lymphomas, breast, lung, and gastric cancers.[8] A small number of chemotherapeutic agents have been orally delivered and have shown promising results such as lower toxicity and clinical efficacy that is equal to or sometimes improved when compared to intravenous treatment.[9–15] Strategies for developing oral delivery of vehicles for doxorubicin have included poly(D,L-lactide-co-glycolide), poly(amido amine) dendrimers, lipid nanocarriers, and surface functionalized chitosan nanoparticles.[16–18] These carriers are focused on transmembrane transport of doxorubicin from the GI tract to the bloodstream to treat a variety of cancers whereas the materials reported here are focused on direct, local delivery of doxorubicin to the colon to treat colon cancers.

Previously, our laboratory has focused on optimizing poly(methacrylic acid-grafted-ethylene glycol) (P(MAA-*g*-EG)) hydrogels for the oral delivery of proxyphylline, insulin, insulin–transferrin bioconjugates, vitamin B12, theophylline, bleomycin, vancomycin, and other compounds.[19–27] Utilizing the P(MAA-*g*-EG) hydrogel, we modified it with poly (*n*-butyl acrylate) (PBA) to form interpenetrating polymer networks (IPNs). In IPNs, two homopolymers or copolymers are physically entangled within one another, but are not chemically bound; this allows the combinatorial expression of properties from each individual polymer network. For our IPNs, the hydrophobic PBA would preferentially associate with hydrophobic chemotherapeutics, whereas the P(MAA-*g*-EG) hydrogel would provide the pH-responsiveness for loading and release of the encapsulated chemotherapeutic agent.

The synthetic technique used in this work allows incorporation of varying amounts of PBA into the P(MAA-*g*-EG) hydrogel resulting in IPNs with a distribution of physical properties. These IPNs were previously synthesized, and there physical properties extensively characterized as well as how they interacted with fluorescein, a model solute with properties similar to chemotherapeutics.[28] This article is focused on elucidating PBA's effect on the loading and release of doxorubicin, a common chemotherapeutic used in cancer treatment. The transition from the stomach to the upper small intestine may be used as a trigger for release and is tested. Biocompatibility of materials was assessed against cell lines modeling the GI tract and a cell line modeling colon cancer.

Materials and Methods

Materials

Methacrylic acid (MAA), *n*-butyl acrylate (BA), tetraethylene glycol dimethacrylate (TEGDMA), 1-hydroxycyclohexyl phenyl ketone (Irgacure® 184), 3,3-dimethylglutaric acid, Nile Red, dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) medium, fibronectin, and ethanol were purchased from Sigma–Aldrich (St. Louis, MO). 10× phosphate-buffered saline (PBS), sodium chloride, and hydrochloric acid were purchased from Fisher Scientific (Fair Lawn, NJ). Poly(ethylene glycol) monomethyl ether monomethacrylate (PEGMMA; 1000 g/mol) was from Polysciences (Warrington, PA). Doxorubicin was purchased from Selleck Chemicals (Houston, TX). Fetal bovine serum (FBS) and trypsin with ethylenediaminetetraacetic acid (EDTA) were obtained from Hyclone (South Plainfield, NJ). 1× PBS without calcium or magnesium along with penicillin and streptomycin were from MediaTech (Manassas, VA). The cell proliferation [3-(4,5-dimethylthiazol-2-yl)-5-(3-(carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) assay was purchased from Promega (Madison, WI). Caco-2 and SW620 cells were obtained from American Type Culture Collection (ATCC, Rockwell, MD), and HT29-MTX cells were a gift from Dr. Thecla Lesuffleur, INSERM, Paris, France. All chemicals were used as received except for MAA, which was vacuum distilled at 54°C and 25 mm Hg prior to use to remove the inhibitor hydroquinone. Double distilled water was used in all studies.

