World Cancer Research Journal WCRJ 2020; 7: e1462

HERBAL EXTRACT INCORPORATED CHITOSAN BASED NANOFIBERS AS A NEW STRATEGY FOR SMART ANTICANCER DRUG DELIVERY SYSTEM: AN *IN VITRO* MODEL

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Abstract – *Objective:* *Despite the anticancer effect of Berberine (BBR), low aqueous solubility and poor gastrointestinal absorption can make its therapeutic usage difficult. However, chitosan/polyethylene oxide (CH/PEO) nanofibers scaffold eliminate this problem. This study has been conducted to recognize CH/PEO/BBR nanofibers effect on cancer cell lines.*

Materials and Methods: *CH/PEO solution was prepared at different ratios for achieving optimal nanofibers. CH/PEO/BBR nanofibers were provided via electrospinning. Internal structure and 3-D morphology of fibers were studied using TEM and AFM, respectively. Functional groups were analyzed by a Fourier Transform Infrared (FTIR) spectroscopic device. Characterization of electrospun nanofibers was done by SEM. BBR released from nanoscaffolds was detected within 2 weeks by a UV-Visible device.*

The growth and proliferation of human breast cancer cell lines (MDA-MB-468, BT474 and MCF7), human HeLa cervical cancer cells and fibroblast cells in cultured medium were investigated by an inverted microscope. The cytotoxic effect of CH/PEO/BBR nanofibers against mentioned cell lines was characterized by MTT assay. Statistical analysis was done by SPSS-18 software. $p<0.05$ was considered as significant.

Results: *Nanoscaffolds containing 0.5-20 wt.% BBR concentrations inhibited cell growth compared to the control group in HeLa, BT474, MCF7 and MDA-MB-468 cell lines. The cell viability of cancer cell lines was significantly decreased after exposure with CH/PEO/BBR in a time dependent manner (HeLa, BT474, MCF7 ($p=0.000$) and MDA-MB-468 ($p=0.001$)).*

Conclusions: *Our results suggested that CH/PEO/BBR nanofiber has the potential to be developed as co-chemotherapeutic agent for human breast and cervical cancer therapy. However, its molecular mechanisms need to be further explored.*

KEYWORDS: *Chitosan/Polyethylene oxide, Cancer, Drug delivery system, Natural compounds, Therapeutic agent.*

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INTRODUCTION

Cancer is one of the most common causes of morbidity and mortality worldwide and a major public health issue, especially in women[1]. Disability and early death from breast or cervical cancer are preventable disaster for thousands of women and their families each year. In 2012, more than half a million women lost their life due to these two cancers[2].

Despite advances in medicine and the use of common therapies such as chemotherapy, radiotherapy and surgery, incidences of these cancers are increasing. These treatments are costly, invasive and toxic, with serious side effects to normal cells. One way to overcome these challenges is to use other treatments, such as herbal remedies[2,3].

Natural compounds have been used for centuries as an effective complementary and alternative medicine for various diseases such as cancer[4]. Plants with a wide range of biological and medicinal properties are highly safe, widely available, low cost, high efficiency, and display a number of possessions including antioxidant, antibacterial, anti-inflammatory, and even anticancer activity[5].

Berberine (BBR), an isoquinoline alkaloid, has a history of usage in Ayurvedic, Iranian and Chinese medicine since time immemorial [6]. BBR possesses antiviral, antimicrobial, anti-inflammatory properties and other pharmacological effects and is used for the treatment of many diseases, including hyperglycemia, metabolic syndrome, gastrointestinal infections, diarrhea, obesity and coronary heart disorders[5,7,8]. The anticancer activity of BBR has been revealed in various cancers, such as leukemia, lung, breast, ovarian and colorectal cancers[8-11].

BBR can suppress promotion, invasion and metastasis of tumor and inhibits cancer cells proliferation through induction of the cell cycle arrest and cellular apoptosis[7,12,13]. Furthermore, BBR exhibits little resistance and low toxicity to normal tissues during cancer treatment[14].

Despite the benefits of BBR, there are some limitations in its therapeutic applications, the most notable of which is its low aqueous solubility and poor absorption by the gastrointestinal tract[15,16]. To overcome these drawbacks, many approaches have been designed to employ nanostructure carriers to improve solubility and bioavailability of BBR and ultimately, increase its effectiveness and safety[16].

Different materials are used to produce nanofibers. Chitosan (CH), for example, is a natural polysaccharide mainly obtained from shrimp and crab shells with non-toxic, degradability and biocompatibility properties[17]. It has been utilized for drug delivery recently.

However, CH electrospinning is not possible alone because of its poor solubility in water and its high viscosity in aqueous solutions, and it can be carried out in the presence of other polymers. However, the addition of polyethylene oxide (PEO) can solve this problem. Based on previous research, the matrix made with CH/PEO (90:10 ratio) retains its fiber structure integrity in the water[18].

