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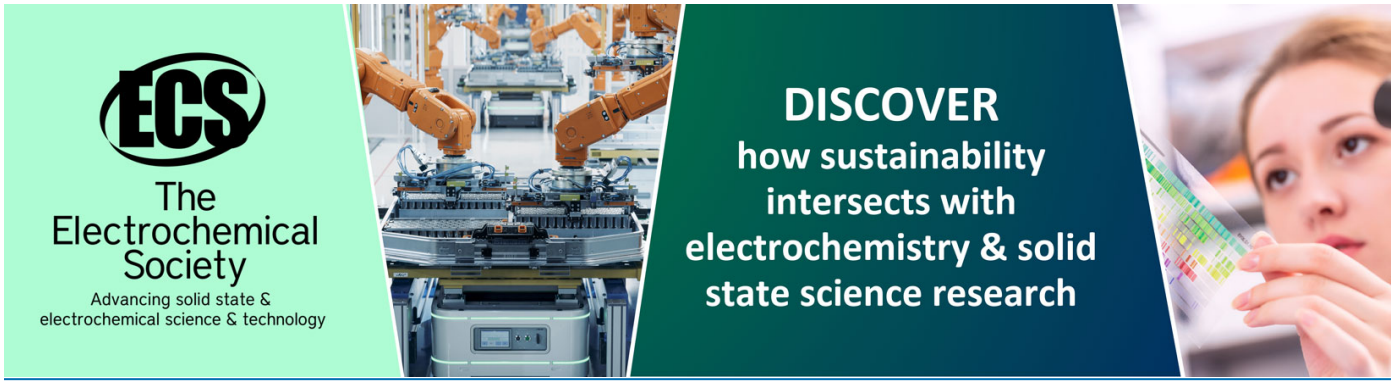

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Cytotoxicity Test of Chitosan Solution as Acrylic Denture Cleansers Solution

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Abstract. Removable denture is the easiest and most affordable management for tooth loss. Denture cleanser solution is needed in daily care procedure of removable denture but the price is relatively expensive. Chitosan is a solution derived from chitin marine animals. The objective of the study is to test cytotoxic effects of Chitosan in various concentrations. We used 13 samples treated with chitosan and acetic acid in different concentration. Cytotoxicity test was done with MTS assay method in-vitro on BJ cells (normal skin cells). The results showed that percentage of cell death (cytotoxicity effect) in chitosan solution increased as the concentration raised, with IC_{50} values of 0.032% chitosan solution. We concluded that chitosan is cytotoxic and unsuitable to be used as removable denture cleanser solution.

1. Introduction

The most common dental and oral diseases are dental caries and periodontal disease, both of which cause permanent tooth loss in adults. The consequence of tooth loss is the disruption of various functions of the stomatognathic system, such as chewing or mastication function, aesthetic function, and speech function. To restore various functions of the stomatognathic system a rehabilitative device called dental prosthesis is developed. Dental prosthesis that is widely used because it is applicable to wide variety of clinical situation, affordable, non invasive that do not require extensive modification of abutment teeth is removable partial denture.[1,2]

The base of removable denture is the part of dental prosthesis that contacts the oral mucosal tissue, especially the alveolar lining mucosa and the palate. This part is made of poly-methyl-methacrylate resin (PMMA) or commonly known as acrylic resin [2-4], which has a porous surface texture on a micro scale. This structure causes easy attachment of plaque and food debris that triggers the growth of microorganisms as the cause of denture stomatitis.[5-7] Denture stomatitis can be prevented through cleansing of removable denture base as preventive measures. Commonly used methods are mechanical methods and immersion methods.[8-9]

Chitosan is a chitin derivat, made from the shells of marine animals. Chitosan has antifungal and antimicrobial properties derived from the cation groups in the polymer (amino (NH2)) are able to bind to the anion group on the bacterial cell wall which will then synthesize the bacterial cell wall and inhibit mass transfer between bacterial cells and accelerate the process of bacterial death. Chitosan is extensively used in medical field due to its biodegradable properties. [10-13]

2. Methods

To test cytotoxicity effect of chitosan solutions and acetic acid as its solvent, we prepared thirteen solutions which consists of nine different concentrations of chitosan solution (0.01%, 0.02%, 0.03%, 0.1%, 0.2%, 0.3%, 1%, 2%, and 3%), four different concentrations of acetic acid solutions (0.02%, 0.2%, 2%, and 0%). Chitosan solutions was prepared by dissolving chitosan powder into acetic acid solution up to 100 ml volume, stirred using magnetic stirrer until chitosan dissolved without precipitate.

