Chitosan-ellagic acid composite films induce apoptotic death in human melanoma cells

Sungwoo Kim,[1] Y Liu,[1] MW Gaber,[1] JD Bumgardner,[2] WO Haggard,[2] Y Yang[1]
[1]Department of Biomedical Engineering and Imaging, University of Tennessee Health Science Center, Memphis, TN 31863
[2] Department of Biomedical Engineering, University of Memphis, TN

Statement of Purpose: Chitosan, which is a well known cationic natural polymer, has special characteristics such as biocompatibility, biodegradability, and enzymatic depolymerization for rebuilding of other biological events and provides a wide range of biomedical applications. Ellagic acid, a naturally occurring bioactive compound extracted from grapes, is a polyphenol compound. It has been reported to inhibit cancer cells growth. Various investigations has evaluated that the ellagic acid has potential cytotoxic, anti-proliferative activities and induction of apoptosis in cancer cells as a therapeutic approach. In this study, chitosan-ellagic acid composite films were developed, and the effect of their unique properties on malignant human melanoma was evaluated as a new effective therapeutic approach to treat cancer. The objective of the present study was to evaluate the effects of chitosan-ellagic acid composite films on viability of melanomas and fibroblasts.

Methods: Chitosan-ellagic acid 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 the distilled water. Composite films were characterized using Fourier transform infrared (FTIR), differential scanning calorimetry (DSC), scanning electron microscope (SEM), and contact angle measurement. Melanoma as a model cancer cell and fibroblast as a control were used to investigate cell-composite films interaction. Direct and indirect cell cultures were performed to evaluate cell viability. In the direct-contact cell culture test, 60,000 cells/well were seeded directly on chitosan-ellagic acid films cast in 48-well plates. In the indirect cell culture tests, 60,000 cells/well were seeded into inserts (BD BioCoat™, 0.45 micron pore size) in 12-well plates to hold cells above the composite films to test the effect of the released or dissolved products from the films on the cells. The number of viable cells (melanomas and fibroblasts) was assessed using the Cell Titer 96AQueous One solution cell proliferation assay. The apoptotic cells were determined by Annexin V-PE and 7-AAD (7-Amino-actinomycin D) staining.

Results/Discussion: In the present study, chitosan-ellagic acid composite films were made and the effect of their unique properties on melanoma as an alternative cancer treatment was evaluated in vitro. Ellagic acid contains hydroxyl and carboxyl groups on its structure associated with benzene ring and has potential to form new bonds with chitosan. The results measured by FTIR, DSC and contact angles showed that the chitosan-ellagic acid composite films have amide and ester linkages between functional groups of chitosan and ellagic acid as well as increased hydrophilicity and thermal stability associated with increased ellagic acid. Chitosan-ellagic acid composite films induced apoptotic cell death in the number of melanomas within few hours only when cells interact directly with material's surface. In addition, they showed a selective anti proliferative activity in melanomas without toxic effect on the viability of fibroblasts depending on a certain range of component ratios of ellagic acid. (Fig. 1)

Conclusions: Chitosan-ellagic acid composite films displayed increased hydrophilicity and thermal stability, as well as reduced the number of viable melanomas within few hours. In addition, they showed a selective anti proliferative activity in melanomas compared to fibroblasts depending on a certain range of component ratios of ellagic acid. Animal study is in progress to determine the in vivo efficacy and efficiency of chitosan-ellagic acid composite materials on melanoma treatment.

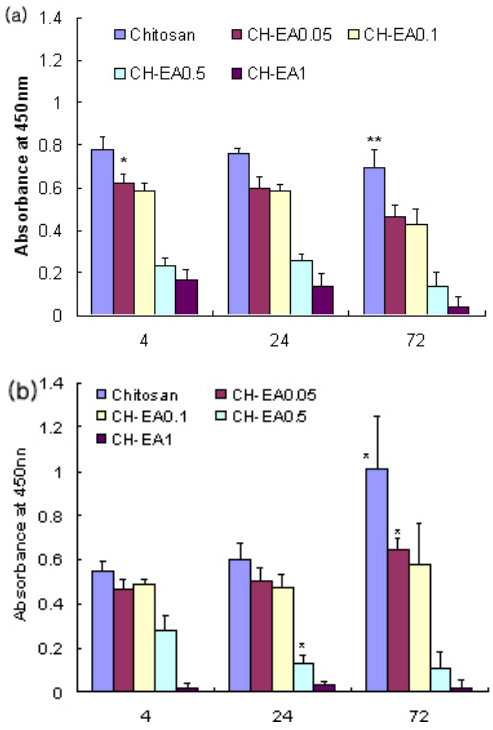

Figure 1. Cell viability on different coating surfaces for 3 days incubation: (a) melanoma cells; *p<0.05 compared with chitosan only at 4 hours. **p=0.069 compared with chitosan at 4 hours (b) fibroblasts; *p<0.05 compared with each group at 4 hours.

References:

1. Muzzarelli. R.A. CMLS, Cell. mol. life sci.1997; 53:131-140.
2. Bhagavathi A. Cancer Letter. 1999;136:215-221.
3. Hussein MR. J Pathol. 2003;119:275-288.