The aim of the present study was to investigate the anticancer activity of CH/PEO nanofibers containing BBR on cancer cell lines. For this purpose, nanocomposite fibers, including CH/PEO and BBR, were successfully prepared using electrospinning. Our data provides clues to this nanocomposite as a novel effective agent for cancer treatment.

MATERIALS AND METHODS

Chemicals and instruments

Medium molecular weight CH (85% of deacetylation), PEO with molecular weight of 900 kD, Berberine (BBR), DMEM/F12, RPMI-1640 and MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide)] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, dimethyl sulfoxide (DMSO) and trypsin were prepared from Merck (KGaA, Darmstadt, Germany).

Electrospinning instrument (ES100; Fanavaran nano-meghyas, Tehran, Iran), Scanning electron microscope (SEM) (AIS 2100; Uiwang-si, Korea), Fourier Transform Infrared (FTIR) device (Nexus 670; USA), UV-Visible (UV-Vis) spectrometer (Analytik Jena AG, Germany), Atomic force microscope (AFM) (NanoWizard-II; Berlin, Germany), Transmission electron microscope (TEM) (Ziess EM900; Jena, Germany), Incubator (JTSL 40; Jal Tajhiz, Tehran, Iran) and multi-mode reader (Synergy HTX; BioTek Instruments, VT, USA) were used.

Preparation CH/PEO polymeric solution at different ratios

To determine the optimal mixing ratio of CH/PEO solution, these two polymers were initially mixed at 50:50, 70:30, and 90:10 ratios, respectively. After analyzing the images of nanofibers obtained with SEM, the ratio of 90:10 was selected as the optimum ratio.

Preparation of CH/PEO polymeric solution (90:10) without and with BBR

The CH/PEO solution was prepared using the method described by Rahimi et al[18] with minor modification. After that, a solution containing 15 wt.% of

dimethyl sulfoxide (DMSO) was mixed with CH/PEO, and the mixtures were stirred overnight to yield a milky homogeneous solution.

Then, BBR with percentages of 0.5-20 wt.% were slowly added to polymeric solutions of CH/PEO and stirred well for 6 h at 37°C and centrifuged to remove the air bubbles before use.

Electrospinning process

The solutions were supplied into a 2-mL syringe. The air inside the syringe was completely removed. The electrospinning was performed at room temperature. In this study, feed rate, voltage and tip-to-target distance have been chosen on the basis of trial and error testing.

Aluminum plates were placed on an aluminum collector to easy collecting of the nanofibers. In order to obtain nanofiber with the appropriate thickness, electrospinning was performed for several hours. For complete removal of water and solvent from nanofibers, the samples were placed at room temperature for 24 h.

Characterization of electrospun nanofibers (SEM, TEM, AFM)

Electrospun nanofibers collected onto aluminum plates were sputter-coated with gold (Au). Then, SEM images with different magnifications were prepared.

Three SEM photos were selected randomly to measure the average fiber diameter and standard deviation, using ImageJ software program. Fiber diameter was measured at more than 10 different points from each SEM image. To investigate the internal structure and 3-D morphology of nanofibers, TEM and AFM were used, respectively. To prepare the sample for TEM, a groove was created on an aluminum foil. Then a 200-mesh grid was placed inside the groove. Electrospinning was then performed for 1-2 minutes on it.

Cross linking of prepared nanofibers

In order to improve the nanofibers hydrophilic properties, prepared nanofibers were cross linked by glutaraldehyde. For this purpose, electrospun nanofibers were placed on the top of the aqueous glutaraldehyde in a desiccator for 24 h at room temperature to be exposed under glutaraldehyde vapour. After that, the nanofibers were dried at room temperature for 24 h to remove the unreacted glutaraldehyde[19,20].

Fourier transform infrared (FTIR) spectroscopy

Samples were crushed with potassium bromide (KBr) in opal mortar and pestle to achieve powder particles with a diameter less than 2 μm. Then, a powder mixture was compacted by pressing to form a 0.25 mm thick tablet. Finally, the spectra were recorded and analyzed using a FTIR device[19,21].

Plotting calibration curve on the environment

A UV-Vis spectrometer is used for drawing a calibration curve to study BBR release. At first BBR was dissolved in phosphate-buffered saline (PBS) and its absorbance measured in 200-800 nm wavelength. The λ_{max} of BBR was calculated by this manner. Then different concentrations of BBR were prepared and their calibration curves were drawn by evaluating absorbance in the λ_{max} wavelength by UV-Vis spectroscopy based on the Beer-Lambert law.