Synthesis of IPNs of P(MAA-g-EG) and PBA

IPNs were formed by sequential free radical UV-initiated polymerization of P(MAA-*g*-EG) then PBA in the presence of the P(MAA-*g*-EG) and previously discussed in detail.[28] Briefly, P(MAA-*g*-EG) was formed by combining MAA, PEGMMA, TEGDMA, and Irgacure® 184 in ethanol and water. The solution was sonicated, purged with N_2, placed between glass slides, and exposed to UV light (Dymax 2000-EC Light Curing System, Torrington, CT). The resulting P(MAA-*g*-EG) film was washed, punched into disks, or dried and crushed into particles (75–500 μm) for future use. Both particles and disks were dried in vacuum at 30°C for 1 week.

The dried P(MAA-*g*-EG) disks were then swollen in the presence of BA, TEGDMA, Irgacure® 184, and a cosolvent of ethanol and water for 24 h under constant mixing, then removed, placed on an open face Teflon sheet, and exposed to UV light to form the PBA network. The solvent of ethanol to water was adjusted to 80:20, 75:25, and 70:30 (w/w) to produce IPNs with increasing PBA content: 80/20 IPN, 75/25 IPN, and 70/30 IPN. The amphiphilic IPN was washed in 30% (w/w) ethanol in water, punched into disks, or dried and crushed into particles (75–500 μm) for future use. Both particles and disks were dried in vacuum at 30°C for 1 week.

Loading doxorubicin in P(MAA-g-EG) and IPNs

Doxorubicin was loaded by equilibrium partitioning in the following manner: a stock solution of doxorubicin was prepared in 2 wt % DMSO in 1× PBS (pH 7.4) at a concentration of 0.25 mg/mL. A 5 mg/mL concentration of P(MAA-*g*-EG) or IPN crushed particles (75–500 μm) to doxorubicin stock solution was allowed to stir slowly for 2 h. The doxorubicin loaded particles were filtered and rinsed with water to remove any surface absorbed doxorubicin. A fluorescent plate reader (Biotek Synergy-HT, Winooski, VT), operating at a 485 nm excitation and 590 nm emission wavelengths, determined the concentration levels and calculated the loading efficiency as follows

$$\text{Loading Efficiency} = \frac{C_o - C_f}{C_o} \times 100 \quad (1)$$

where C_o is the initial doxorubicin concentration and C_f is the final doxorubicin concentration remaining in the solution.

Doxorubicin release from P(MAA-g-EG) and IPNs

Release experiments were performed on a rotary mixer (Glas-Col, Terre Haute, IN) operating at 15 rpm, which was placed in a dry oven (Fisher Scientific Isotemp Incubator

Model 525D, Pittsburgh, PA) thermostated to 37°C. For all doxorubicin release experiments, 1.5 mg of doxorubicin loaded P(MAA-*g*-EG) or IPN microparticles was added to 3 mL of 1× PBS (pH 2.0 or 7.4). For doxorubicin release in neutral pH, 1× PBS (pH 7.4) was used and over the duration of 6 h, samples were taken and replaced to maintain sink conditions. Doxorubicin release in low pH was conducted in the same manner, as neutral pH except 1× PBS was adjusted to a pH of 2.0 using 1N HCl.

To mimic the physiological conditions and residence time in the stomach and small intestine, doxorubicin was released using a two-step pH change from low pH (2.0) to high pH (7.0).[28] Doxorubicin loaded microparticles were first placed in 1× PBS at pH 2.0. After 90 min, 5N NaOH was added to increase the pH to 7.0 where release continued for 6 h. Samples were obtained as earlier. The mass of doxorubicin released was determined by the fluorescent plate reader and reported as follows

$$\text{Mass Released} = M_t/M_\infty \times 100 \quad (2)$$

where M_t is mass released at a given time and M_∞ is total mass released.

Cytocompatibility

Caco-2 and HT29-MTX cells were maintained in DMEM and are used as GI tract cell models. SW620 cells were maintained in RPMI media and were used as a colon cancer cell model. Both media were supplemented with heat-inactivated FBS, penicillin, and streptomycin. Cytocompatiblity experiments were performed in fibronectin coated 96-well plates (Nunc, Rochester, NY). Caco-2, HT29-MTX, and SW620 cells were seeded at a density of 2.0×10^3, 3.0×10^4, and 1.5×10^4 cells/cm^2, respectively, and incubated for 48 h before testing.

