

# Chitosan Membrane in Combinations with Nanoparticles and Adriamycin as a Treatment to Inhibit Glioma Growth and Migration

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## ABSTRACT

Chitosan exhibits antimicrobial activities through its interaction(s) with microbial cell surface thereby altering their gene expression and cellular function, leading to cell death. Nanometal particles exert many effects not previously expected on biological systems and thus can be explored for diverse biomedical applications. Our previous and on-going studies indicate that U87 cells cultured on chitosan film/membrane exhibited significantly slower growth and proliferation kinetics compared to U87 cells cultured alone. In this study we tested the hypothesis that the inhibitory effect of chitosan is enhanced if combined with nanometals and Adriamycin, a common anticancer drug. Our results showed that combinations of metal nanoparticles, Adriamycin and chitosan induced decreased survival of U87 glioma cells at different rates, more marked than those with chitosan alone. Thus, our results have pathophysiological implications in inhibiting human brain glioma invasion and migration.

**Key words:** Chitosan, nanoparticles, Adriamycin, cancer therapy, U87 cells

## 1 INTRODUCTION

Chitosan, a polysaccharide biopolymer that combines a unique set of physicochemical and biological properties has increasingly gained popularity in biomedical applications [1, 2]. Our previous and on-going studies indicate that U87 cells (human brain glioblastoma cell line) cultured on chitosan film/membrane exhibited significantly slower growth and proliferation kinetics compared to U87 cells cultured in the absence of chitosan film/membrane [3]. Chitosan exhibits antimicrobial activities through its interaction(s) with microbial cell surface thereby altering their gene expression and cellular function and leading to cell death [4].

Nanometal particles, such as nanosilver and nanogold, are important material being utilized frequently in biomedical treatment. Recent studies of nanometal particles have revealed many properties that were not previously expected in biological systems and thus can be explored for various applications in biomedicine, such as tissue engineering [5] and wound dressing [6].

In this study, we hypothesized that the inhibitory effect of chitosan would be greatly modulated if we combine chitosan with nanometals and Adriamycin, a common drug for cancer therapy. Similar treatments with a different cancer (PANC-1, human pancreatic cancer cell line) and normal cells (BJ, human fibroblast cell line) were also used in this investigation.

## 2 MATERIALS AND METHODS

### 2.1 Materials

Human astrocytoma (astrocytes-like) U87 cells, human pancreatic PANC-1 cells and human fibroblast BJ cells were obtained from ATCC (Manassas, VA, USA). Chitosan (from crab shells, minimum 85% deacetylated), thiazolyl blue tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and Adriamycin were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA, USA). Tetrachloroauric (III) acid ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ), trisodium citrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) and silver nitrate ( $\text{AgNO}_3$ ) were purchased from Fisher Scientific (Pittsburgh, PA, USA). All chemicals were of analytical grade unless otherwise stated.

### 2.2 The Preparation of Nanosilver, Nanogold Particles

To prepare nanosilver,  $\text{AgNO}_3$  and  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$  solutions were filtered through a  $0.22 \mu\text{m}$  microporous membrane filter, and nanosilver was prepared according to the literature [7] by adding  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$  solution to boiling  $\text{AgNO}_3$  aqueous solution.

To prepare nanogold,  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  and  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$  solutions also need to be filtered through a  $0.22 \mu\text{m}$  microporous membrane filter prior to use. Nanogold was prepared according to the literature [8] by adding  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$  solution to boiling  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  aqueous solution.

### 2.3 Cell Culture

Chitosan membrane was prepared as described previously [3]. A certain amount of nanosilver or nanogold solution was added into each well of 24-well culture plates in which a sterile chitosan membrane had already been placed. After 12 hours, nanosilver or nanogold solution was aspirated and sterile phosphate-buffered saline (PBS) was added into each well to wash the membrane. We thereby refer the chitosan membrane coated with nanometal particles as metal CytoMem.

U87 cells were seeded with equal density in each well of the 24-well plates and cultured in an incubator at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS). Adriamycin with a final concentration of  $0.1 \mu\text{m}$  was added after cells were seeded. Culture of PANC-1 cells and BJ cells were similar with U87 cells whereas PANC-1 cells were grown in the RPMI 1640 medium.

### 2.4 MTT Assay

Cell survival and growth was determined using the MTT assay [9]. Cells (U87, PANC-1, and BJ) were cultured in 24-well plates as described in the preceding subsection. At the end of the incubation period (4, 7, 10, or 14 days), MTT dye (0.5%, w/v, in PBS) was added to each well and the plates were incubated for an additional 4 hours at  $37^\circ\text{C}$ . Purple-color insoluble formazan crystals in viable cells were dissolved using dimethyl sulfoxide, and the subsequent absorbance of the content of each well was measured at 570 nm using a Bio-Tek Synergy HT Plate Reader (Winooski, VT, USA) [10].

### 2.5 Statistical Analysis of Data

Results are presented as mean  $\pm$  standard error of the mean (S.E.M.) of 6 determinations in each experiment.

## 3 RESULTS AND DISCUSSION

As shown in Figure 1, all the treatments induced decrease in cell survival of U87 cells. There were apparent differences in the effects exerted by different treatments. After being treated for 14 days, chitosan with nanosilver (Ag CytoMem) and  $0.1 \mu\text{m}$  Adriamycin was the most effective. Thus, when metal CytoMem was used in combination with Adriamycin, the approach may enable lower doses of Adriamycin to be used, hence reduced toxicity. Chitosan with nanogold (Au CytoMem) and  $0.1 \mu\text{m}$  Adriamycin was the second most effective; chitosan alone was the least effective. Ag CytoMem showed greater effect than Au CytoMem. Therefore, these results provided some support for our hypothesis that a combination of metal CytoMem with Adriamycin was more effective than chitosan, Adriamycin alone or metal CytoMem only.

