Dual effect of F-actin targeted carrier combined with antimitotic drug on aggressive colorectal cancer cytoskeleton: Allying dissimilar cell cytoskeleton disrupting mechanisms

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ABSTRACT

A recent approach to colon cancer therapy is to employ selective drugs with specific extra/intracellular sites of action. Alteration of cytoskeletal protein reorganization and, subsequently, to cellular biomechanical behaviour during cancer progression highly affects the cancer cell progress. Hence, cytoskeleton targeted drugs are an important class of cancer therapy agents. We have studied viscoelastic alteration of the human colon adenocarcinoma cell line, SW48, after treatment with a drug delivery system comprising chitosan as the carrier and albendazole as the microtubule-targeting agent (MTA). For the first time, we have evaluated the biomechanical characteristics of the cell line, using the micropipette aspiration (MA) method after treatment with drug delivery systems. Surprisingly, employing a chitosan-albendazole pair, in comparison with both neat materials, resulted in more significant change in the viscoelastic parameters of cells, including the elastic constants \(K_1\) and \(K_2\) and the coefficient of viscosity \(\mu\). This difference was more pronounced for cancer cells after 48 h of the treatment. Microtubule and actin microfilament (F-actin) contents in the cell line were studied by immunofluorescent staining. Good agreement was observed between the mechanical characteristics results and microtubule/F-actin contents of the treated SW48 cell line, which declined after treatment. The results showed that chitosan affected F-actin more, while MTA was more effective for microtubules. Toxicity studies were performed against two cancer cell lines (SW48 and MCF10A1h) and compared to normal cells, MCF10A. The results showed cancer selectiveness, safety of formulation, and enhanced anticancer efficacy of the CS/ABZ conjugate. This study suggests that employing such a suitable pair of drug-carriers with dissimilar sites of action, thus allaying the different cell cytoskeleton disrupting mechanisms, may provide a more efficient cancer therapy approach.

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1. Introduction

Colorectal cancer is known to be one of the major causes of cancer-related deaths worldwide. In view of its high growth rate of incidence of 5–6%, a great deal of research has been undertaken to develop therapeutic strategies for colon cancer. These efforts have mainly focused on producing specific drugs therapy in its metastatic stage (Dong et al., 1994; Kline and El-Deiry, 2013). A wide range of these specific chemotherapeutic agents act as inhibitors for the assembly of microtubules (MTs), an important part of the cell cytoskeleton, by favouring the curved protofilaments that are not able to associate laterally to form microtubules (Amos, 2011). The cytoskeleton comprises protein fibres inside eukaryotic cells (mainly microfilaments, intermediate filaments and MTs). The cytoskeleton is comparable to tensegrity structures...
that are self-stabilizing via the equilibrium of tension forces (inside microfilaments and intermediate filaments) and compression forces (inside microtubules) (Tanaka-Kamioka et al., 1998; Matthews et al., 2008).

Several important roles have been addressed for MTs in scientific reports (Ramlaho et al., 2007). It is known that MTs, by attaching to the ends of cellular structure such as chromosomes, mitotic spindles and other organelles, act to transport the cellular structure components around inside cells (Howard and Hyman, 2003). Meanwhile, MTs, as the second major cellular structural components, bind to the other filament biopolymers to stabilize the cytoskeleton against compression loadings (Pachenari et al., 2014). Moreover, it has been confirmed that involvement of the tubulin pathway is effective also in suppressing growth of Paclitaxel-resistant tumour cells (Chu et al., 2009). These characteristics, in addition to the dynamic structure of microtubules, make microtubule-targeting agents (MTAs) one of the most promising classes of drugs in cancer therapy.

