Ellagic acid-chitosan based local delivery system has an anti-tumor effect on brain cancer both *in vitro* and *in vivo*.

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Statement of Purpose: In recent years, implantable drug delivery systems have gained special attention for treating localized diseases or cancers in specific anatomical sites. Especially, local delivery of therapeutic agents to the brain can overcome many limitations associated with the presence of the blood-brain-barrier (BBB). We have attempted to locally deliver a phenolic compound, ellagic acid (EA), to treat brain cancers. Ellagic acid has been reported to exhibit anti-carcinogenic properties including cell cycle arrest, induction of apoptosis, and inhibition of tumor formation and growth [1]. Chitosan has been chosen as a delivery carrier because it can be readily formed to film-, gel- and porous scaffold-type materials. In addition, chitosan is also less soluble in aqueous solution at normal pH value but enzymatically degradable [2]. The objective of the present study was to evaluate the anti-tumor effect of a chitosan based delivery system with ellagic acid against brain cancers such as U87 human glioblastomas and rat C6 gliomas both *in vitro* and *in vivo*.
Methods: Chitosan (190- 310 KDa, 85% DDA) based composite films were prepared by solution casting 1%(w/v) chitosan with 0, 0.05, 0.1, 0.5, or 1% (w/v) of ellagic acid in distilled water for the cell culture study, and 1%(w/v) chitosan films with 20%(w/v) of ellagic acid (Ch-EA20) were also prepared for animal study. Composite films were characterized using Fourier transform infrared (FTIR) and X-ray diffraction (XRD). The number of viable cells (U87 and C6) was determined with the Cell Titer 96AQueous One Solution Cell Proliferation Assay (MTS assay, Promega Corp.). In this study, direct and indirect-cell cultures were performed to evaluate cell viability. In the direct cell culture, 30,000 cells/well were seeded directly on chitosan-ellagic acid films cast in 48-well plates. In the indirect-cell culture tests, 36,000 cells/well were seeded into inserts (BD BioCoat™, 0.45 micron pore size) in 12-well plates which were coated with the composite films to test the effect of the released or dissolved products from the films into medium on the cell viability. In the animal study, GFP (green fluorescent protein) tagged rat C6 glioma cells were implanted subcutaneously at a density of 4×10^6 cells in 200µl of PBS in the right flank region of 5 female nude mice per group and allowed to grow until tumor size reaches 2cm in a diameter. Treatment was initiated by implanting films (chitosan or Ch-EA20) subcutaneously onto the tumor sites at 5 days after tumor injection. For control group, tumors were left untreated. Tumor growth was evaluated by measuring tumor volume using a caliper, an ultrasound machine, and an optical imaging system.
Results/Discussion: The XRD patterns demonstrate that the crystallinities of the chitosan-ellagic acid films increased with higher EA composition. The results of FTIR of the chitosan-ellagic acid films exhibit new bands for amide and ester, suggesting that hydroxyl and carboxyl groups of ellagic acid formed new linkages with amine and hydroxyl groups of chitosan. It is suggested that the ellagic acid formed covalent binding with chitosan in addition to physically embedding into chitosan polymer network. The chitosan-ellagic acid films (Ch-EA 0.5 and Ch-EA1) were found to have an anti-proliferative effect on U87 and C6 glioma cells in the direct cell culture test. Indirect-cell culture test revealed the slow release of ellagic acid into cell culture medium reduced toxic effect on cell viability. This result indicates the local delivery effect of ellagic acid-chitosan composite materials on the growth of U87 and C6 glioma cells. The animal study demonstrated that Ch-EA20 group significantly inhibited tumor growth compared with the untreated group and chitosan control group (Fig.1). However, no significant differences in tumor growth were observed between the untreated group and the chitosan group. Weight loss more than 10% before treatment was not observed in any group, indicating no severe side toxic effects on animals during the period of experiments.
Conclusions: Ellagic acid -chitosan based local delivery systems can significantly inhibit glioma tumor growth both *in vitro* and *in vivo*. The less toxic side effect using local delivery system is also demonstrated in this study.

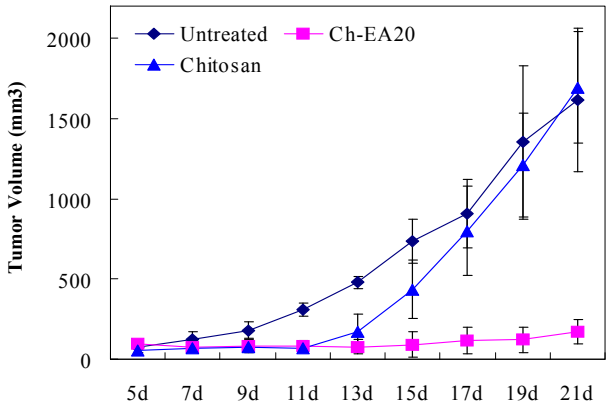

Figure 1. *In vivo* tumor growth over time in absence and presence of implant biomaterials. Untreated (closed diamond, n=5); chitosan (closed triangle, n=5), and Ch-EA20 (closed square, n=5). Tumor volumes were measured by a caliper. Ultrasound and optical imaging measurements (not shown) have similar results.

References:

1. Narayanan BA, Geoffroy O, Willingham MC, Re GG, Nixon DW. Cancer Letters 1999;136: 215-221.
2. Jaworska M, Sakurai K, Gaudon P and Guibal E. . Polymer International 2003;52:198-205.