

Chitosan Film/Membrane as a Surface to Alter Brain Glioma Growth and Migration

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ABSTRACT

A hydrophilic polysaccharide derived from chitin, chitosan has gained increasing popularity in biomedical applications such as tissue engineering, wound dressing, and drug delivery because of its presumed biocompatibility. These applications were founded on chemical similarities between chitosan and cell surface carbohydrate moieties and certain extracellular materials. Human brain glioblastoma U87 cells modify their plasma membranes to facilitate migration and invasion through complex and as yet poorly understood interactions with the extracellular matrix of brain tissue. Because chitosan exhibits anti-microbial activities through its interaction(s) with microbial cell surface thereby altering their gene expression and cellular function and leading to cell death, we hypothesized

that the properties of chitosan can be exploited to inhibit human brain glioma migration and invasion. We have designed a cell model to test this hypothesis. We cultured human glioblastoma U87 cells on a film/membrane of chitosan and compared their growth and proliferation kinetics with U87 cells not cultured on chitosan film/membrane (i.e., the control). Our results of on-going studies indicate that U87 cells cultured on chitosan film/membrane exhibited significantly slower growth and proliferation kinetics compared to U87 cells cultured in the absence of chitosan film/membrane. Thus, they may have pathophysiological implications in glioma migration and invasion.

Keywords: Chitosan film, chitosan membrane, tumor migration and invasion, tissue engineering

1. INTRODUCTION

A hydrophilic polysaccharide derived from chitin, chitosan has gained increasing popularity in biomedical applications such as tissue engineering, wound dressing, and drug delivery because of its presumed biocompatibility [1-3]. These applications were founded on chemical similarities between chitosan and cell surface carbohydrate moieties and certain extracellular materials [4-6].

Glioblastoma multiforme is a malignant primary brain tumor. It is one of the most aggressive brain tumors and humans diagnosed with this disease usually have extremely poor prognosis and survival [see 7, 8 and references therein].

Although these tumors rarely metastasize out of the central nervous system, their invasion away from the tumor mass makes it difficult for surgical resection of the tumor and for the treatment of these tumors [7]. Because current therapies for this disease are largely ineffective in curing it, it is imperative to find new approaches to manage this disease even if a cure is not yet in sight [see 7 & 8 for additional discussion].

Human brain glioblastoma U87 cells modify their plasma membranes to facilitate migration and invasion through complex and as yet poorly understood interactions with the extracellular matrix of brain tissue [5-8]. Because chitosan exhibits anti-microbial activities through its interaction(s) with microbial cell surface thereby altering their gene expression and cellular function and leading to cell death [4], we hypothesized that the properties of chitosan can be exploited to inhibit human brain glioblastoma migration and invasion. We have therefore designed a cell model to systematically investigate this hypothesis.

2. MATERIALS AND METHODS

2.1 Materials

Human astrocytoma (astrocytes-like) U87 cells were obtained from ATCC (Manassas, VA, USA). Chitosan (from crab shells, minimum 85% deacetylated), thiazolyl blue tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA, USA). Other chemicals were of analytical grade and were usually from

2.2 Culture of U87 Cells with and without Chitosan Film/Membrane

A certain amount of chitosan was weighed and dissolved in 100 ml 1% (v/v) acetic acid solution. The solution was filtered, poured onto a plastic plate, and then oven-dried at a constant temperature of 40° C for 24 hours to form solid film. The films were then washed with 5% NaOH aqueous solution until neutral and then repeatedly washed with distilled water. The dried chitosan films were punched out (in the forms of circles with ~15-mm in diameter). Subsequently, the circular membranes were sterilized with 75% (v/v) ethanol overnight and then exposed to ultraviolet light for 40 min on each side. Finally each circular piece of chitosan film was rinsed extensively with sterile phosphate-buffered saline (PBS) and then placed into one well of 24-well culture plates.

U87 cells were seeded with equal density in each well (with or without the chitosan film on the bottom of the well) of 24-well plates and cultured in an incubator at 37° C and 5 % CO₂ in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) as described previously [9,10].

2.3 MTT (Cell Survival/Growth) Assay

Cell survival and growth was determined using the MTT assay [9,10]. U87 cells were cultured in 24-well plates as described in the preceding subsection. At the end of the incubation period (24, 36, 48, 60 or 72 hours), 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°C. The purple-colored 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) [9,10].

2.4 Statistical Analysis of Data

Results are presented as mean \pm standard error of the mean (S.E.M.) of 6 determinations in each experiment as described previously [9]. Linear regression analysis of data was performed using the software KaleidaGraph version 4 (Synergy Software, Reading, PA, USA).

3. RESULTS AND DISCUSSION

To test our hypothesis that the properties of chitosan can be exploited to inhibit human brain glioblastoma migration and invasion, we have employed a cell model in vitro. We cultured human glioblastoma U87 cells on a film/membrane of chitosan and compared their growth and proliferation kinetics with U-87 cells not cultured on chitosan film/membrane (i.e., the control) because growth and proliferation of cancer cells will determine their migration and invasion.