Among MT targeting drugs, albendazole (ABZ) is considered as a highly effective therapeutic agent in disrupting tubulin polymerization in metastatic cells. ABZ is a benzimidazole that is usually employed for disrupting the microtubule cytoskeleton; it is a potent inhibitor of cell proliferation, angiogenesis and tumour growth (Saeheb et al., 2012). It has been shown that bioavailability of the drug is raised by opening the tight junctions of epithelial cell layers and also by reducing the rate of mucociliary clearance. These characteristics can be obtained using bioavailable drug carriers such as chitosan (CS) (Gulbake and Jain, 2012; Hsu et al., 2013). CS, a linear polysaccharide, is composed of randomly distributed d-glucosamine and N-acetyl-glucosamine units, and benefits from the ability to open intercellular tight junctions (TJs) in a pH dependent manner (Hejazi and Amiji, 2003; Taranejoo et al., 2011; Allaghaie et al., 2012; Alamdarnejad et al., 2013; Patel, 2014). CS derivatives can also be employed for a variety of cancer therapies to improve their safety and efficacy. Also, it has been reported that employing CS nanoparticles as anticancer drug carriers resulted in an enhanced anticancer effect of the therapeutic agent (Li et al., 2009).

Juliano et al. have found that the cytoskeletal architecture of Caco-2 cells is strongly affected by treatment with chitosan malate, but only for a high level of the CS derivative concentration. Such a dose-dependent and partially reversible redistribution of the cytoskeletal proteins, tubulin and actin under the action of employing chitosan malate has been reported elsewhere (Juliano et al., 2011).

More interestingly, it is known that CS and its derivatives can induce a redistribution of the tight junction protein ZO-1 and cytoskeletal F-actin that results in the opening of cellular tight junctions and increases the paracellular permeability of the epithelium (Smith et al., 2005). The actin microfilaments, as the most important structural reinforcing components of a cell, control the cytoskeleton response to the extracellular forces (Chhabra and Higgs, 2007).

We previously reported that the mechanical properties of cancer cytoskeleton depend on the relative content of actin microfilaments to MTs (Pachenari et al., 2014). However, the effects of employing drug delivery systems compared to neat chemotherapeutic drugs on the cytoskeleton of cancerous cells have not yet been clear.

The mechanical behaviours of cells and especially viscoelastic properties, which evidently are regulated by cytoskeletal biopolymers, have been introduced as a reliable and reproducible measure to study cancer cell progression, circulating tumour cell isolation, and drug efficacy evaluation. Understanding mechanical behaviour changes in tumour cells by anticancer drugs can enhance the profiling of tumours and tailored therapy (Seyedpou et al., 2015; Krishnan et al., 2016).

So far, two major quantitative approaches have been developed to evaluate the mechanical behaviour of the cells. In the first approach, such as micropipette aspiration (MA), the whole-cell deformability is assessed. In the second method, such as atomic force microscopy (AFM), only some points of the cells are considered (Kasza et al., 2007; Suresh, 2007). Compared with the AFM approach, the MA approach seems to be more reliable in representing the whole cell mechanical properties (Lim et al., 2006).

To the best of our knowledge, this is the first study that evaluates the viscoelastic alteration of cancer cells by the MA method incorporating treatment by a drug delivery system, composing of CS as a carrier and ABZ as an anticancer drug. In the present study, we investigated the effects of ABZ encapsulated CS nanoparticles, which are two cytoskeleton disturbing agents with different sites of action, on the mechanical behaviour of SW48, a human colon adenocarcinoma cell line. Finally, we evaluated the effect of ABZ and CS/ABZ on viability of multiple cell lines (including both cancer cell lines and normal cells).

2. Material and methods

2.1. Materials

Chitosan (low molecular weight, 75–85% deacetylated) was obtained from Aldrich. Sodium tripolyphosphate (TPP) was obtained from Merck (Germany). Albendazol was a gift from Damloran Co. (Iran). All other chemicals used were of analytical reagent grade.