P(MAA-*g*-EG) and IPNs were incubated with all cell lines for 2 h at concentrations ranging from 1 to 5 mg/mL. P(MAA-*g*-EG) and 75/25 IPN were also evaluated for long-term exposure using a 5 mg/mL concentration for 6, 12, and 24 h for all cell lines. To determine cell viability, the microparticles were aspirated after the timed exposure, cell lines rinsed three times with 1× PBS, and MTS assayed (CellTiter 96® Aqueous One Solution Cell Proliferation Assay).

Mucoadhesion

Mucoadhesion testing was carried out on P(MAA-*g*-EG) and 75/25 IPN disks using a texture analyzer (TA.XT plus, Stable Micro Systems, UK) and a mucoadhesive testing rig (Stable Micro Systems, UK). Sample disks were allowed to swell and equilibrate in 1× PBS adjusted to a pH of 7.0 for 24 h and attached to a cylindrical probe.

Fresh porcine upper small intestine tissue was obtained immediately after slaughter at the local slaughterhouse and used within 2–4 h. Rectangle tissue samples were placed on the mucoadhesive rig, submerged in 1× PBS (pH 7.0) and thermostated to 37°C. The probe and disk were attached to the texture analyzer, the polymer sample was lowered at a rate of 5 mm/min until a force of 5 g was sensed between polymer sample and tissue, then proceeded to lower at 0.1 mm/min until a force of 50 g was applied. After 5 min of static loaded force, the probe was withdrawn at a rate of 0.1 mm/min, until it was fully detached from the tissue sample. Using texture analyzer software (Texture Exponent 32), the work of adhesion (W_{ad}) was determined and used to compare mucoadhesive properties between samples. W_{ad} was calculated as the area under the curve of a force vs. distance plot, where force was the force required to deadhere samples from porcine tissue and distance was how far the sample traveled during deadhesion.

Table 1. Doxorubicin Loading Efficiency and Weight Percent Loading for P(MAA-*g*-EG) and IPNs (75–500 μm)

Formulation	Loading Efficiency (%)	Wt. loading (%)
P(MAA-*g*-EG)	47 ± 1	2.3
80/20 IPN	30 ± 5	1.5
75/25 IPN	56 ± 2	2.8
70/30 IPN	40 ± 3	2.0

Results and Discussion

Synthesis of IPNs of P(MAA-g-EG) and PBA

IPNs were formed by sequential free radical UV-initiated polymerization of P(MAA-*g*-EG) then PBA in the presence of the P(MAA-*g*-EG). During polymerization of PBA, the ethanol and water were varied to develop IPNs with different degrees of hydrophobicity. Extensive material characterization, particularly for hydrophobic and swelling properties, was previously completed and indicated that each IPN expresses a unique set of properties that will affect loading and release properties of hydrophobic therapeutic agents.[28]

Loading doxorubicin in P(MAA-g-EG) and IPNs

The loading of doxorubicin was dependent on the hydrophobic and hydrophilic ratio among the IPNs and P(MAA-*g*-EG) (Table 1). P(MAA-*g*-EG) had a loading efficiency of 48 ± 1%, and the 75/25 had the highest at 55 ± 2%. The 75/25 IPN possesses the appropriate amount of hydrophobic PBA content while still swelling appreciably to imbibe the highest amount of doxorubicin. P(MAA-*g*-EG), which displays the greatest swelling ratio,[28] allows it to still have a high loading level as compared to the other IPNs, but does not possess the hydrophobic content to reach the loading levels of the 75/25 IPN. The 80/20 IPN has the smallest hydrophobic content, and the 70/30 IPN has the lowest swelling level and these reasons contribute to their reduced loading levels. Weight percent loading ranged from 1.5 to 2.8% and is defined as the mg of doxorubicin per mg of polymer.