For cytotoxicity test, BJ cultured cell from the American Type Culture Collection (ATCC, USA) in adequate amount (80-90% of the culture bottle polystyrene substrate) was washed with 1 mL 1X PBS twice. One mL of trypsin EDTA was added to the solution and incubated for 3 minutes until the cell is released. The cells were then transferred into a Falcon tube containing 5 mL of culture medium and centrifuged at 1200 rpm for 4 min. The supernatant was discarded, the cells were resuspended with a culture medium of 1 mL, then calculated using a hemocytometer.

The viability of cells was determined with formazen by MTS assay test. The cells were planted on 96 well plates with standard curve series (6 replication) and 5000 cells per well for treatment (3 replication). The addition of 100 µL of medium was performed before the incubation in overnight temperature of 37^{o}C, 5% CO_2. The medium was then replaced with 180 µL medium, 20 µL chitosan extract, 24 hour incubation, 37^{o}C, 5% CO_2. 20 µL of MTS was added, followed by 3 hours incubation period in temperature of 37^{o}C, 5% CO_2, and absorbance was then measured at 490 nm with spectrophotometer.

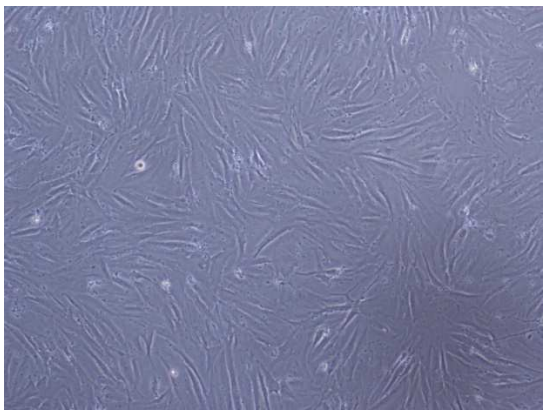

Figure 1. BJ cells (100X magnification)

The standard curve that was made by planting BJ cells with different cell counts as much as six replicates in a 96 well plate, was used to count the number of cells on day 0 (D0) planted for treatment and to count the number of cells after treatment. IC50 value counted by statistical analysis using SPSS software, to find the most adequate concentrations of chitosan solutions without toxic effect on BJ cells.

3. Result and Discussion

As mentioned before, chitosan solutions with different concentration were analyzed for their cytotoxic effects on the BJ cell line with MTS assay. The BJ cells standard curve used for calculation of regretion index values as shown in figure 2. Cells viability (y) counted using formula from BJ cells standard curves ($y = 0.0001x + 0.0238$). Results shows, the greater the concentration of chitosan solutions the greater number of death cells observed, with IC_{50} values respectively at 0.032% chitosan.

Tabel 1. Cytotoxicity Test Result

Groups	Cell counts Average	SD	Absorbance Average	SD	Viability (%) Average	SD	Inhibition (%) Average	SD
Chitosan 3%	-181	59	0.0057	0.0059	-2.20	0.72	102.20	0.72
Chitosan 2%	-153	21	0.0085	0.0021	-1.85	0.25	101.85	0.25
Chitosan 1%	-103	45	0.0135	0.0045	-1.25	0.55	101.25	0.55
Chitosan 0.3%	11	94	0.0249	0.0094	0.13	1.13	99.87	1.13
Chitosan 0.2%	131	74	0.0369	0.0074	1.58	0.89	98.42	0.89
Chitosan 0.1%	219	90	0.0457	0.0090	2.65	1.09	97.35	1.09
Chitosan 0.03%	4608	200	0.4846	0.0200	55.90	2.42	44.10	2.42
Chitosan 0.02%	5947	260	0.6185	0.0260	72.13	3.16	27.87	3.16
Chitosan 0.01%	8001	312	0.8239	0.0312	97.05	3.79	2.95	3.79
Acetic Acid 2%	-152	66	0.0086	0.0066	-1.85	0.80	101.85	0.80
Acetic Acid 0. 2%	-66	21	0.0172	0.0021	-0.80	0.26	100.80	0.26
Acetic Acid 0.0 2%	7192	214	0.7430	0.0214	87.23	2.60	12.77	2.60
Medium (Acetic Acid 0%)	8244	559	0.8482	0.0559	100.00	6.78	0.00	6.78

