Effectiveness of Controlled *MDM2* Inhibitor Release on Brain Tumor Cells *in vitro*

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***Abstract*— Cancer is a significant health issue all over the world causing severe decline in life expectancy of patients and their life standards. Traditional cancer treatment methods such as chemotherapy, surgical intervention and radiotherapy lead to critical side effects such as systemic toxicity and drug resistance development. In the last decades, controlled drug delivery systems have been studied to overcome those limitations. The study aims controlled drug release treatment on brain cancer cells. Therefore, chitosan microspheres were potentially used as drug carriers, and *MDM2* inhibitory molecule was selected as a model drug to assess the cell viability of T98G brain tumor cell line. *MDM2* inhibitor-loaded chitosan microspheres were produced by the emulsion/cross-linking method. *MDM2* inhibitory molecule release profile *in vitro* was carried out in phosphate-buffered saline at pH 7.4. Moreover, effects of drug-loaded microspheres on brain tumor cells were tested by MTT assay.**

Keywords — chitosan microspheres, controlled drug delivery, MDM2 inhibitor

I. INTRODUCTION

Cancer is an important health problem which can be defined as an abnormal proliferation of cells in an uncontrolled way. There are clinically used conventional techniques such as chemotherapy, surgery and radiation therapy for its treatment. However, those methods have serious side effects on the human body. For instance, chemotherapeutic agents are systemically given to body, and they are non-specific for tumor tissues. Therefore, they have harmful effects on healthy cells. The studies on controlled drug delivery aim to control the release of anti-cancer agents to specific cells and tissues, to reduce the side effects of drugs and to increase the therapeutic impacts.

Chitosan as a natural polymer has been widely used in the field of drug delivery systems, cell culture, gene transfer and tissue engineering due to its biocompatible, biodegradable and non-toxic behaviors [1]. Therefore, chitosan microspheres as drug carriers can be used for improving the efficiency of anti-cancer agent delivery in a controlled manner.

The purpose of this study is the synthesis of drug-loaded chitosan microspheres and determination of effectiveness of controlled drug release treatment on the brain tumor cells in vitro. *MDM2* inhibitor was used as a model drug to investigate the release property of chitosan microspheres and the effects of cell viability of T98G cells.

I. METHODS

In the first part of the study, chitosan microspheres were produced by the emulsion/cross-linking method. Firstly, chitosan was dissolved in acetic acid solution (0.1M). Then, the polymer was added dropwise into a 50 ml mineral oil containing 1 ml span 80. After 30 minutes, 400 µl glutaraldehyde (25% aqueous solution) was added and stirred continuously for 1 hour. After, the mixture waited at 60°C in the incubator, and then the chitosan microspheres were washed three times with hexane. Finally, the obtained microspheres were dried at 37°C and characterized by light microscopy.

In the second part of this study, novel model drug which is an *MDM2* inhibitor was loaded into the chitosan microspheres. The chitosan microspheres and 1 ml *MDM2* inhibitor (20uM) were rotated at 100 rpm for 48 hours. Then, the release of *MDM2* inhibitor from the microspheres was analyzed in PBS (pH 7.4) at 37°C. The amount of released *MDM2* inhibitor was determined by a UV-spectrophotometer at 555 nm. Later, to determine the efficacy of drug-loaded chitosan microspheres and their capacity to affect the cell viability of T98 cells, MTT assay was performed. T98 cells were seeded on a 24 well plate and when they reached 30K cells per well, drug treatment was performed. Untreated, only sphere, only drug and drug-loaded spheres were experiment groups studied in triplicate. Cells were treated with 100uM of *MDM2* inhibitor and *MDM2* inhibitor-loaded chitosan microspheres. Cells were tracked for 120 hours. MTT solution was added at 24hr, 48hr, 72hr, 96hr and 120hr onto the cells. After addition of the MTT solution, cells were incubated at 37°C for 2-4 hours. Later, SDS containing solubilization buffer was added on the cells and they were incubated at 37°C for another 15 mins. Cell viability was measured by spectrophotometer at 570 nm. Absorbance values were interpreted by the use of Prism 8.

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I. RESULTS

The sizes of chitosan microspheres were determined by light microscopy analysis. It was found that the average size of the microspheres was 40.55 µm ±14 (Figure 1).