Doxorubicin release from P(MAA-g-EG) and IPNs

Release studies were completed in neutral pH, low pH, and two-step pH conditions. In neutral pH, 86–90% of doxorubicin released occurred within 4 h for P(MAA-*g*-EG), 80/20 IPN, 75/25 IPN, and 70/30 IPN (Figure 1). In neutral pH conditions, the polymer carriers are capable of developing a porous structure due to polymer swelling that allows doxorubicin to diffuse out and into the surrounding solution.

To simulate the GI pH conditions and transit time, a two-step pH model was used. First, doxorubicin loaded particles were released in pH 2.0 (1× PBS) for 90 min and then increased to pH 7.0 (1× PBS) for an additional 6 h. IPNs released approximately 30–70% of doxorubicin in the low pH conditions as compared to P(MAA-*g*-EG)'s 20% (Figure 2). After transitioning from the low pH to neutral pH conditions, the remaining amount of doxorubicin loaded in the IPNs and P(MAA-*g*-EG) were slowly released over 4 h

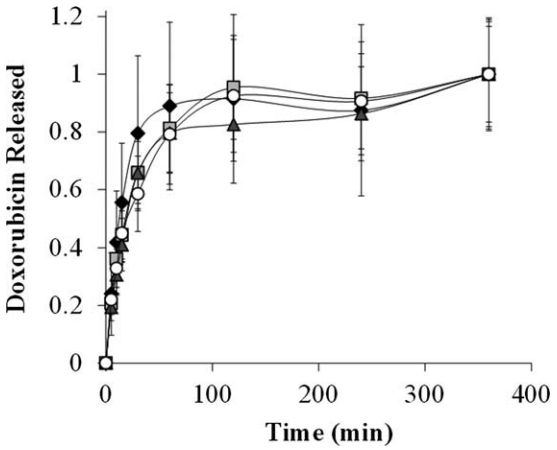

Figure 1. Doxorubicin release of P(MAA-*g*-EG) and IPNs in neutral pH conditions.

Doxorubicin loaded P(MAA-*g*-EG) (◆), 80/20 IPN (■), 75/25 IPN (▲), and 70/30 IPN (○) crushed particles (70–500 μm) were released in 1× PBS (pH 7.4) for 6 h. Doxorubicin release is expressed as M_t/M_∞. Curves generated are $n = 3$, and error bars represent error propagation due to ratio of M_t/M_∞.

(Figure 2). The large amount of doxorubicin released in the low pH conditions could be harmful for *in vivo* conditions and appeared to be dependent on the degree of hydrophobicity. The 70/30 IPN had the highest degree of hydrophobicity, the 75/25 IPN the next highest, and the 80/20 IPN the lowest. In the low pH conditions of the two-step pH release of doxorubicin, the 80/20 IPN demonstrated the least amount of released (30%), whereas the 75/25 IPN and 70/30 IPN released 64 and 70%, respectively. These large burst effects for the IPNs as compared to the P(MAA-*g*-EG) initially indicated surface loading of doxorubicin instead of uniform distribution through the core of the polymer carrier. To further investigate, a 75/25 IPN doxorubicin loaded sample was exposed to a continuous drip of 1× PBS for 30 min, which

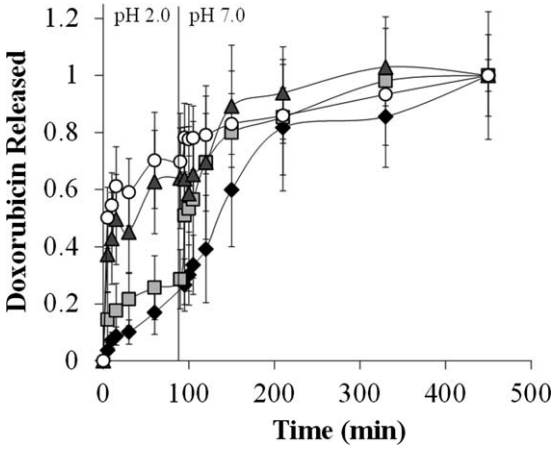

Figure 2. Doxorubicin release of P(MAA-*g*-EG) and IPNs in two-step pH conditions.

