Effects of Chitosan and Nanoparticles on Survival of Schwann Cells and Dorsal Root Ganglion Neurons

James C.K. Lai*, Wenjuan Gao**, and Solomon W. Leung***

*Biomedical & Pharmaceutical Sciences Department and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, email: lai@pharmacy.isu.edu

**Civil & Environmental Engineering Department, Idaho State University, Pocatello, ID 83209, USA, email: gaowenj@isu.edu

***Civil & Environmental Engineering Department, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA. Fax: 208-282-4538; Tel: 208-282-2524; email: leunsolo@isu.edu

ABSTRACT

We have systematically examined the effects of chitosan film or chitosan in combination with nanometal particles (nanogold or nanosilver particles) on the growth of Schwann cells (S16) and DRG neurons (50B 11). Our results strongly suggest chitosan and chitosan in combination with nanogold particles had no effects on the growth kinetics of Schwann cells but had slightly slowed those of DRG neurons. These findings suggest that chitosan and chitosan in combination with nanogold particles are more biocompatible with Schwann cells than with DRG neurons. However, chitosan in combination with nanosilver particles depressed the growth kinetics of both Schwann cells and DRG neurons. Thus, nanosilver particles are much less biocompatible compared to nanogold particles. Our results suggest that chitosan film and chitosan in combination with nanogold particles may have the potential to be developed as suitable biocompatible materials for the fabrication of artificial nerve graft.

Key words: Schwann cell, DRG neuron, chitosan, nanoparticle, biocompatible

1 INTRODUCTION

Peripheral nerve injuries are common in a variety of chronic and degenerative disease (e.g., diabetes). A promising alternative for the repair of peripheral nerve injuries is the bioartificial nerve graft, comprised of biocompatible materials pre-seeded with neuronal supporting cells, such as Schwann cells [1]. Chitosan is the fully or partially deacetylated form of chitin [2]. Due to its biocompatibility, biodegradation, and other favorable biological properties, chitosan has been tested for its suitability as a scaffolding material for nerve regeneration [3]. Nanometal particles possess many properties that were not previously expected in biological systems are also introduced into the construction and/or modification of the bioartificial nerve graft.

Current cell models involving primary cultures of peripheral nervous system (PNS) neural cells employed to investigate the mechanisms underlying nerve degeneration and regeneration have significant limitations because primary cultures of PNS neurons have a very limited life-span.

In this study, we have overcome such limitations by employing immortalized Schwann cells (S16) and DRG neurons (50B11), which have much longer life-span in culture, to develop PNS neural cell models *in vitro*. These models are suitable for nerve degeneration and regeneration studies. Employing the models, we have studied the effects of chitosan film or chitosan in combination with nanometal particles (nanogold or nanosilver particles) on the growth of Schwann cells (S16) and DRG neurons (50B 11) for periods of 5 and 14 days.

2 MATERIALS AND METHODS

2.1 Materials

S16 Schwann cells were obtained from ATCC (Manassas,VA, USA). 50B 11 DRG neurons obtained from Dr Hoke's Laboratory at Johns Hopkins School of Medicine. 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). Tetrachloroauric (III) acid ($HAuCl_4 \cdot 3H_2O$), trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), and silver nitrate ($AgNO_3$) were purchased from Fisher Scientific (Pittsburgh, PA, USA). All chemicals were of analytical grade unless otherwise stated.

2.2 The Preparation of Nanogold and Nanosilver Particles

To prepare nanogold particles, $HAuCl_4 \cdot 3H_2O$ and $C_6H_5Na_3O_7 \cdot 2H_2O$ solutions were filtered through a 0.22 µm membrane filter prior to use. Nanogold particles were prepared according to the literature [4] by adding $C_6H_5Na_3O_7 \cdot 2H_2O$ solution to boiling $HAuCl_4 \cdot 3H_2O$ aqueous solution.

To prepare nanosilver particles, $AgNO_3$ and $C_6H_5Na_3O_7 \cdot 2H_2O$ solutions were filtered through a 0.22 µm microporous membrane filter and nanosilver particles were prepared according to the literature [5] by adding $C_6H_5Na_3O_7 \cdot 2H_2O$ solution to boiling $AgNO_3$ aqueous solution.

2.3 Cell Culture

Chitosan film was prepared as described previously [6]. A specified amount of nanogold or nanosilver suspension was added into each well of 24-well culture plates in which a sterile chitosan film had already been placed. After 12 hours, nanogold or nanosilver suspension was aspirated and sterile phosphate-buffered saline (PBS) was added into each well to wash the membrane. We thereby refer the chitosan film coated with nanometal particles as metal CytoMem.

S16 Schwann cells or DRG neurons were seeded with equal density in each well of the 24-well plates and cultured in an incubator at 37˚ C and 5 % CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS.

