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Synthesis and anticancer properties of bacterial cellulose-magnesium oxide bionanocomposite

Mohsen Safaei[1*], Mojtaba Taran[2], Razieh Rezaei[3], Kamran Mansouri[3], Hamid Reza Mozaffari[3,4], Mohammad Moslem Imani[5], Roohollah Sharifi[6]

[1] Oral and Dental Sciences Research Laboratory, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Department of Nanobiotechnology, Faculty of Science, Razi University, Kermanshah, Iran
[3] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Department of Oral and Maxillofacial Medicine, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
[5] Department of Orthodontics, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
[6] Department of Endodontics, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

Given the increase in global mortality rate due to various types of cancer, the present study aimed to develop optimal conditions for the synthesis of cellulose-magnesium oxide nanocomposite with favorable anticancer activity. For this purpose, the Taguchi method was used to design nine experiments with varied ratios of cellulose biopolymer, magnesium oxide nanoparticles and different stirring times. The scanning electron microscopy (SEM) images confirmed the formation of cellulose-magnesium oxide nanocomposite. The anticancer activity level of nine nanocomposites studied was evaluated using MTT assay on Michigan Cancer Foundation-7 (MCF-7) cell line. The nanocomposite synthesized in experiment 9 (8 mg/ml of magnesium oxide, 2 mg/ml of cellulose and stirring time of 60 min) showed the highest growth inhibitory activity on the cancer cells. Based on the attained results,e cellulose-magnesium oxide nanocomposite synthesized in optimal conditions can be used as an eligible anticancer agent.

INTRODUCTION

Finding effective treatments for cardiovascular diseases [1], autoimmune diseases [2,3], antimicrobial resistance [4,5], diabetes [6], chronic pain [7-9] and cancer [10,11] are, today, among the greater challenges of human society. Despite significant advances in medical science, cancer continues to be one of the most important diseases of the present century and the second leading cause of death behind cardiovascular diseases [12]. The World Health Organization (WHO) reported that more than 8.8 million people die every year from the cancer diseases. This figure is increasing every year. Breast cancer is one of the major malignant cancers, affecting one in eight females. Notwithstanding new treatments, the cancer deaths are rising every year, and the breast cancer is the first cause of death among women in the world [13,14].

The use of nanotechnology and nanoparticles in recent years has opened new promising windows in the treatment of various diseases. Metal oxide nanoparticles with high specific surface area have many desireable features [15]. Magnesium oxide is now widely used as a very important metal oxide. The magnesium oxide nanoparticles can be used as a potent effective anticancer agent [16-18]. However, one of the most important limitations of using metal nanoparticles is their intense tendency to accumulate. Therefore, the use of stabilizers such as polymers can prevent the unwanted accumulation of nanoparticles [19].

Cellulose is of particular importance in various industries such as wood and paper, textile, food, drug and medicine. Cellulose polymer forms the main cell wall of plants, but it contains some impurities such as lignin, pectin and hemicellulose. Bacterial cellulose is a promising alternative to plant cellulose in certain applications such as medicine. The replacement of conventional sources of cellulose production with the microbial source has the benefits of time, energy and cost reduction in the cellulose production processing. In addition, the cellulose produced with a microbial source has desired properties in terms of strength and durability, and is free of impurities present in the natural cellulose. Furthermore, it is possible to produce cellulose

* **Corresponding author**
e-mail: mohsen_safaei@yahoo.com

derivatives with targeted capabilities. The microbial cellulose is a polysaccharide produced by a variety of different bacteria, among which *Gluconacetobacter xylinus* is the most important cellulose producing bacterium [20,21].

The purpose of this research was to create optimal conditions for the synthesis of nanocomposites containing MgO inorganic nanoparticles in the sodium hyaluronate biopolymer matrix and to examine its anticancer properties.