2.2. Preparation of free and ABZ loaded CS nanoparticles

ABZ loaded CS nanoparticles were fabricated via a method we reported before (Alamdarnejad et al., 2013). 100 mg of ABZ along with 200 mg of CS were dissolved in 50 mL acetic acid (50%), under vigorous stirring for 24 h, at room temperature. 20 mL of aqueous solution of TPP (10 mg/ml) was then added dropwise to 50 mL of the drug–polymer solution under sonication power of 45 W. After 30 min, the resultant ABZ loaded CS nanoparticles (CS/ABZCS/ABZ) were collected by centrifugation at 13200 rpm for 1 h, washed in deionizer water, dispersed in distilled water and finally lyophilized at −60 °C for two days. The amount of the ABZ remaining in the supernatant was analysed using the UV–vis spectrophotometer at wavelength of 291 nm. The CS nanoparticles were prepared in a similar manner, but without using ABZ.

The drug entrapment efficiency (EE) can be obtained according to the following equation:

\[
\text{EE} = \frac{\text{Total amount of the ABZ} - \text{Amount of ABZ remaining in the supernatant}}{\text{Total amount of the ABZ}}
\] (1)

2.3. Fourier transform infrared (FTIR) spectroscopy

The FT-IR spectra of ABZ and CS/ABZ were recorded using an attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) (PerkinElmer, USA) in the range of 500–4000 cm⁻¹ at an average of 40 scans with a resolution of 4 cm⁻¹ over the spectral region 4000–500 cm⁻¹.

2.4. Morphology study, zeta potential and size measurement

A Philips SEM XL30 at 15 kV accelerating voltage was employed to examine the morphology of the free and the ABZ-loaded
nanoparticles. All the samples were previously sputter-coated with an ultra-thin layer of gold. Zeta potential and size measurement of the nanoparticles was done by a Zetasizer Nano-ZS-90 (Malvern Instruments) in 0.1 mM KCl solution at 25°C under automatic mode. Different pH conditions were applied for Zeta potential measurement.

2.5. MTT assay

SW48, MCF10CA1h and MCF10A cells were cultured in DMEM/F12 medium with 10 mM HEPE (Sigma), supplemented with 2.2 g/l sodium bicarbonate, 5% horse serum, 100 ng/ml chlorella toxin (Sigma), 0.5 μg/ml hydrocortisone, 10 μg/ml bovine insulin and 20 ng/ml human epidermal growth factor (EGF) (GIBCO). All cells were grown at 37°C in 5% CO2. Cells were seeded onto the 96 wells for viability assay prior 24 h. Various concentrations of free ABZ and CS/ABZ were added to the cells for estimating their toxicity against cancer cell lines (SW48 and MCF10CA1h) and a normal cell line, MCF10A. After 24 h incubation with ABZ and CS/ABZ, the cells were washed twice with 1× PBS and 20 ul of CellTiter 96 (Promega). Non-radioactive cell proliferation assay reagent was added to each well. Following incubation at 37°C in 5% CO2, absorbance reading was taken at 490 nm for different time points.

2.6. In vitro release studies

In vitro release of ABZ from the CS/ABZ was studied in a glass apparatus containing 50 mL of buffered saline (PBS, pH = 7.4) solution, as the release medium, at 37°C. 10 mg of ABZ-loaded nanoparticles were suspended in the medium and kept in a horizontal laboratory shaker, maintaining constant temperature and stirring (300 rpm). Samples (0.5 mL) were periodically removed and the volume of each sample was replaced by the same volume of fresh medium. The amount of released ABZ was analysed by the UV-spectrophotometer at 291 nm. The drug-release studies were performed in triplicate for each of the samples, and the quantity of ABZ was determined using a standard calibration curve, obtained under the same conditions.

2.7. Cell transfection

Culturing of SW48 cells was performed on a standard 96-well plate at a density of 10⁴ cells per well. After incubating for 24 h, the culture medium was replaced by fresh medium containing specific concentrations of ABZ (200 nM) and its equilibrium amount with the same encapsulated ABZ (2.7 mg/ml) and then again incubated for 72 h. Before adding drug (ABZ) or CS/ABZ, we have eliminated the serum for all of our experiments. So, we minimized the serum effects on cells.