Evaluation of BBR Release

To assess BBR release, first 5×5 cm^2 segments of the composite nanofibrous scaffold were placed inside a 12 kD dialysis bag, which is filled with PBS. Next, the dialysis bag was hung inside a beaker containing 20 mL of PBS. The solution was kept under continuous stirring at 37°C. After a certain time, 2 mL of the solution inside the beaker was removed to measure the absorption. This volume was replaced again. The absorption of the samples was studied with the UV-Vis spectrophotometer at the λ_{max} wavelength. In the next step, the degree of release was estimated using the standard curve. The release kinetics of CH/PEO/BBR can be described using Korsmeyer–Peppas, based on the results obtained from all the samples. It was calculated by using the following formula:

$$M_t/M_\infty = Kt^n$$

Where, M_t is the cumulative amount of drug released at time t, M_∞ is the initial drug loading, K is a kinetic constant characteristic of the drug/polymer system, t is the release time and n is the diffusion exponent suggesting the nature of release mechanism.

Cell culture

Human breast cancer cell lines (MDA-MB-468, BT474 and MCF7) and also fibroblast cells were

cultured in Dulbecco's modified Eagle's medium (DMEM). Cervical cancer cell (HeLa) cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% penicillin/streptomycin (Sigma-Aldrich; St. Louis, MO, USA) at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

In vitro biocompatibility of nanofibers

Fibroblast cells were used for the investigation of biocompatibility and bioactivity of the nanofibers. Before starting the cell culture experiments, the nanofibers were sterilized by UV for 45 min (each side). Cells were expanded in DMEM/F12 supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified atmosphere (37°C, 5% CO_2). The CH/PEO/BBR nanofibers were placed in plates and soaked in cell culture medium for 24 h. Cells were allowed to adhere for 30 min in the incubator before filling up the wells with additional medium. Pretreated cells were seeded at a density of 5×10^3 cells/well in a 96-well plate in standard culture condition. After 24 h, the viability of cells was checked by trypan blue. On the second, fourth and sixth days the growth and proliferation of fibroblasts cells were checked and each of the plates of cells was investigated by an inverted microscope.

Cell viability assay

The cytotoxic effects of CH/PEO/BBR nanofibers against MDA-MB-468, BT474, MCF7 and HeLa cells were determined by MTT assay. Cells (5×10^3/well) were seeded in a 96-well plate in different percentages of BBR for 24, 48 and 72 h at 37°C in a CO_2 incubator. Thereafter, 20 µL of a 5 mg/mL MTT solution was added to each well. After 4 h incubation at 37°C, the cell supernatants were removed and replaced with 100 µL DMSO to dissolve formazan crystals. The absorbance of the samples was measured using a multi-mode reader at 570 and 630 nm, respectively. The percentage of cell viability was calculated by using the following formula:

$$\text{Cell viability (\%)} = (OD_{samples} / OD_{control}) \times 100$$

Swelling percent of nanofibers

The swelling percent or water uptake of samples was calculated by using following formula, where

W_0 is initial weight of the dry sample and W_s is a swollen weight of the sample at equilibrium.

$$\text{Swelling percent} = [(W_s - W_0) / W_0] \times 100$$

Statistical analysis

Statistical analysis was done by SPSS-18 software (SPSS Inc., Chicago, Il, USA). Differences were determined by one-way ANOVA, followed by Dunnett and Duncan (post-hoc) comparison. *p*-values inferior to 0.05 were considered significant. In addition, IC_{50} values and selectivity index (SI) were calculated by GraphPad Prism 6.07 (San Diego, CA, USA) software.

RESULTS

Evaluation of CH/PEO nanofiber with a ratio of 90:10

The results of the electrospinning process for voltage, distance between the nozzle and the collector plate, and the feed rate were 14.5 kV, 10 cm and 0.2 mL/h, respectively.

Then, the speed of the collector plate was adjusted to 500 rpm. Based on images of CH/PEO nanofibers, the prepared nanofibers were bead-free and regular, and their mean diameter was calculated (Figure 1).

Electrospinning of CH/PEO/BBR nanofibers

The perfect, uniform, oriented, and bead-free nanofibers were achieved in 0.5-20 wt.% BBR concentrations. SEM images and analysis by ImageJ software showed that the mean diameter of prepared nanofibers was 97±13 nm. The images obtained with the SEM show the sample surface and generally it cannot reveal information from within the sample, but it can lead to a greater understanding of the sample's inner structures. In order to investigate the internal structure and 3-D morphology of the nanofibers, TEM and AFM images were obtained. The images of TEM and AFM confirmed the core-shell structure and 3-D scaffold morphology of the prepared nanofibers, respectively (Figure 2).

Analysis of nanofibers stability

As observed in the SEM images, the stability of cross-linked nanofiber scaffolds is related to joining CH molecules. Therefore, water molecules can hardly penetrate into the scaffolding networks.