As shown in Figure 2, all the treatments showed an inhibitory effect on cell survival of PANC-1 cells. Similarly, after being treated for 14 days, Ag CytoMem and  $0.1 \mu\text{m}$  Adriamycin was the most effective; chitosan alone was the least effective. However, the effect was stronger on PANC-1 cells than on U87 cells. Compared with U87 cells, PANC-1 cells were considerably more sensitive to the effects of Adriamycin alone.

Figure 3 showed the effect of different treatments on the survival of BJ cells. Ag CytoMem and  $0.1 \mu\text{m}$  Adriamycin was the most effective, the same as U87 cells and PANC-1 cells. By day 14, however, there was no significant difference in the survival rate of cells treated with chitosan alone and Au CytoMem as compared with control.

## 4 CONCLUSIONS

Our previous and ongoing studies demonstrate that combinations of Adriamycin with metal CytoMem induced cell death at different rates, with reference to U87 cells (control) and with chitosan alone. Ag CytoMem and  $0.1 \mu\text{m}$  Adriamycin showed the greatest cell survival reduction on all three cell lines. Ag CytoMem showed greater effect than Au CytoMem. Taken together, these results suggest potentials for pathophysiological applications in inhibition of human brain glioma migration and invasion as well as in treatment of pancreatic cancer.

## 5 ACKNOWLEDGMENTS

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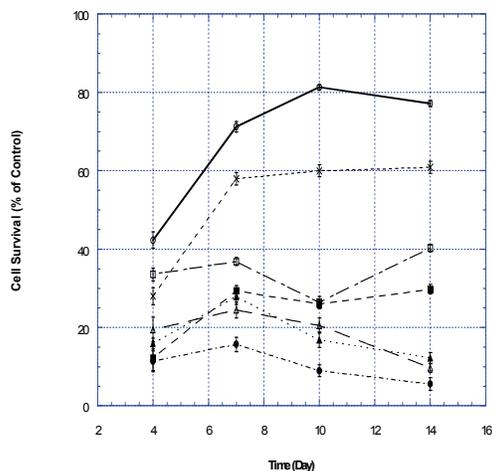


Figure 1: Effect of chitosan, metal CytoMem and 0.1 μm Adriamycin on survival of U87 cells. Circles: chitosan; Cross signs: Au CytoMem; Squares: 0.1 μm Adriamycin; Dark squares: chitosan with 0.1 μm Adriamycin; Dark triangles: Ag CytoMem; Triangles: Au CytoMem and 0.1 μm Adriamycin; Dark circles: Ag CytoMem and 0.1 μm Adriamycin. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± SEM.

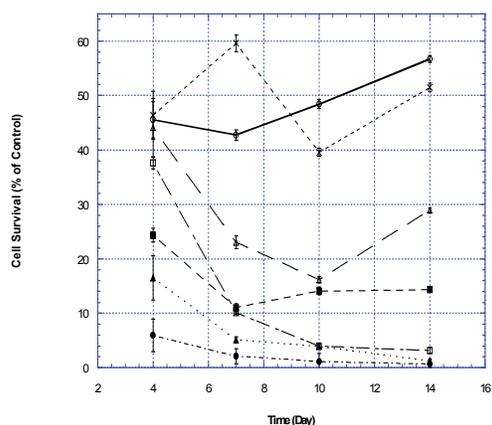


Figure 2: Effect of chitosan, metal CytoMem and 0.1 μm Adriamycin on survival of PANC-1 cells.

Circles: chitosan; Cross signs: Au CytoMem; Triangles: Au CytoMem and 0.1 μm Adriamycin; Dark squares: chitosan with 0.1 μm Adriamycin; Squares: 0.1 μm Adriamycin; Dark triangles: Ag CytoMem; Dark circles: Ag CytoMem and 0.1 μm Adriamycin. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± SEM.

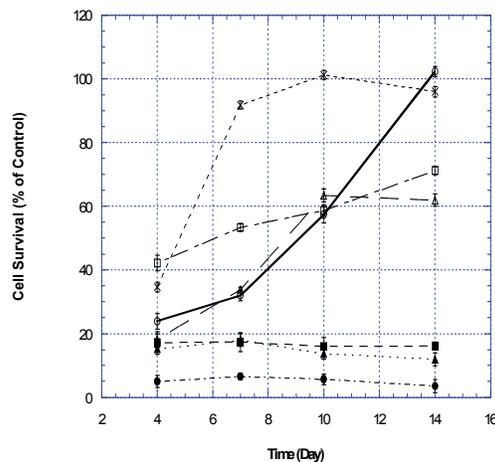


Figure 3: Effect of chitosan, metal CytoMem and Adriamycin on survival of BJ cells. Circles: chitosan; Cross signs: Au CytoMem; Squares: 0.1 μm Adriamycin; Triangles: Au CytoMem and 0.1 μm Adriamycin; Dark squares: chitosan with 0.1 μm Adriamycin; Dark triangles: Ag CytoMem; Dark circles: Ag CytoMem and 0.1 μm Adriamycin. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± SEM.

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