2.8. Micropipette aspiration

Quantitative assessment of viscoelastic properties of cells was performed by the micropipette aspiration (MA) technique. MA experiments were performed similar to our previous studies (Jammaleki et al., 2016). Briefly, by adding 0.25% trypsin (Invitrogen), cells were detached from their substrate and then resuspended in new culture medium. The procedure was performed at room temperature at around 20–22°C. Then the cells were aspirated into the micropipette (internal diameters of micropipette within the range of 4.5–5 μm) through applying controlled suction pressure on the cell surface. Investigation of the leading edge of each cell surface was done by employing an inverted microscope (Nikon Eclipse) equipped with a digital camera (Nikon DXM1200). To avoid adhesion of cells to their inner walls, the micropipettes were coated with Sigmolute chemical agent (Sigma). Image analysis was done using Axiomvision LE Software (Zeiss).

The measured values using this method were used to calculate the parameters of the Maxwell model, which included a spring (k₁) in parallel with series of a damper (μ) and a spring (k₂) (Sato et al., 1996; Guilak et al., 2002; Seyedpour et al., 2015). The theoretical model had been previously reported for extracting the mechanical properties of cells.

The generalized Maxwell model includes a spring (k₁) that provides the restoring force necessary to recover the initial shape after the release of the stress in parallel with series of a damper (μ) and a spring (k₂). These parameters are all assumed to be constant. Cells are assumed to be homogeneous, half-space, incompressible and viscoelastic materials influenced by a uniform axisymmetric aspiration pressure 1. The relation between elastic and viscous parameters is described by Eq. (2), in which the boundary condition of no axial displacement of the cell at the micropipette end is considered.

\[ L(t) = \frac{2Δp}{μ\pi} \left( 1 + \frac{k_1}{k_1 + k_2} - 1 \right) c(t) + h(t) \]

Here, Δp is the applied pressure, μ is the inner radius of the micropipette, and h(t) and L(t) are the time-dependent unit step function and aspirated length, respectively. The time constant parameter (τ) of the introduced viscoelastic model can be obtained using following equation:

\[ τ = \frac{μ}{k_1(1 + \frac{k_1}{k_2})} \]

The viscoelastic parameters of cells are obtained through curve fitting of experimental data y vs time by employing the least square method in Matlab software, Release R2013b according to the following equations:

\[ y = ae^{-bx} + c, \]

\[ a = \frac{2Δp}{k_1τ} \left( \frac{k_1}{k_1 + k_2} - 1 \right), \quad b = \frac{1}{τ}, \quad c = \frac{2Δp}{k_1τ} \]

Both elastic constants are related to standard elasticity coefficients by following equations. E₀ and E∞ are the instantaneous and the equilibrium Young modulus, respectively.

\[ E_0 = \frac{3}{2}(k_1 + k_2), \]

\[ E_∞ = \frac{3}{2}k_1. \]

2.9. Fluorescence labelling for microscopy

Phalloidin, Fluorescein isothiocyanate labeled (Sigma, P5282), was used to visualize actin filaments. After washing with phosphate buffered saline (PBS), the cells were fixed for 5 min with (3.7%) paraformaldehyde (dissolved in PBS buffer). Then, after several rinses in PBS, the cells were permeabilized with (0.1%) Triton-X100 in PBS and washed again in PBS. Actin staining was achieved in a 50 mg/ml fluorescent phalloidin conjugate solution in PBS for 40 min. For detection and localization of MTs. Monoclonal Anti-β-Tubulin-Cy3 (Sigma, C4585) was used. Diluted antibody conjugate in PBS containing (1%) BSA was added to cover actin-stained cells and incubated for 60 min. Washing several
times with PBS, samples were left to dry. Images were captured with an inverted fluorescent microscope (Olympus, BX51 with DP72 camera) and were processed with ImageJ software. The level of fluorescence intensity was measured in terms of the corrected total cell fluorescence (CTCF):

\[ \text{CTCF} = \text{integrated density of pixels for one cell} - (\text{area of the selected cell} \times \text{mean fluorescence of background}) \] (8)