Doxorubicin loaded P(MAA-*g*-EG) (◆), 80/20 IPN (■), 75/25 IPN (▲), and 70/30 IPN (○) crushed particles (70–500 μm) were released in 1× PBS (pH 2.0) for 90 min. Then, the pH was increased to 7.0 by adding 5N NaOH and release continued for 6 h. Doxorubicin release is expressed as M_t/M_∞. Curves generated are $n = 3$, and error bars represent error propagation due to ratio of M_t/M_∞.

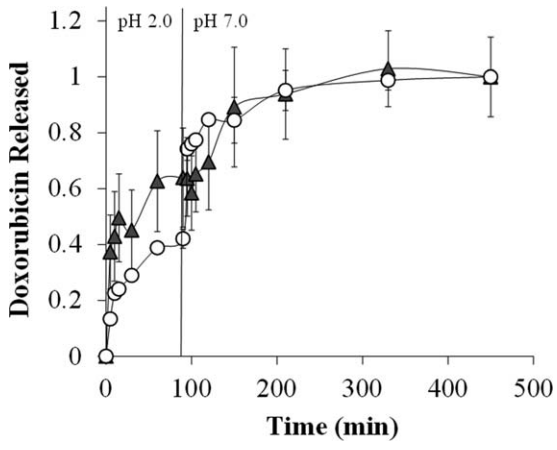

Figure 3. Doxorubicin release of 75/25 IPN with and without washing in two-step pH conditions.

Doxorubicin loaded 75/25 IPN (▲) and 75/25 IPN washed with water for 30 min (○) were released in 1× PBS (pH 2.0) for 90 min. Then, the pH was increased to 7.0 by adding 5N NaOH and release continued for 6 h. Doxorubicin release is expressed as M_t/M_∞. Curves generated are $n = 3$, and error bars represent error propagation due to ratio of M_t/M_∞.

is sufficient enough to remove surface adsorbed doxorubicin but insufficient enough to fully release all doxorubicin from the core of the particle. The results of the 75/25 IPN with and without the 30 min washed are illustrated in Figure 3. 75/25 IPN particles washed for 30 min released 42% of doxorubicin as compared to 64% of unwashed 75/25 IPN particles. These results indicated that the adsorption of doxorubicin to the surface of the particles is a leading cause of burst effects in the low pH conditions. These materials may be viable options for the oral drug delivery of chemotherapeutics with further improved washing procedures for removing surface adsorbed doxorubicin.

Another hypothesis for burst release in low pH conditions is considered based on how the IPNs are synthesized. During the formation of the second hydrophobic PBA network, the P(MAA-*g*-EG) network is swollen in a ratio of ethanol to water. As the amount of water is increased in this ratio, the P(MAA-*g*-EG) network exhibits a higher degree of swelling resulting in a higher degree of hydrophobicity on polymerization of the butyl acrylate monomer. However, as a result of this degree of swelling and polymerization, permanent pores could have formed into the polymer structure of the PBA network. These pores would be formed by the presence of the PBA network and its ability to inhibit the first P(MAA-*g*-EG) network from fully collapsing on itself in low pH conditions. Thus, with increasing PBA content, larger pores or increased number of pores may be formed during the synthesis technique. Therefore, even in the collapsed state of the IPN structure, there still could be a porous structure that would allow doxorubicin to prematurely release in low pH conditions as a function of hydrophobicity. The premature release of doxorubicin in low pH conditions may not be beneficial for colon cancer, but may be effective for stomach cancers or targeting chemotherapeutics to the upper small intestine for transport for systemic circulation. This hypothesis is reconfirmed by dynamic swelling experiments where IPN disks demonstrated more water uptake in 5 min as compared to P(MAA-*g*-EG) and increased with increasing hydrophobic content.[28]