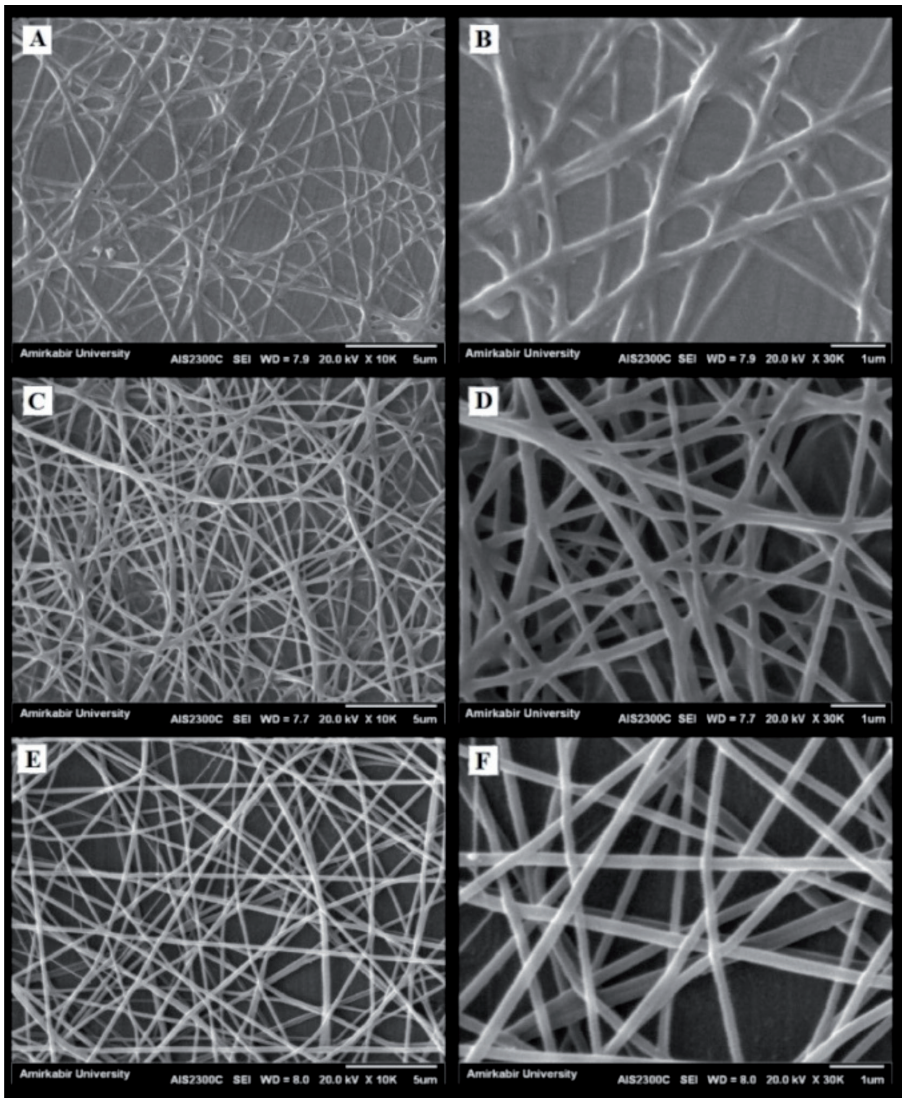

Fig. 1. SEM images of CH/PEO electrospun nanofibers at different concentrations; (*A*, *B*) 50:50, (*C*, *D*) 70:30 and (*E*, *F*) 90:10.

Swelling percent of nanofibers

The lowest and the highest water uptake was 55.4% and 93.5% in 0.5 and 20 wt.% of prepared nanofibers, respectively.

Drug Releasing template of BBR from nanofiber scaffold

After drawing the calibration curve, the relationship between absorption and concentration of the drug was calculated. BBR release was detected in 4 stages: 1st stage was the burst release of BBR from CH/PEO/BBR nanofiber during which drug release was done at a high rate; 2nd stage was the drug slow release; 3rd stage was the drug release from scaffold depth, and finally the 4th stage was the reduction drug release and extreme decrease in curve slope. Half and two-fifths of the drug release were characterized after 16 h and 24 h, respectively. In addition, the study with UV-Vis indicated that the BBR released from nanoscaffolds is done within 2 weeks.

FTIR analysis of prepared nanofibers

The compositional and chemical properties of the nanoscaffolds produced by electrospinning were investigated using FTIR analysis. For this purpose, the FTIR spectrum of CH/PEO and CH/PEO/BBR was studied. In the present study, functional groups in the nanofibers of scaffold were determined using a range of 400-4000 cm^{-1}. Hydrogen bond existence between CH and PEO was CS/PEO nanofiber FTIR analysis outcome. Further, methoxyl group was detected in BBR in CH/PEO/BBR nanofibers FTIR analysis (Figure 3).