Relative fluorescence of MTs to F-actin microfilaments (RFMA) per cell for each cell line was calculated to evaluate the change in the cytoskeletal elements (Pachenari et al., 2014; Seydpour et al., 2015; Tavakolinejad et al., 2015):

\[ RFMA = \frac{\text{CTCF(ForF - Tubulins)}}{\text{CTCF(ForF - actins)}} \] (9)

2.10. Statistical analysis

Analysis of variance and Dunnet’s multiple comparisons post-test was done to compare the results. The Dunnet method compares mean difference of each test group with the control e.g. Control vs. 6 h, instead of including other groups which enhances to detect differences by reducing the number of comparisons. To appraise the difference between treated CS, ABZ and CS/ABZ cells and the control group, a \( P < 0.05 \) was applied. Furthermore, a post-test for trend was performed to determine if the means of the viscosity and elasticity decreased systematically with drug treatment time. All statistical tests and graphs were performed using the version 6.0 of GraphPad Prism.

3. Results and discussion

3.1. Chemical structure of nanoparticles

The chemical structures of ABZ and CS/ABZ were identified with Fourier transformation infrared spectroscopy (FTIR) and the results are presented in Fig. 1. The bonds at 898, 930 and 1053 cm\(^{-1}\) are assigned for C—H in plane deformation of ABZ (Gunsekara et al., 2008). Characteristic peaks are Amide IV at 1232 cm\(^{-1}\), Amid III at 1287 cm\(^{-1}\) and Amide I at 1698 cm\(^{-1}\) of ABZ. The bonds at 1639 cm\(^{-1}\) and 3367 cm\(^{-1}\) of CS/ABZ represent the Amide II and —C—H— stretching of CS.

3.2. Morphological study

The nanoparticles have pseudo-spherical shape and have uniformly distributed size and structure (Fig. 2). The average size of the nanoparticles, obtained by a Zetasizer, is 92.2 ± 5.6 nm.

3.3. Particle stability

Zeta potential studies at different pH (3, 7.4) and time points (0, 3 days) were performed to evaluate the stability of the fabricated

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**Fig. 1.** The FTIR spectra of ABZ and ABZ/CS.

**Fig. 2.** SEM morphological image of CS nanoparticles obtained from (a) high concentration and (b) low concentration of CS nanoparticle suspension.
nanoparticles (Fig. 3). The highest Zeta potential value (+55 mv) was obtained for the NPs fabricated and dispersed at pH = 3. Unsurprisingly, fabricating and dispersing nanoparticles at higher pH resulted in reducing net positive charge, and a decreased Zeta potential (+14 mv). No significant changes in Zeta potential values of the nanoparticles were observed after three days of immersion. It proves that no significant nanoparticle aggregation occurred during the study that caused stability of the fabricated nanoparticles.

3.4. Micropipette aspiration results

Viscoelastic parameters ($K_1$, $\mu$, $K_2$ and $\tau$) of SW48 treated before and after treatment with albendazole (ABZ) 200 nM, Chitosan (CS) and CS/ABZ with the same amount of encapsulated ABZ (200 nM) are presented in Fig. 4. $K_1$, correlating with elastic modulus value (E), represents the restoring force necessary to recover the initial shape after the release of the stress after treatment with ABZ, CS and CS/ABZ (Howard and Hyman, 2003). As shown in Fig. 4(a), the change in the values of the $K_1$ is related more closely with the deformation of actin microfilaments, as the major structural reinforcing cytoskeleton part. F-actin microfilaments are responsible for providing the highest resistance to deformation up to a critical value of local strain, and hence have the major role for determining the $K_1$ value (Jannney et al., 1991).