As shown in Figure 1, typical of aggressive cancer cells, U87 cells grew rapidly, with a doubling time of approximately 22 hours under our study conditions, with a minimal lag period. However, our initial observation that when we cultured these cells on a chitosan film/membrane, their growth rate appeared to slow down prompted us to investigate more fully this apparently growth-slowing effect of the chitosan film/membrane.

Employing the MTT assay we systematically studied the growth of U87 cells cultured on chitosan film/membrane of various compositions (from 0.25 to 1.5 %) (Figure 1). Our results allowed us to tentatively draw two conclusions (Figure 1). (i) When U87 cells were cultured on a chitosan film/membrane, their rate of growth was significantly slowed. (ii) Although there were some apparent differences in the effects exerted by various concentration of chitosan in forming the film/membrane (from 0.25 to 1.5%), their difference is unlikely to be biologically significantly.

To more quantitatively assess the results as shown in Figure 1, we performed linear regression analysis on the data. All the U87 growth plots showed linear regression correlation coefficients of greater than 0.95 (Table 1). From the equations (Table 1), evidently the slope of growth plot of control (i.e., untreated) U87 cells was significantly higher than those of U87 cells cultured on chitosan films (0.0032713 versus <0.0016, respectively; Table 1). Thus, the quantitative analysis of our data agrees very well with our conclusions based on visual inspection of the plots shown in Figure 1.

Clearly, it would be important to ascertain if this growth-slowing effect of the chitosan film/membrane only applies to one type of tumor cells (namely U87 glioma cells) or if this effect applies also to other tumor types. Our on-going studies appear to suggest that the chitosan film/membrane we have employed exerts similar effect on the growth of other tumor types. Undoubtedly, this important area merits further investigation.

Effect of Chitosan Film/Membrane on U87 Cell Growth

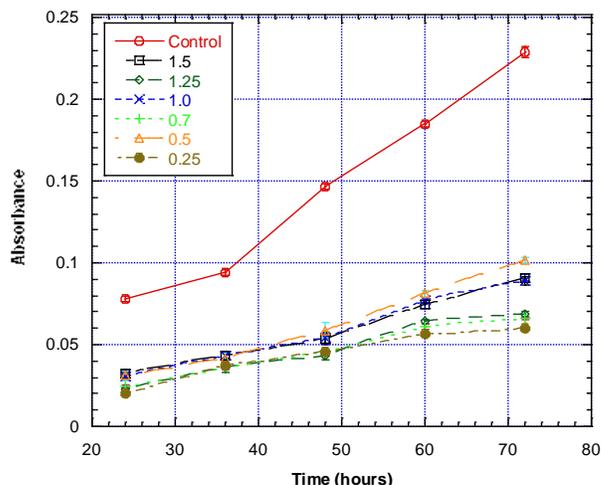


Figure 1. Effect of Chitosan Film on Survival and Growth of U87 cells. U87 cells were cultured alone (red circles) or on chitosan film/membrane of various percentages: black circles, 0.25%; red triangles, 0.5%; green plus signs, 0.7%; blue multiple signs, 1.0%; dark green circles, 1.25%; and open squares, 1.5%. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean \pm SEM; however, in some of the cases the SEM values were too small to be visible.

Treatment Group	Equation	R ²
Control	$y = 0.0032713x - 0.010455$	0.98149
0.25%	$y = 0.00083055x - 0.0042004$	0.95011
0.5 %	$y = 0.0015037x - 0.009289$	0.98499
0.7%	$y = 0.00089166x - 0.0040672$	0.9855
1.0%	$y = 0.0012518x - 0.0010884$	0.98416
1.25%	$y = 0.0009722x - 0.000633$	0.96908
1.5%	$y = 0.001237x - 0.0005784$	0.97894

Table 1. Linear Regression Analysis of the Results of the Effect of Chitosan Film/Membrane on U87 Cell Growth. The data shown in Figure 1 were analyzed using linear regression analysis.

Our results (Figure 1 and Table 1) provide some support for our hypothesis that the properties of chitosan can be exploited to inhibit human brain glioblastoma migration and invasion. To our knowledge, this is the first report of such a chitosan effect on human brain glioblastoma cells.

Our finding that chitosan film/membrane exerts a growth-slowing effect on U87 cells may have pathophysiological implications in glioma migration and invasion. We are currently actively pursuing this important line of research.

4. CONCLUSIONS

Our previous and ongoing studies demonstrate that U87 cells constitute a viable cell model for investigating the effects of agents on glioblastoma growth, proliferation, migration, and invasion [7,8]. Our results of on-going studies indicate that U87 cells cultured on chitosan film/membrane exhibited significantly slower growth and proliferation kinetics compared to U87 cells cultured in the absence of chitosan film/membrane. Thus, they may have pathophysiological implications in glioma migration and invasion. As such they may have relevance to the design of new treatment paradigm for glioblastoma multiforme.

5. ACKNOWLEDGMENTS

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