MATERIALS AND METHODS

The bacterial cellulose was produced using *Acetobacter xylinum* (PTCC 1734) prepared by the Iranian Research Organization for Science and Technology (IROST). For this purpose, the bacterium was cultured in a Hestrin-Schramm broth medium for 7 days at 30°C in an incubator, during which a layer of cellulose was formed while in the air and liquid phase. The attained cellulose after separation was washed several times with deionized water to remove impurities. It was then placed in a water bath at 90°C for an hour in the presence of 0.5 M sodium hydroxide to remove impurities and cell residues. Finally, the obtained cellulose was placed in an oven (EHRET, Germany) at 40°C for three days to provide cellulose powder [22]. Magnesium oxide nanoparticles were synthesized using the co-precipitation method introduced by Krishnamoorthy *et al* [23].

The Qulitek-4 software and Taguchi method was used to design nine experiments with varying ratios of biopolymer, nanoparticles and various stirring times (Table 1). For this purpose, the nine nanocomposites were synthesized using in situ method and levels of 2, 4 and 8 mg/ml of magnesium oxide nanoparticles and 0.5, 1 and 2 mg/ml of the cellulose biopolymer at the stirring times of 30, 60 and 90 min [24].

Table 1. Taguchi design of experiments and results of anticancer activity of cellulose-magnesium oxide nanocomposites

Experiment	MgO (mg/ml)			Cellulose (mg/ml)			Stirring time (min)			Cell growth inhibition (%)
	2	4	8	0.5	1	2	30	60	90	
1		2			0.5			30		38.16
2		2			1			60		52.23
3		2			2			90		43.60
4		4			0.5			60		62.08
5		4			1			90		69.42
6		4			2			30		66.14
7		8			0.5			90		51.06
8		8			1			30		68.28
9		8			2			60		72.11

The MCF-7 cell line was provided from the cell bank of the Pasteur Institute of Iran to evaluate the anticancer activity of the synthesized nanocomposites. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C, in the presence of 5% CO_2 and 95% humidity. The MTT assay was applied to investigate the anticancer effects of the synthesized compounds. Herein, three experiments with three replicates were considered for each of the studied nanocomposites and the control group [25]. The percentage of cancer cell death rate following treatment with the 20 µL of nanocomposites suspension for 48h was calculated by using the following equation:

$$\text{Cell growth inhibition (\%)} = \frac{\text{ODc-ODt}}{\text{ODc}} \times 100\%$$

ODt: optical density of treated cells,
ODc: optical density of control cells.

To investigate the characterizations and confirm the formation of cellulose-magnesium oxide nanocomposite, its scanning electron microscopy (TESCAN, Czech Republic) images were prepared at two magnifications of 100 and 200 nm. In designing this study, the Taguchi method was applied to save time and cost. Qualitek-4 software was also used to determine the effect of studied factors, the interaction of them, and the prediction of optimum conditions for cellulose-magnesium oxide nanocomposite synthesis.

RESULTS

The scanning electron microscopy (SEM) images of cellulose-magnesium oxide nanocomposite showed that magnesium oxide nanoparticles were well placed on the surface of cellulose biopolymer and confirmed the formation of the nanocomposite (Fig. 1).

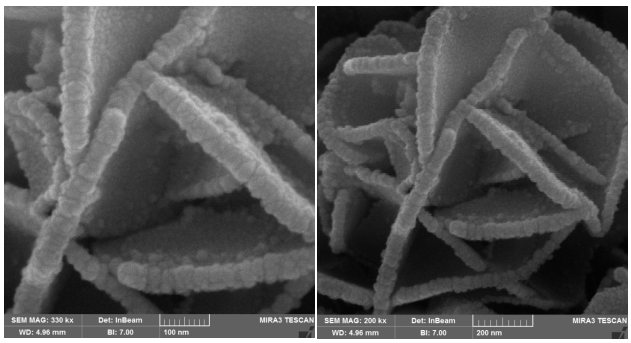

Figure 1. Scanning electron microscopy images of cellulose-magnesium oxide nanocomposite

Table 1 shows the inhibition levels of cancer cell growth by the synthesized nanocomposites in different conditions. The highest rate for the inhibition of cancer cell growth was found to be the nanocomposite synthesized in experiment 9, containing 8 mg/ml of magnesium oxide, 2 mg/ml of cellulose and the stirring time of 60 min.