In the first 6 h of the study, no obvious change was observed in any sample in the case of elastic modulus (P-value > 0.98). It is mainly because of the time-consuming nature of cytoskeleton disrupter mechanisms after treatment by therapeutic agents. ABZ and other benzimidazoles are known to bind to beta-tubulin and disrupt microtubule polymerization (Robinson et al., 2004). For cells that were affected by ABZ, such a disrupting approach needs sufficient time to be effective (Chu et al., 2009). On the other hand, treatment with CS affected the organization of the microfilaments structures; however, tangible alterations were only observed after

Fig. 3. Zeta potential of the fabricated nanoparticles at pH=3 and pH=7.4.

Fig. 4. Viscoelastic parameters of SW48 treated before and after treatment with albendazole (ABZ) 200 nM, Chitosan (CS) and CS/ABZ with the same amount of encapsulated ABZ (200 nM). (a) Elastic constant, $K_1$, (b) Elastic constant, $K_2$. (c) Coefficient of viscosity, $\mu$. (d) Time constant $\tau$. 
specific exposure to CS. Again, such an outcome needs a considerable amount of time in order to reach an irregular arrangement, and consequently to affect the cell modulus. It seems a 6-h treatment was not enough to cause this alteration for the CS-treated cells.

In the second 6-h interval, remarkable decreases in the value of \( K_1 \) were detected in ABZ (P-value < 0.04) and ABZ/CS (P-value < 0.02) affected samples of SW48 cell. Furthermore, using ABZ caused nearly 30.8% decline in \( K_1 \) after 12 h, compared with just 17.8% decrease in \( K_1 \) in the case of the CS treated sample (P-value > 0.2), over a similar interval.

In the first 12-h, employing ABZ-CS and neat ABZ was associated with a similar effect on \( K_1 \). During the second 12-h interval, however, no significant change was observed in the value of \( K_1 \) in the ABZ affected cell line. Through the same time-interval ABZ loaded CS caused a drastic drop off in \( K_1 \) value (approximately 29.6% decline after approximately 24 h of treatment, compared with 12 h). As stated before, CS and ABZ act on the cell cytoskeleton with dissimilar mechanisms. It has been reported that CS mainly influences the actin microfilaments but partially the MTs too, while on the other hand, ABZ and other benzimidazoles are known to bind to beta-tubulin and disrupt microtubule polymerization (Chhabra and Higgins, 2007). ABZ more vigorously affected its cytoskeleton site of action, e.g., MTs. Combining these two dissimilar disrupting mechanisms against cell cytoskeleton, resulted in such a significant change in \( K_1 \) value of the ABZ/CS-treated cells.

During the study, the value of \( \mu \), similar to \( K_1 \), decreased after treating with all different samples (Fig. 4(b)). For CS-treated cells, After 12 h, the value of \( \mu \) reached close to its final level, approximately 3342.97 PaS and remained unchanged. But for ABZ-affected cells the \( \mu \) value continued its decreasing trend and reached from 1324.45 PaS to around 1192.866 PaS in 24 and 48 h, respectively. MTs have the main important role in regulation of the value of \( \mu \). It has been reported that hyperpolymerization and stabilization of microtubules resulted in a time-dependent increase in the viscosity (Yamamoto et al., 1998). At the final time stage, the CS/ABZ resulted in a greater decline in the quantity of \( \mu \) compared to both ABZ and CS. Treatment with CS/ABZ, in comparison with CS and ABZ, caused more reduction in \( K_2 \). But in the term of time constant (\( \tau \)) the difference was not significant (P-value > 0.19).

The normalized value of Young’s Modulus change, \( \frac{\Delta E}{E_0} \), has been shown in Fig. 5. Obviously, SW48 cells reached their final value for Young’s modulus (E) after approximately 12 h of ABZ treatment (Fig. 5). \( \frac{\Delta E}{E_0} \) increased from 0.27 to around 0.5, between 12 h and 48 h of CS/ABZ treatment, respectively. The normalized value of viscosity change, \( \frac{\Delta \mu}{\mu_0} \), after 48 h of cell treatment with ABZ and CS/ABZ increased to approximately a same amount around 0.8.