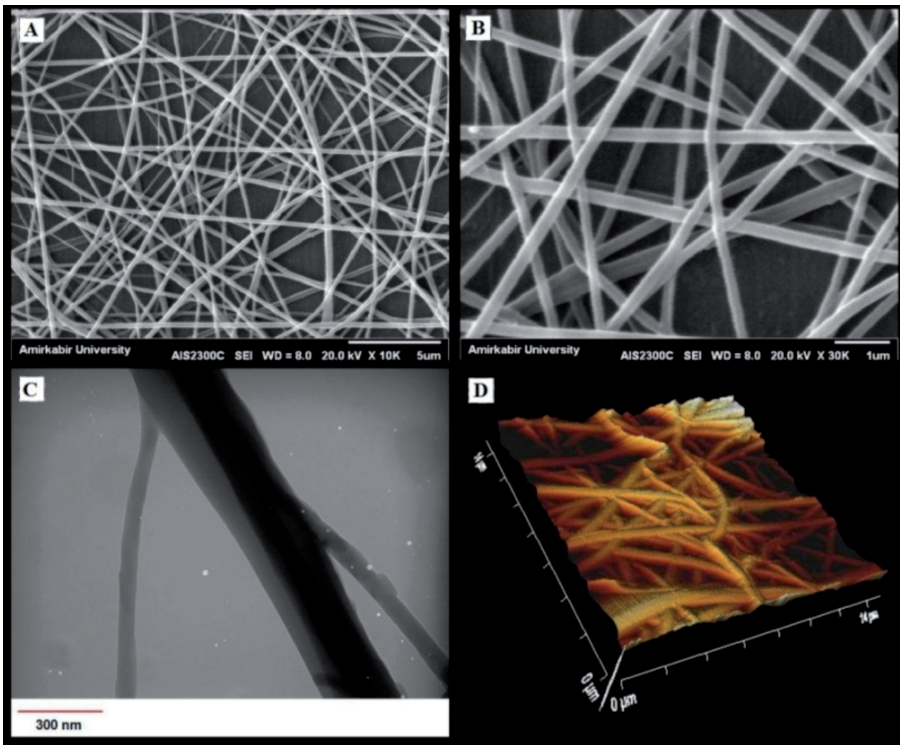

Fig. 2. SEM images of electrospun CH/PEO nanofibers containing; (*A, B*) BBR 1 wt.% in various magnifications, (*C*) TEM image of electrospun CH/PEO/BBR 20 wt.% nanofiber and (*D*) AFM image of 3-D structure of prepared nanofiber after BBR 20 wt.% loading.

IC_{50} and SI results

The IC_{50} values of CH/PEO/BBR 20 wt.% nanofibers were calculated for all cell lines using MTT assay technique. Our results showed breast and cervical cancer cell lines responded differently to the same BBR release from nanoscaffold in a similar situation.

Comparing IC_{50} values, it was showed that MDA-MB-468 cells are more sensitive than other cancer cell lines to BBR release since the IC_{50} of MDA-MB-468 (0.30±0.02, 0.50±0.07 and 0.30±0.01 µg/mL) was less than that for BT437 (1.14±0.06, 5.70±0.02 and 46.50±1.07 µg/mL), MCF7 (2.50±0.12, 15.30±0.58 and 5.94±0.20 µg/mL) and HeLa (0.86±0.01, 1.98±0.07 and 0.47±0.02 µg/mL) after 24, 48 and 72 h, respectively.

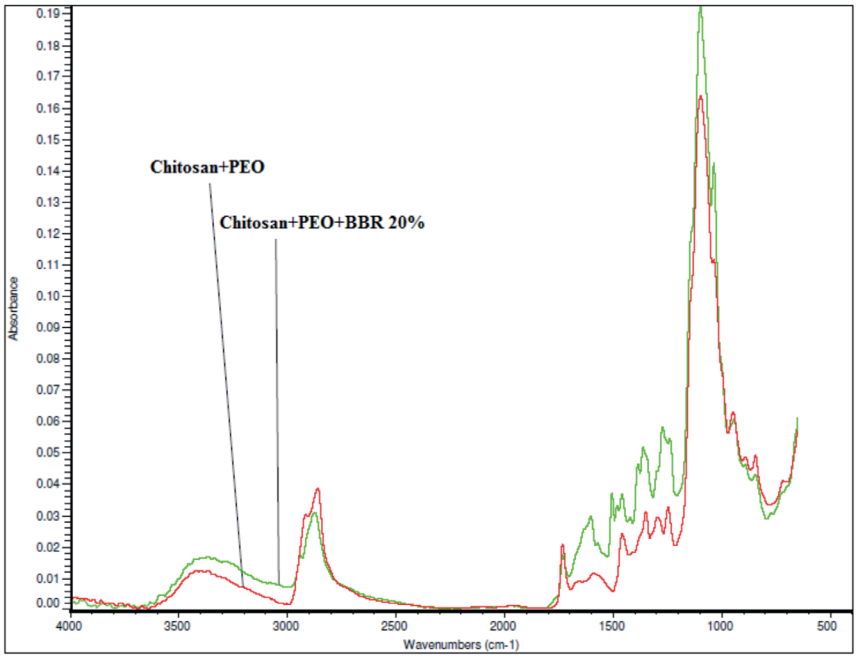

Fig. 3. FTIR spectrum of CH/PEO and CH/PEO/BBR 20 wt.% nanofibers.