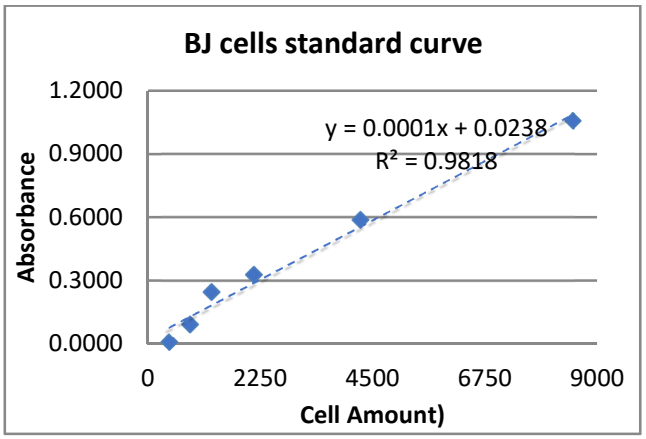

Figure 2. BJ cells standard curve

Photographic observation of cytotoxic test of chitosan on BJ cell line in 96 well plate (5000 cells/well) shows that at 3%, 2%, and 1% chitosan in 2% acetic acid, the cells were not visible due to chitosan particles that precipitate and cover the base of flask. At 2% acetic acid, cells were clearly visible. As seen in figure 3. The number of cells looked less than before treatment. This showed that 2% acetic acid was toxic to BJ cells.

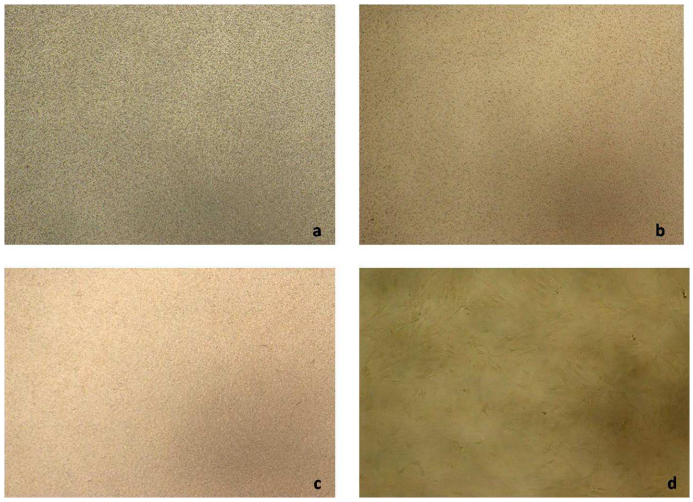

Figure 3. a, b, c: Cells at 3%, 2%, and 1% chitosan in 0.2% acetic acid; d: cells at 2% acetic acid

At 0.3%, 0.2%, and 0.1% chitosan in 0.2% acetic acid the cells were visible in a limited amount. At 0.2% acetic acid more cells were visible compared to 2% acetic acid as seen in figure 4.

At 0.03%, 0.02%, and 0.01% chitosan in 0.02% acetic acid, cells were clearly visible with morphologic changes. At 0.02% acetic acid cells were also visible without morphologic changes. Where as at control group (without chitosan and acetic acid) more cells were visible compared to before treatment as seen in figure 5.