The calculated P-value was < 0.005, confirming that the trends in \( K_1 \) and \( \mu \) were both significant. Although CS/ABZ resulted in a greater drop off in elastic modulus \( K_1(E) \), but its disrupting effect on cell cytoskeleton, and consequently on \( K_1 \) value, was delayed up to the second 12-h interval. The dilatory trend of CS/ABZ arises from a typical characteristic of such drug delivery systems, which somewhat impeded the sudden drug release. Time-dependent diffusion of ABZ onwards the nanoparticles caused the gradual exposure of cells with the drug. This phenomenon postponed the cytoskeleton mechanical effects of cell treatment with CS/ABZ.

The instantaneous Young’s modulus (\( E_0 = \frac{1}{2}(K_1 + K_2) \)) decreased with time after treatment with CS, ABZ, and CS/ABZ samples (Fig. 6). After 12 h, CS-treated SW48 cells, showed a greater change in the value of \( E_0 \) compared with ABZ treated cells (60.80% and 40.09% decreases, respectively). \( E_0 \) represents the mechanical properties of the cell cortex which is the first examined part of the cell in micropipette aspiration experiments. The interaction of positively-charged amine groups on the CS backbone with negatively-charged macromolecules, such as integrin \( \alpha V \beta 3 \) on cell membranes caused rapid disruption of membrane cytoskeleton. (Hejazi and Amiji, 2003; Taranejoo et al., 2011; Aliagaia et al., 2012; Alamdarnejad et al., 2013; Patel, 2014).

The effect of ABZ was less significant for the first 6 h of treatment. However, the final value of \( E_0 \) for ABZ-treated cells was close to the final value of \( E_0 \) after treatment with CS/ABZ (70.37 Pa) and much lower than Final \( E_0 \) of CS-treated cells (232.62 Pa). It was shown that long-term cell treatment with a moderate
concentration of an MTA, like ABZ, can result in significant decline in $E_0$ value of invasive cells, (e.g. SW48) (Seyedpour et al., 2015).

3.5. Drug release characteristics

The cumulative release profiles for the ABZ-loaded CS nanoparticles as a function of time has been presented in Fig. 7. The profile has an initial rapid release with more than 87% release of ABZ within the first 12 h and a very slow and steady release in the following period, reaching a plateau after 84 h. The majority of encapsulated ABZ is released just before the third 6-h interval. This phenomenon confirms the dilatory trend of CS/ABZ. Releasing a considerable amount of ABZ in the second 6-h interval (from 50.2% in the first 6 h to 87.2% after 12 h) provided the opportunity for the delivery system to continue its role to effectively change the cytoskeleton microstructure and hence, $E(K_1)$ and $\mu$ values.

3.6. Cytoskeleton content evaluation and staining

Fig. 8 shows the actin filaments and MTs of the SW48 cells (Untreated, CS-Treated, ABZ-treated and CS/ABZ-treated SW48 cells) visualized via immunofluorescent staining. As shown, treatment with ABZ somewhat resulted in rearrangement and cell content change of MT in SW48. Quantified results of cytoskeleton content evaluation obtained from immunofluorescent staining of SW48 cells are presented as the relative fluorescence of MTs to F-actin microfilaments (RFMA) in Fig. 9.

In comparison with MT, the microfilament content of SW48 underwent a less decline after treatment with ABZ. ABZ treatment, compared to CS treatment, caused a greater drop in the MTs: F-actin microfilament ratio in SW48. Treatment with CS/ABZ delivery system resulted in an alteration in actin microstructure and content. This alteration agrees well with the results that were obtained from the viscoelastic characteristics that showed a huge

![Fig. 7. ABZ cumulative release profiles for the CS/ABZ NPs.](image)