2.4 MTT Assay

Cell survival and growth was determined using the MTT assay [7]. Cells (S16 Schwann or DRG neurons) were cultured in 24-well plates as described in the cell culture subsection. At the end of the incubation period (1, 2, 3, 4, or 5 days; 1, 2, 3, 4, 5, 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°C. Purple-color insoluble formazan crystals in viable cells were dissolved using DMSO, 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) [8].

2.5 Statistical Analysis of Data

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

3 RESULTS AND DISCUSSION

Employing the MTT assay, we systematically studied the growth of S16 Schwann cells cultured on chitosan film or on metal CytoMem [6] for 5 days or 14 days (Figure 1 and Figure 2). As shown in Figure 1, S16 Schwann cells cultured on chitosan film or AuCytoMem exhibited similar growth rates compared to those cells cultured in the absence of chitosan film (i.e., the control). However, when S16 Schwann cells were cultured on AgCytoMem, their rate of growth was significantly slowed (near zero). When S16 Schwann cells were cultured up to two weeks, they showed rates that were similar to those cultured for 5 days (Figure 2).

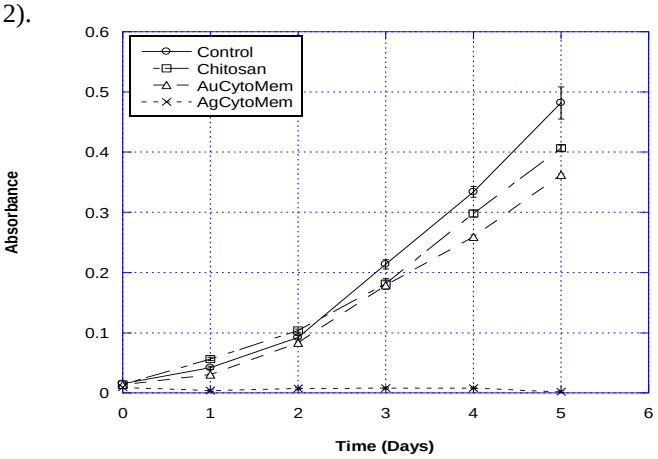

Figure 1: Effect of chitosan, metal CytoMem on the Growth of S16 Schwann Cells for 5 Days. Circles: control; Squares: chitosan treated; Triangles: AuCytoMem treated; Crosses: AgCytoMem treated. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± S.E.M.

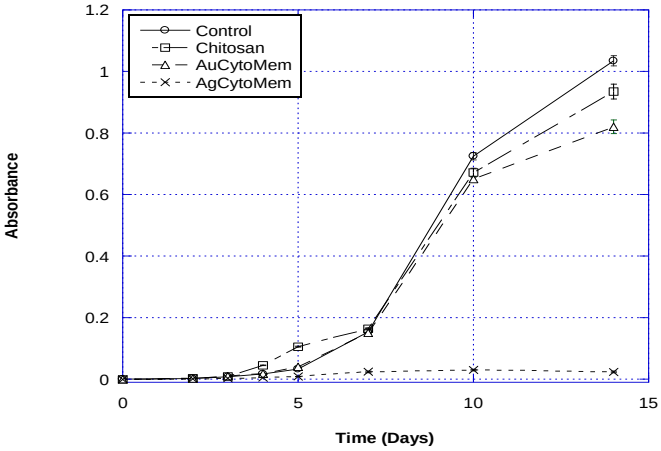

Figure 2: Effect of chitosan, metal CytoMem on the Growth of S16 Schwann Cells for 14 Days. Circles: control; Squares: chitosan treated; Triangles: AuCytoMem treated; Crosses: AgCytoMem treated. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± S.E.M.

Figure 3 and Figure 4 show the effects of chitosan and metal CytoMem on the growth of DRG neuron. As shown in Figure 3, chitosan film and AuCytoMem exerted inhibitory effect on the growth rate of DRG neurons. Similarly, AgCytoMem also depressed the growth rate of DRG neurons.

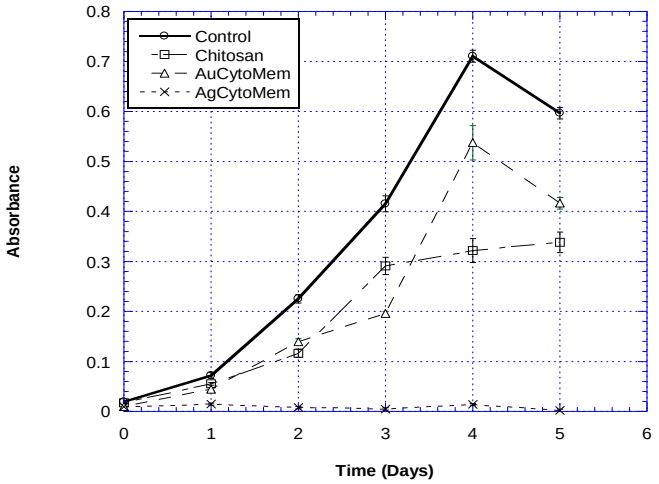

Figure 3: Effect of chitosan, metal CytoMem on the Growth of DRG neurons for 5 Days. Circles: control; Squares: chitosan treated; Triangles: AuCytoMem treated; Crosses: AgCytoMem treated. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± S.E.M.