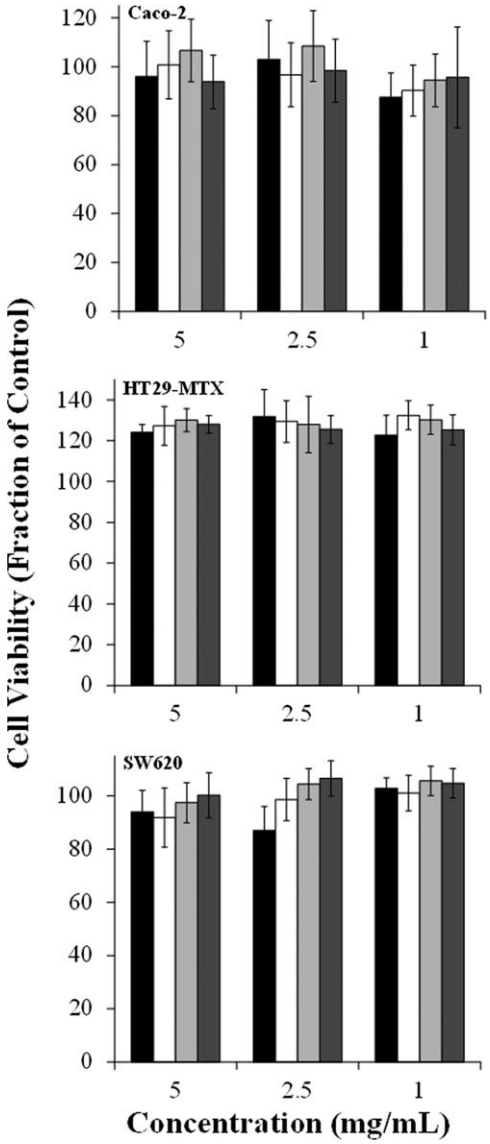

Figure 4. Effect of 2 h P(MAA-*g*-EG) and IPN exposure on Caco-2, HT29-MTX, and SW620 cell proliferation.

P(MAA-*g*-EG) (■), 80/20 IPN (□), 75/25 IPN (▒), and 70/30 IPN (▒) microparticles (75–500 μm) were added to all cell lines and incubated for 2 h. Error bars represent error propagated over control cells. $n = 6–8$.

Release studies in low pH conditions were extended to 2 h to further investigate release profiles for longer residency times in stomach conditions. After 2 h, the 75/25 IPN and P(MAA-*g*-EG) demonstrated no statistical increased in doxorubicin release in longer low pH conditions as compared to the two-step pH release profile (not pictured). These results indicate that variable stomach residency times due to digestion or variation from patient to patient may not play a major role in the effectiveness of oral delivery chemotherapeutics using these polymer carriers. Final concentration levels ranged from 9.11 to 26.66 μg/mL with P(MAA-*g*-EG) and 75/25 IPN being the lowest and highest, respectively.

Cytocompatiblity

Caco-2, HT29-MTX, and SW620 cells were plated in fibronectin coated 96-well plates and exposed to varying concentrations of P(MAA-*g*-EG) and IPNs for 2, 6, 12, or 24 h. For the 2-h study, P(MAA-*g*-EG) and IPNs were tested at the following concentrations: 1, 2.5, and 5 mg/mL. No significant decrease in cell viability was observed for these concentrations (Figure 4).

For exposure times of 6, 12, and 24 h, a concentration of 5 mg/mL of P(MAA-*g*-EG) or 75/25 IPN was used. No cytotoxic effects were observed for these longer residency times (Figure 5). These microparticles are not intended to cross the GI tract into the bloodstream; therefore, investigating long-term exposure relates to the residence time of microparticles in the small intestine and colon. The lack of toxicity indicates that these microparticles should be biocompatible with human physiological systems and appropriate for oral delivery of chemotherapeutics.