The effect of different levels of each of the factors including magnesium oxide nanoparticles, cellulose and stirring time on inhibiting the growth of cancer cells is presented in Table 2. The highest effect on the level of cell growth inhibition was observed in the magnesium oxide nanoparticles at the second level (65.88%) and the lowest yield was found in the first level (44.66%). The cellulose biopolymer and the stirring time also showed the best efficacy in the second level (63.31% and 62.14%, respectively). The lowest efficacy of the cellulose biopolymer and the stirring time were related to the first level (50.43%) and the third level (54.69%), respectively.

Table 2. Effect of different levels of factors on anticancer activity of cellulose-magnesium oxide nanocomposite

Factors	Level 1	Level 2	Level 3
MgO	44.66	65.88	63.82
Cellulose	50.43	63.31	60.62
Stirring time	57.53	62.14	54.69

The interaction effect of the studied factors on the level of MCF-7 cell growth inhibition is shown in Table 3. The strongest interaction was reported for the cellulose biopolymer in the third level and the stirring time in the second level (58.86%). The weakest interaction was observed for the magnesium oxide nanoparticles and the cellulose biopolymer in the third levels (9.91%). The interaction of the magnesium oxide nanoparticles in the third level and the stirring time in the second level was 26.70%.

Table 3. Interaction of investigated factors on anticancer activity of cellulose-magnesium oxide nanocomposite

Interacting factor pairs	Column	Severity Index (%)	Optimum conditions
Cellulose × Stirring time	2×3	58.86	[3,2]
MgO × Stirring time	1×3	26.70	[3,2]
MgO × Cellulose	1×2	9.91	[3,3]

Table 4 exhibits the analysis of variance of the factors affecting the cancer cell growth inhibition. The magnesium oxide nanoparticles had the greatest effect on inhibiting cell growth by 66.95%. The lowest inhibitory effect was related to the stirring time (5.59%). The cellulose biopolymer also inhibited the growth of MCF-7 cancer cells by 21.59%.

Table 4. Analysis of variance of factors affecting anticancer activity of cellulose-magnesium oxide nanocomposite

Factors	DOF	Sum of Squares	Variance	F-Ratio (F)	Pure Sum	Percent (%)
MgO	2	821.25	410.63	46.59	803.63	66.95
Cellulose	2	276.76	138.38	15.70	259.14	21.59
Stirring time	2	84.76	42.38	4.81	67.14	5.59

DOF – degree of freedom

After reviewing the studied factors and their interactions using Taguchi method and Qualitek-4 software, the optimal conditions for the synthesis of cellulose-magnesium oxide nanocomposite with the highest anticancer activity are proposed in Table 5. All three factors including magnesium oxide nanoparticles, cellulose biopolymer and stirring time in the second level had the highest efficacy. The magnesium oxide nanoparticles had the highest contribution (7.76%) and the stirring time had the lowest contribution (4.02%) on the inhibition level of cancer cell growth. Moreover, the contribution rate of cellulose biopolymer in inhibiting the cell growth was 5.19%. The mean inhibitory level of cell growth in the nine designed experiments in exposure to the synthesized nanocomposites was equal to 58.12%, while it is expected that the synthesized nanocomposite in the optimal conditions suggested by the Taguchi method would prevent the growth of MCF-7 by 75.09%.

Table 5. Prediction of optimal conditions for the synthesis of cellulose-magnesium oxide nanocomposite with the highest anticancer activity

Factors	Level	Contribution
MgO	2	7.76
Cellulose	2	5.19
Stirring time	2	4.02
Total contribution from all factors		16.97
Current grand average of performance		58.12
Cell growth inhibition at optimum condition		75.09

DISCUSSION

The current treatment methods for cancer include various treatments based on alkylating agents, antimetabolites, biological agents, and so on. However, the main concern is the side effects from failure to distinguish correctly between the cancerous and normal cells, resulting in systemic toxicity [26]. One of the ways to find new strategies for cancer treatment is through the use of nanomaterials. The development of nanostructures has led to the creation of novel antitumor therapies. Therefore, there is a growing research field in using nanoparticles due to their potential antitumor effects to impede tumor onset, development, and progression [27]. In this study, the nine cellulose-magnesium oxide nanocomposites were synthesized using the Taguchi method under different conditions and then their anticancer activity levels were compared using MTT assay. The results demonstrated that the combination of the cellulose biopolymer and the magnesium oxide nanoparticles culminates in the formation of cellulose-magnesium oxide nanocomposite so that the synergistic effect of the components could improve the synthesized nanocomposite properties. The synthesized nanocomposites showed an optimal anticancer activity. The inhibition level of cancer cell growth in different experimental conditions varied from 38.16% to 72.11%, indicating the effect of concentration of the components, their ratio and the stirring time on the anticancer ability of the synthesized nanocomposites.