TABLE 1. CH/PEO/BBR 20 wt.% nanofibers selectivity index against breast and cervical cancer cells.

Time (Hours)	*Selectivity index (SI)**			
	MDA-MB-468	*BT437*	*MCF7*	*HeLa*
24	23	6.05	2.76	8.02
48	6.8	0.59	0.22	1.71
72	11.66	0.075	0.58	7.44

*Significance between different studied cell lines (p=0.047).

TABLE 2. Cell viability of breast and cervical cancer cell lines after 24, 48 and 72 h nanofibers exposure.

*Cell Viability (%)**	*24 h*	*48 h*	*72 h*
HeLa	32.8±7.2	5.50±1.07	4.7±1.1
BT437	47.5±5.4	18.9±3.5	4.2±0.9
MCF7	37.9±6.1	28.1±8.4	5.6±2.1
MDA-MB-468	25.0±6.3	10.8±2.4	0.40±0.07

*Data are expressed as the mean ± SD (n=3).

In contrast, BT437cells were more resistant to BBR since a higher concentration of BBR was needed to kill 50% of the cells. Following nanofiber exposure, IC_{50} values against MDA-MB-468 cell line were around 0.3, 0.5 and 0.3 after 24, 48 and 72 h, respectively, using GraphPad Prism software.

The SI of CH/PEO/BBR 20 wt.% nanofibers against breast and cervical cancer cells is displayed in Table 1. The SI of CH/PEO/BBR 20 wt.% nanofiber against MDA-MB-468 cell line was 23, 6.8 and 11.6 after 24, 48 and 72 h, respectively.

Cell viability

Cell viability on the CH/PEO/BBR 20 wt.% after 24, 48 and 72 h incubation, is shown in Table 2 and Figure 4.

Anticancer Activity of Electrospun CH/PEO/BBR Nanofibers

Nanoscaffolds containing BBR (0.5-20 wt.%) concentrations inhibited cell growth compared to the control group in HeLa, BT474, MCF7 and MDA-MB-468 cell lines (p=0.000). CH/PEO nanofibers containing 0.5 wt.% BBR had the lowest growth inhibition concentration on four studied cell lines. There were significant differences between drug exposure time and cancer cell lines growth inhibition in HeLa, BT474, MCF7 (p=0.000) and MDA468 (p=0.001). The lowest and highest nanoscaffold growth inhibition concentrations on cell lines are shown in Table 3. There were no significant differences between highest concentration and higher doses.

IC_{50} values were not significant in different groups (p=0.469) and between different cell lines over time (p=0.379). SI differences were statistically significant between different studied cell lines (p=0.047). There were no differences between HeLa cell line and others in term of SI, but the differences were significant between MDA-MB-468 and BT474 (p=0.019) as well as MDA-MB-468 and MCF7 (p=0.013). There were no differences between SI and different time points (Figure 5).

DISCUSSION

Herbal remedies have been used as effective complementary and alternative medicine for the treatment of various diseases since ancient time. BBR is a well-known phytochemical with pharmacological

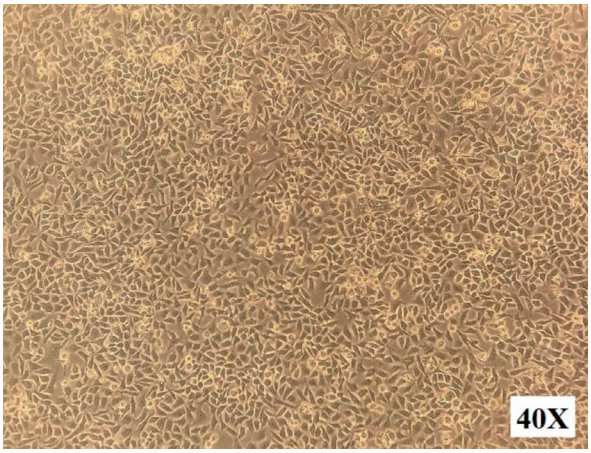

Fig. 4. Inverted microscopic image of HeLa cells cultured in RPMI-1640 after 24 h.

TABLE 3. The lowest and highest nanoscaffold growth inhibition concentration on cell lines.