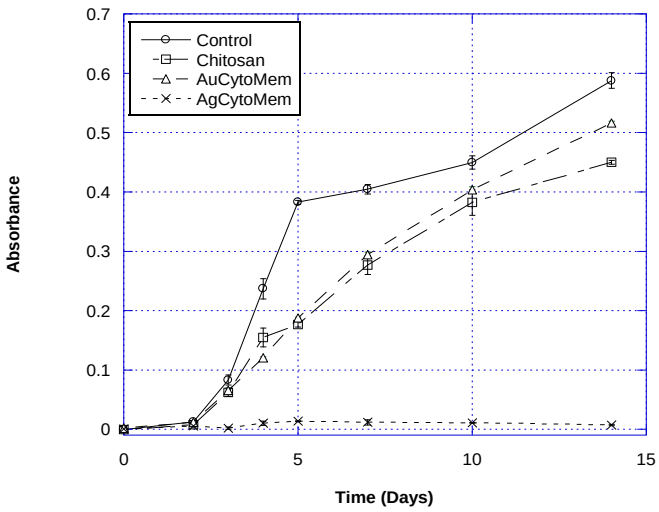

Figure 4: Effect of chitosan, metal CytoMem on the Growth of DRG neurons for 14 Days. Circles: control; Squares: chitosan treated; Triangles: AuCytoMem treated; Crosses: AgCytoMem treated. At the end of the specified incubation time, cell survival and growth was determined using the MTT assay. Values are the mean ± S.E.M.

4 CONCLUSIONS

Our ongoing studies demonstrate that chitosan and AuCytoMem had no effects on the growth kinetics of Schwann cells. However, chitosan and AuCytoMem appeared to exert a small negative impact on the growth kinetics of DRG neurons. These findings suggest that chitosan and AuCytoMem are more biocompatible with Schwann cells than with DRG neurons. On the other hand, AgCytoMem depressed the growth kinetics of both Schwann cells and DRG neurons. Thus, nanosilver particles are much less biocompatible compared to nanogold particles. Taken together, our results suggest that chitosan film and AuCytoMem may have the potential to be further developed as suitable biocompatible materials for the fabrication of artificial nerve graft.

5 ACKNOWLEDGMENTS

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REFERENCES

[1] Rutkowski GE, Heath CA, "Development of a bioartificial nerve graft. II. Nerve regeneration *in vitro*," Biotechnol. Prog., 18(2): 373-379, 2002.

[2] Sundararajan VM, Howard WTM, "Porous chitosan scaffolds for tissue engineering," Biomaterials, 20(12):1133-1142, 1999.

[3] Kim IY, Seo SJ, Moon HS, Yoo MK, Park IY, Kim BC, Cho CS, "Chitosan and its derivatives for tissue engineering applications," Biotechnology Advances, 26(1): 1-21, 2008.

[4] Turkevich J, Stevenson PC, Hillier J, "A study of the nucleation and growth processes in the synthesis of colloidal gold," Discuss. Faraday Soc., 11: 55-75, 1951.

[5] Kamat PV, Flumiani M, Hartland GV, "Picosecond Dynamics of Silver Nanoclusters. Photoejection of Electrons and Fragmentation," J. Phys. Chem. B, 102(17): 3123-3128, 1998.

[6] Gao WJ, Wang YH, Gu HY, Jandhyam S, Dukhande VV, Lai MB, Leung SW, Bhushan A, Lai JCK, "Chitosan Film/Membrane as a Surface to Alter Brain Glioma Growth and Migration," In Proceeding of the 12th NSTI (Nano Science and Technology Institute) Nanotech, Houston, USA, Volume 2, pp. 302-305, 2009.

[7] Mossman T, "Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays," Journal of Immunological Methods, 65(1-2): 55-63, 1983.

[8] Dukhande VV, Malthankar-Phatak GH, Hugus JJ, Daniels CK, Lai JCK, "Manganese induced neurotoxicity is differentially enhanced by glutathione depletion in astrocytoma and neuroblastoma cells," Neurochem Res., 31(11): 1349-1357, 2006.