![Fig. 8. The actin filaments and MTs of the SW48 cells (Untreated, CS-Treated, ABZ-treated and CS/ABZ-treated SW48 cells) visualized via immunofluorescent staining.](image)
Some research studies showed that a large decrease in actin stability is associated with inducing cell death (Gourlay et al., 2004; Gourlay and Ayscough, 2005). It was previously shown that early stages of apoptosis are associated with depolymerization of actin and degradation of intermediate filaments (Bursch et al., 2000). On the other hand, MTs have a key role in the formation process of the mitotic spindles, disruption of which would result in cell death. It is well known that employing microtubule-disrupting agents for chemotherapy induces cellular apoptosis (Pourgholami et al., 2005). As shown, the most vigorous change occurred through employing ABZ. (ABZ and CS/ABZ treatments decreased the RFMA of SW48 cells by approximately 30% and 16%, respectively. ABZ was mainly effective on MTs contents rather than actin microfilaments contents. Hence, treatment with ABZ caused decreasing the

**Fig. 9.** Microtubule: F-actin content ratio obtained from fluorescence staining. Treatment with ABZ 200 nM and CS/ABZ (200 nM) led to a decrease in this ratio.

**Fig. 10.** The viability of SW48, MCF10CA1h and MCF 10A after 6, 12, 24 and 48 h exposures to different concentrations of ABZ and ABZ loaded CS NPs at 37 °C.
relative fluorescence of MTs to F-actin microfilaments (RFMA). But CS/ABZ, alloying two stated dissimilar cell cytoskeleton disrupting mechanisms, significantly decreased the cell content of both MTs and actin.

3.7. Cancer selectivity and anticancer efficacy

The main aim of the study was to determine the anticancer efficacy and cancer selectivity of ABZ and CS/ABZ conjugation. Human colon and breast cancer cell lines (SW48, MCF10A) and a non-tumorigenic epithelial cell line, MCF10A, were chosen for in-vitro toxicity evaluation of ABZ and CS/ABZ. Fig. 10 displays the viability of SW48, MCF10AC1h and MCF 10A after 6, 12, 24 and 48 h exposures to different concentrations of ABZ and ABZ loaded CS NPs at 37° C. In vitro treatment of these cell lines with various concentrations of ABZ and CS/ABZ showed that toxicity of the anticancer drug and its CS conjugation was both dose and time dependent. Results proved that for both cancer cell lines, anticancer efficacy of CS/ABZ conjugate was significantly higher than neat ABZ (P = 0.01). For example, almost no cell viability was found for both SW48 and 0MCF10AC1 h after 12 h treatment with 400 nM CS/ABZ. However, at the same time interval (12 h) and ABZ concentration level (400 nM), around 12% and 25% cellular viability values were observed for SW48 and MCF10A1h, respectively. In comparison with these two cancer cell lines, MCF10A treated with different doses of both ABZ and CS/ABZ showed cell viability levels of more than 60% after 48 h, indicating low toxicity of ABZ and CS/ABZ conjugate toward this typical normal cell line. Selective cytotoxicity of ABZ to other cancer cell lines (e.g., melanoma cells), as compared to normal cells, has been reported elsewhere (Patel, 2011). However, it is the first study that represents this characteristic for CS/ABZ conjugate. These outcomes confirm cancer selectiveness, safety of formulation, and enhanced anticancer efficacy of the CS/ABZ conjugate.

4. Conclusion

The biomechanical properties and cytokinetics of a typical cancer cell, SW48, was studied before and after treatment with albendazole encapsulated chitosan nanoparticles. This study showed that employing albendazole carried by chitosan nanoparticles can be considered to be an effective approach to ally both stated cell cytoskeleton disrupting mechanisms. Microtubule-targeting agents form a most promising class of drugs in cancer therapy. Employing chitosan-based nanoparticles as the drug carrier, added to give more efficient drug delivery with controlled release profile, is a suitable approach to more effectively target the other parts of the cell cytokinetics, e.g., actin microfilaments. Cell viability studies showed enhanced anticancer efficacy of CS/ABZ compared to ABZ for two cancer cell lines. Moreover, CS/ABZ displayed anticancer cell selectivity characteristics.

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