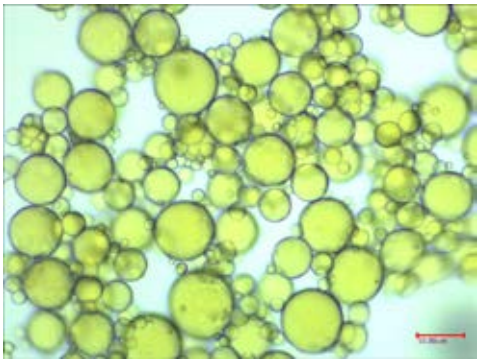

Figure. 1. Light microscopy image of chitosan microspheres (20X)

The encapsulation efficiency (EE) of *MDM2* inhibitor in chitosan microspheres was determined by equation (1).

$$EE\% = \left(\frac{\text{Weight of initially added drug} - \text{Weight of free drug in supernatant}}{\text{Weight of initially added drug}}\right) * 100 \quad (1)$$

The encapsulation efficieny was found as 80.7 %. The release profile of *MDM2* inhibitor from chitosan microspheres was given in Figure 3. The release studies were continued up to 138 hours and 63.98 % of the drug was released within this period.

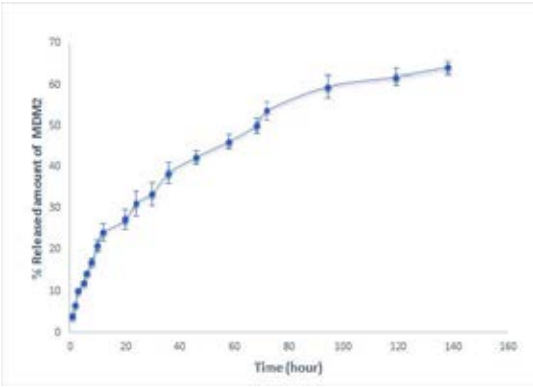

Figure. 3. Release profile of *MDM2* inhibitor

Cell viability was followed for 5 days by performing MTT assay. It was shown that controlled drug release showed a prolonged effect on the tumor cells when the MTT results of the drug-loaded microsphere treated cells and only drug treated cells compared. The differences between control and experimental groups in cell viability were analyzed by ANOVA test. Differences were considered significant at $p < 0.05$. A statistically significant difference was found only between the untreated and sphere+drug at 72hrs.

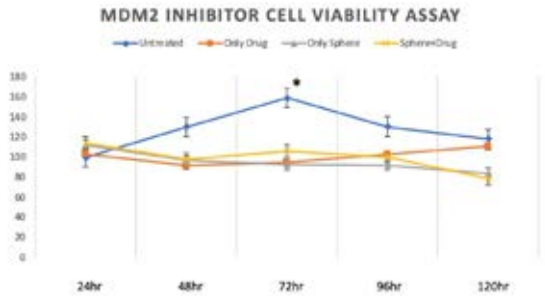

Figure. 4. Cell viability of T98 cells after treatment with *MDM2* inhibitor and drug-loaded chitosan microspheres; *$p < 0.05$.

It was also shown that *MDM2* inhibitor begins to affect the brain tumor cells after 72 hours whether it is applied directly or loaded into microspheres (Figure 4). Moreover, apoptotic cells were seen starting from 24 hours after treatment with drug-loaded microspheres (Figure 5).

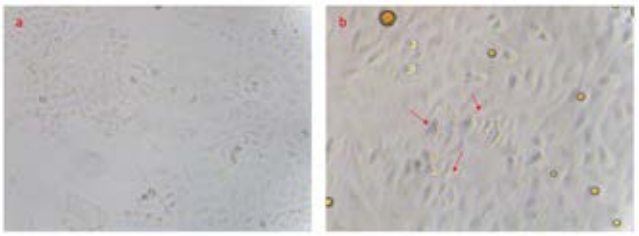

Figure. 5. Microscope images of T98 cells at 24hr after treatment with *MDM2* inhibitor and *MDM2*-loaded microspheres. a) T98 cells treated with 100uM naked drug (10X). b) T98 cells treated with 100uM drug-loaded chitosan microspheres showing apoptotic cells (20X).

I. CONCLUSION

As a result, *MDM2* inhibitor was loaded in the microspheres successfully and 63.98 % of the drug was released within 138 hours. Drug is more effective on brain tumor cells when it is released gradually from chitosan microspheres for longer time period and they trigger death in T98 cells. This study suggests that controlled and prolonged release of potential cancer drugs from chitosan microspheres trigger cell death and can be an effective way to treat malignant tumors.

REFERENCES

[1] Kato, Y., Onishi, H., and Machida, Y. "Contribution of Chitosan and its Derivatives to Cancer Chemotherapy", 2005.