In line with our results, previous studies also reported anticancer activity for the magnesium oxide nanoparticles [16-18]. Patel *et al*. [16] investigated the effects of magnesium oxide nanoparticles on cervical cancer cells and concluded that the concentrations greater than 300 μg/ml had potential anticancer activity. Sugirtha *et al*. [17] extracted the magnesium oxide nanoparticles from the water extract of cauliflower and pomegranate peels, examined their effects on HeLa cells and reported that the use of an extract containing 31 μg/ml caused the destruction of 50% of HeLa cells. In another study, Karthik *et al*. [18] investigated the effect of anticancer activity of the magnesium oxide nanoparticles produced by green synthesis against the MCF-7 cell line and reported potent anticancer activity. Ge *et al*. [28] conducted a study aiming to evaluate the toxicity of magnesium oxide nanoparticles on human umbilical vein endothelial cells, and stated that the magnesium oxide nanoparticles had no toxicity on these cells.

The physicochemical properties of nanoparticles that establish their anticancer activity can be ascribed to the potential features of the nanoparticles, such as antioxidant activity, or activities based on the use of external stimuli.

Further, the nanoparticles may affect the surrounding environment of the tumor, such as blood vessels or stroma, and thus prevent the spread of tumor mass. Indeed, according to studies, the nanoparticles are known to reduce the rate of tumor progression using the antioxidant capacity [29]. Electrostatic interactions are another characteristic of nanoparticles that, due to their small size and surface properties, increase their enhanced permeability and retention (EPR) in tumor cells.

Given the rapid and uncontrolled growth of tumors, blood and lymphatic vessels do not expand properly into these tissues. Cell-cell communication in a tumor cell is also poor due to incorrect adhesion. The blood vessels passing through the tumor have a pore size of about 1 micrometer to 100 nanometers. Because of these tumor stroma properties, the nanoparticles can easily distribute into the tumor cells through blood vessels, resulting in the selectivity of nanoparticles in tumor cells.

Considering the inappropriate lymphatic system in the tumor cells, the nanoparticles are not removed from the tumor tissue and more retention time is provided for the nanoparticles in cancer tissues. Therefore, increasing the EPR effect causes the release of nanoparticles to specific sites in the tumor cell so as to affect them [26,30]. The cancer cells have high concentrations of anionic phospholipids (negative charge) in their outer membranes, which can lead to electrostatic adsorption of positively charged nanoparticles. Localized selectivity by the nanoparticles can be increased by modifying and engineering their surface [31].

Still, despite the fact that the nanoparticles have been reported to exhibit selective toxicity against cancer cells, the exact mechanism of this selective toxicity is still unclear. ROS may be a possible explanation for the selective toxicity response of the nanoparticles for the cells with a high proliferative potential. It has been observed that the ROS production is relatively higher in the cancer cells after treatment with the nanoparticles than in normal cells [32].

CONCLUSION

According to the results obtained from the present study, given the high predictive power of the Taguchi method, this approach can be applied as a useful tool to save time and cost for performing various experiments to detect the maximum level of anticancer activity for the compounds studied. The findings also indicated that the levels of all three factors, cellulose biopolymer, magnesium oxide nanoparticles and stirring time, are effective on enhancing the anticancer activity of the synthesized nanocomposites. optimizing these parameters can, therefore, improve the anticancer activity of the synthesized nanocomposite.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID iDs

Mohsen Safaei https://orcid.org/0000-0003-3885-6640
Hamid Reza Mozaffari http://orcid.org/0000-0001-9351-1499
Kamran Mansouri https://orcid.org/0000-0002-5184-4583
Mohammad Moslem Imani https://orcid.org/0000-0002-3982-5216

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