Cell line	Lowest concentration (wt.%)	p-value	Highest concentration (wt.%)	p-value
HeLa	0.5	p=0.000	8	p=0.047
BT437	0.5	p=0.000	10	p=0.049
MCF7	0.5	p=0.000	10	p=0.015
MDA-MB-468	0.5	p=0.000	8	p=0.04

activities including, anti-inflammation, antimicrobial, anti-cardiovascular diseases and anti-tumor, etc.[7,22]. The anticancer properties of BBR have been indicated in various cancers[13]. Chemo drugs exerted their anticancer action through antiproliferative and pro-apoptotic effects by numerous molecular mechanisms like p53 dependent pathway, activation of reactive oxygen species (ROS), mitogen-activated protein kinase (MAPK) and caspase dependent cell death or inhibition of Wnt/β-catenin, vascular endothelial growth factor (VEGF), nuclear factor κB (NF-κB), signal transducer and activator of transcription 3 (STAT3) signaling [23]. The anticancer effects of BBR are also mediated by its inhibition of cancer cell proliferation, apoptosis induction, suppressing cell migration[5], invasion and metastasis, and inducing cell cycle arrest in cancer cells[13]. In association with apoptosis promotion, BBR exerts its activity by the suppression of MMP-2, the Bcl-2/Bax, and Bcl-xL signaling pathways, downregulating c-FLIP and Mcl-1 and modulation of the JNK/p38 signaling pathway[5,24-26]. Besides, previous studies have shown that BBR suppresses the cancer cell migration and metastasis through decreasing the expressions of prostaglandin E2, prostaglandin E2 receptors and cyclooxygenase-2, activating AMP-activated protein kinase (AMPK) signaling, downregulating EGFR-MEK-ERK signaling pathway and restraining matrix metalloproteinases 1, 2, 9 (MMP-1, -2, and -9) and NF-κB pathway[16,24-27].

It was also observed that BBR contributes to cell cycle arrest in cancer cells by PI3K/Akt cell line and caspase activation, ROS production and releasing cytochrome c (Cytc)[5,27].

In addition to therapeutics properties, BBR exhibits little resistance and low side effects. Disadvantages of BBR, such as its low solubility and bioavailability are eliminated by the use of nanostructure carriers, lead to increased solubility and enhanced efficacy and safety of BBR[28].

Nowadays, nanofibers and nanoparticles offer promising delivery solutions for the treatment of human diseases as biocompatible, biodegradable and immune-stimulating compounds[29]. CH- based nanofibers are controllable diameter fibers from a few microns down to 40 nm. CH is a natural polymer with different properties such as: enzymatic degradability, biocompatibility, lack of toxicity, antigenicity and coagulability. However, it has a poor electrospinning potential and cannot synthesize nanofibers[20]. In this study, PEO has been used for optimum nanofibers due to its biocompatibility. PEO is a non-toxic, odorless, neutral and lubricating agent used in various drugs as solvents, spreading agent, ointment base and an absorption agent in various pills. Moreover, it is used in nanotechnology to reduce the viscosity of CH and prepare nanofibers. The reason for a better nanofiber construction is a hydrogen bond between CH and PEO, which is also proved by FTIR. Bhattarai et al[20] reported that the matrix with a CH/PEO ratio of 90:10 retained excellent integrity of the fibrous structure in water. Our results, in terms of the synthesis of nanofibers and cell culture on it, were similar to their study.

In this study, the evaluation of CH/PEO nanoscaffold was shown bead-free in nanofiber in a 90 to 10 ratio using SEM. This result is similar to the findings of Rahimi et al[18], which believed increased CH proportion leads to nanofiber diameter reduction.

In this research, half and two-fifths of BBR release were identified at 18 and 24 h, respectively. Indeed, burst drug release was done in 24 h. In some studies, initial burst drug release from nanofiber was detected around 30% (one third) in the first 24 h. These differences may occur due to the nanofiber structure, its diameter and drug type[30-32]. In our study, cell death in 4 studied cell lines was above 90% and maximum cell death occurred in MDA-MB-468 cell line after 72 h obtained after nanofibers exposure. Abasian et al[33] have reported maximum cell death of Doxorubicin loaded PLA/chitosan/NaX/Fe3O4 nanofibers on H1355 human lung epidermoid carcinoma cell line 82% after 7 days. Aboutalebi et al[34] and Radmansouri et al[35] have explained the effect of prepared nanofibers on cell death of A549 and B16F10 carcinoma cell lines 65-92% and 58-78%, respectively. Differences in studies done in this field were due to drug type, drug concentration and exposure time.