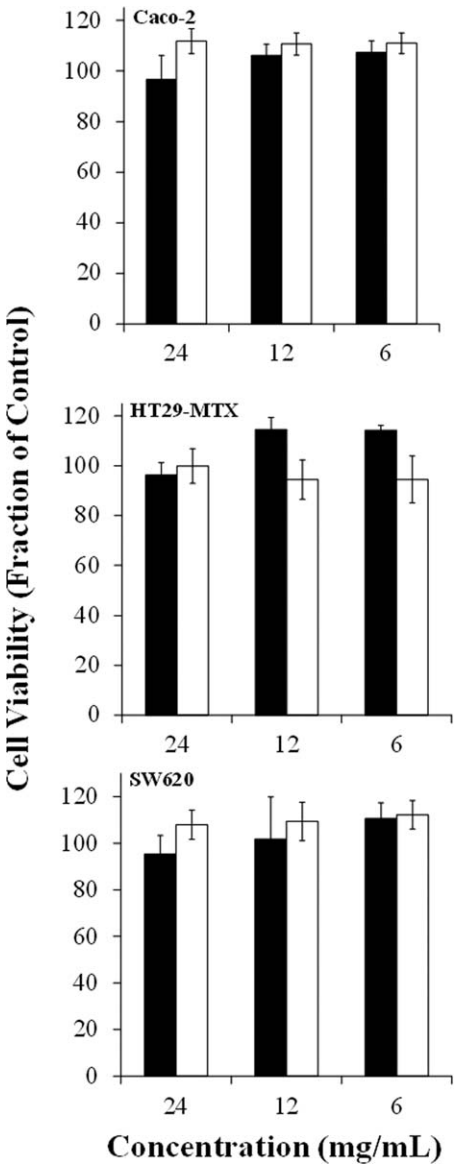

Figure 5. Effect of 6, 12, or 24 h P(MAA-*g*-EG) and IPN exposure on Caco-2, HT29-MTX, and SW620 cell proliferation.

P(MAA-*g*-EG) (■) and 75/25 IPN (□) microparticles (75–500 μm) were added to all cell lines and incubated for 6, 12, or 24 h. Error bars represent error propagated over control cells. $n = 6–8$.

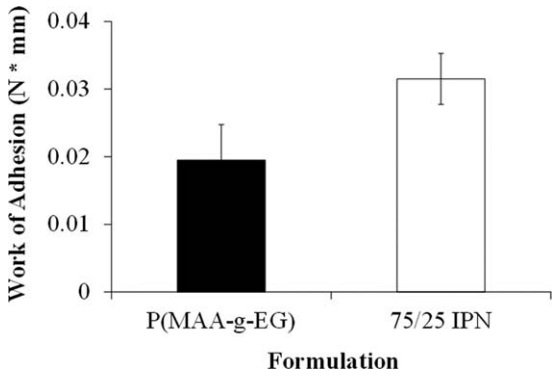

Figure 6. Mucoadhesion for P(MAA-*g*-EG) and 75/25 IPN.

P(MAA-*g*-EG) (■) and 75/25 IPN (□) disks were brought into contact with fresh porcine small intestine for 5 min, retracted slowly, the force measured with a tensile tester, and the work of adhesion computed. Results demonstrate statistical significance ($P < 0.05$). $n = 3–4 \pm$ SD.

P(MAA-*g*-EG)'s biocompatibility has been extensively studied in previous research, and these results are in agreement at concentrations less than 10 mg/mL.[26,29–31] However, the original P(MAA-*g*-EG) hydrogel has been modified with poly(butyl-acrylate) to form the IPN. The incorporation of these new materials could have influenced their cytotoxicity. From these studies, it can be determined that the addition of PBA at these concentration levels did not harm the cells. PBA's use in the medical community is significantly reduced as compared to other hydrophobic biomaterials such as poly(methyl methacrylate). Limited information is known about PBA and its biocompatibility with biological systems. However, that does not mean that PBA's potential for use in the medical community should be eliminated. PBA has been utilized for drug delivery applications in the formation of matrices for paclitaxel release or to form shape-memory suture wound closures and have also been shown to be biocompatible.[32,33]

Mucoadhesion

The mucoadhesion of P(MAA-*g*-EG) and 75/25 IPN were determined using a texture analyzer and mucoadhesive rig. The 75/25 IPN was chosen, because it demonstrated the highest loading efficiencies and release profiles appropriate for oral drug delivery. In the results obtained from these experiments (Figure 6), the IPN demonstrated the highest degree of adhesion and the P(MAA-*g*-EG) the lowest (*t*-test, $P < 0.05$). The incorporation of hydrophobic properties was expected to decrease the interaction of the polymer systems with the mucin surface, which is dominantly hydrophilic due to the presence of carbohydrates.

However, the 75/25 IPN demonstrated increased adhesion as compared to the pure P(MAA-*g*-EG) despite possessing hydrophobic properties. It is hypothesized that the increased adhesion of the 75/25 IPN is due to mobile ethylene glycol tethers. During the formation of the PBA network in the IPN, the first P(MAA-*g*-EG) network is swollen in a solution of water and butyl acrylate monomer and then exposed to UV light. The presence of this second PBA network can cause a porous network to form as well as limit the hydrogen bonding interaction between ethylene glycol chains and unionized hydroxyl groups on the MAA. More mobile tethers and decrease in potential bonding gives ethylene glycol tethers an improved chance for diffusion into the mucin layer and, thus, increase adhesion as compared to the unmodified P(MAA-*g*-EG) counterpart. Increased mucoadhesion means that these IPN microparticles would spend more time in the upper small intestine and, thus, be more suitable for trying to deliver chemotherapeutics to the bloodstream for system circulation.

Conclusions

IPNs and P(MAA-*g*-EG) hydrogels were subjected to the loading of doxorubicin, a low molecular weight, and hydrophobic chemotherapeutic. Release studies were extensively utilized in neutral, low, and two-step pH conditions to properly evaluate these polymer carriers' potentials as oral chemotherapeutic delivery vehicles.

For the IPN system, doxorubicin loading efficiencies ranged from 47 to 56% and were dependent on the IPNs ability to swell in combination with the proper amount of hydrophobic PBA content. The 75/25 IPN demonstrated the highest loading levels. In these experiments, loading efficiency is still considerably low for such costly drugs. Fine tuning loading experiments such as decreasing crushed particle size to enhance surface area to volume ratio and increasing total particle weight in the doxorubicin solution could improve efficiency and make this oral drug delivery device more conceivable.

Release studies show IPNs release more doxorubicin than P(MAA-*g*-EG) at low pH. For the IPN system, as the amount of hydrophobic content increased, the amount of doxorubicin released in low pH increased. This observation is due to doxorubicin adsorbed to the microparticle's surface instead of the core and due to the polymerization process when forming IPNs. The inability to reduce hydrophobic agent release in low pH is not advantageous for local, direct colon cancer delivery, but may be advantageous for stomach cancers or transporting the chemotherapeutic from the GI tract to the bloodstream through the upper small intestine.

Cytotoxicity and mucoadhesive experiments were conducted as *in vitro* assessments of P(MAA-*g*-EG) and IPNs. Cytotoxicity was completed on Caco-2 and HT29-MTX cell lines that represented a GI tract model whereas SW620 operated as a tumor model. For all polymer systems, 2 h exposure at varying concentrations, as well as a 6, 12, and 24 h exposure for P(MAA-*g*-EG) and 75/25 IPN, was completed. MTS assays indicated that all materials in all experimental conditions were biocompatible. Mucoadhesion experiments indicated that the 75/25 IPN possessed the greatest adhesion due to increased ethylene glycol tether mobility and decreased hydrogen bonding. Overall, these IPNs demonstrated potential for the oral delivery of chemotherapeutics to the stomach or the upper small intestine for transport into the bloodstream for metastatic cancers.

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