IC_{50} result analysis showed that 0.3 µg/mL of BBR released from prepared nanofibers can inhibit 50% MDA-MB-468 cell line growth. It means that this nanofiber can inhibit MDA-MB-468 cell line better than other cells in this study. Zhang et al[30]

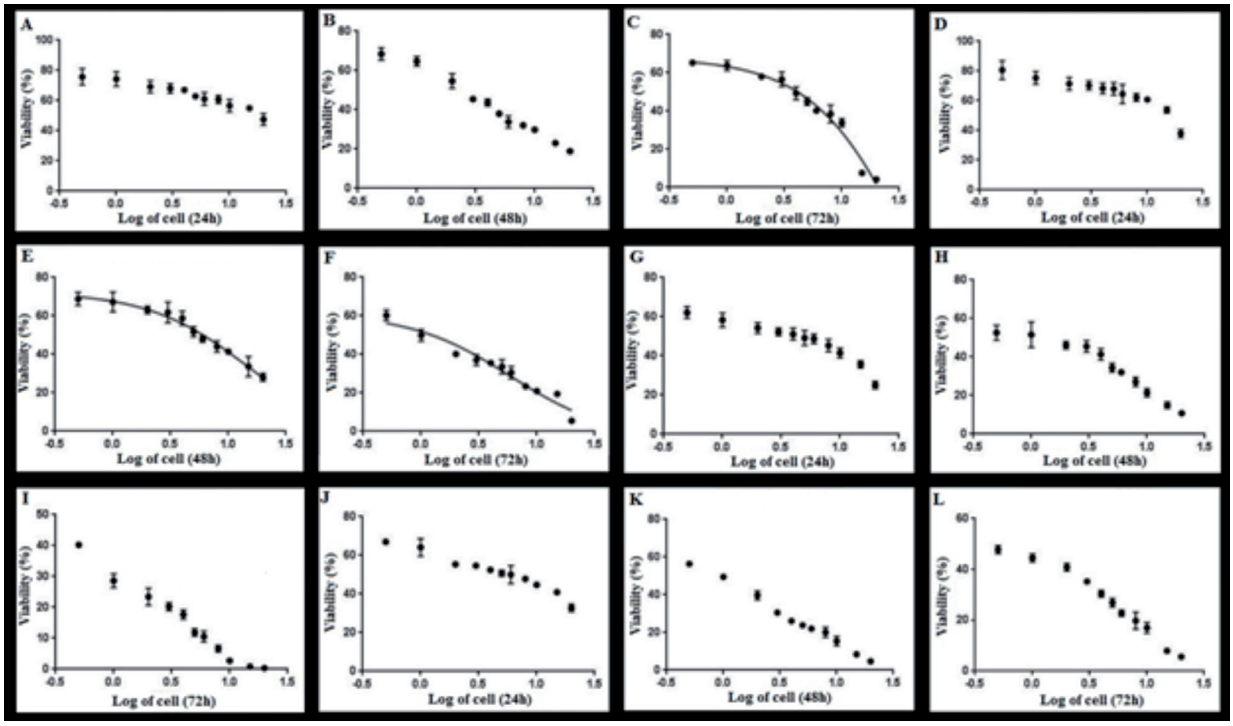

Fig. 5. Diagram of CH/PEO/BBR 20 wt.% nanofibers effect on cancer cell lines, (*A-C*) BT474, (*D-F*) MCF7, (*G-I*) MDA-MB-468 and (J-L) HeLa.

reported that the inhibition rate of oxaliplatin/5-fluorouracil/PLLA nanofiber on human colorectal cell lines (HCT-8 cells) at 120 h was 87.3% (0.1 mg/mL). Also, the SI result showed more efficacy of nanofiber in the inhibition of MDA-MB-468 cell line.

The antimicrobial, tissue regeneration and wound healing effects of different drugs and extracts loaded on CH/PEO nanofibers were confirmed during researches[36-40]. This means that the influence of CH/PEO nanoscaffolds in drug delivery is remarkable.

In general, it is claimed that CH/PEO/BBR nanofibers have a good improving ability against breast and cervical cancer cells *in vitro* condition, and they can be applied in later studies as an ideal drug vehicle system with therapeutic powers on laboratory animals and clinical studies.

No testing of obtained nanofiber on animal models and humans was a limitation of this study. However, to our knowledge, it is the first study of herbal extracts loaded on nanofibers and their effect on cancer cell lines.

CONCLUSIONS

In this study, CH based nanofibers, which were mixed with PEO, were prepared by electrospinning. To overcome the limitation of BBR in therapeutic applications, CH/PEO nanofibers were used as drug delivery system. In this regard, some methods such as SEM, TEM, AFM and FTIR were applied to study the morphology and investigate the structure of the nanoscaffolds. In the present study, the new achieved nanofiber composite revealed high stability and showed a good anticancer effect against the breast and cervical cancer cells. Further, the high porosity of the resulting nanofibers makes it possible to apply it as a suitable drug delivery system on cancer patients.

Acknowledgement:

This study is related to the Project NO 1397/68938 From Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. We also appreciate the "Student Research Committee" and "Research & Technology Chancellor" in Shahid Beheshti University of Medical Sciences for their financial support of this study. The authors wish to thank Mrs. Faezeh Hamidi for her kind cooperation.

Conflict of Interest:

The Authors declare that they have no conflict of interests.

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