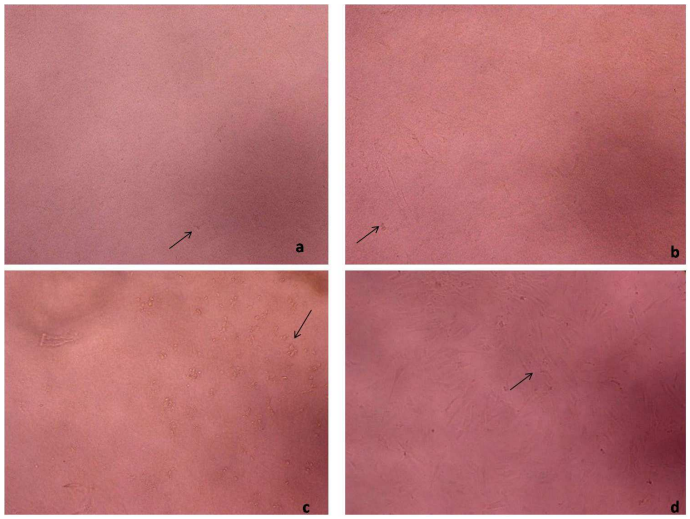

Figure 4. a, b, c: Cells at 0.3%, 0.2%, and 0.1% chitosan in 0.2% acetic acid; d: cells at 0.2% acetic acid

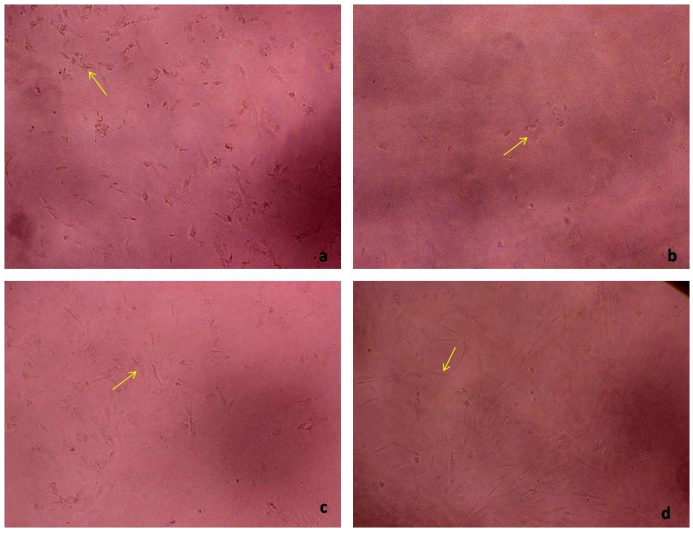

Figure 5. Cells at 0.03%, 0.02%, and 0.01% chitosan in 0.02% acetic acid; cells at 0.02% acetic acid

Toxicity tests are designed with the following elements: (1) a test organism (cells), (2) a specific response or biological endpoint, (3) an exposure or test period, and (4) a dose or a dose curve. There are several pathways through which harmful substances are likely to enter the body. Denture cleansers may enter the body through ingestion. Ingestion of potentially harmful substances is not as great a concern as is exposure through skin and lungs. [4]

Cytotoxicity is the property of a compound that causes toxic effects on cells. Cytotoxic compounds mainly cause carcinogenic, mutagenic, and teratogenic effects. Cytotoxicity test used in this study was based on BJ cell (normal skin cell) viability with MTS assay. MTS assay is one of proliferation test based on cell viability by observing cell metabolic activity. MTS test principal is to indirectly measure viabel cell product.

This study has shown that chitosan solution on acetic acid has potential cytotoxic effects on BJ cells. Cytotoxicity test of chitosan on BJ cells showed that chitosan 1%, 2%, and 3% in 2% acetic acid, and chitosan 0.1%, 0.2%, and 0.3% in 0.2% acetic acid had toxic effect on BJ cells in a 24-hour incubation period. Chitosan with concentration of 0.01%, 0.02%, and 0.03% in 0.02% acetic acid caused 3%, 27%, and 44% cell death respectively. Compared with the solvent-only group (2% acetic acid, 0.2% acetic acid, and 0.02% acetic acid), we concluded that the chitosan toxicity in acetic acid were mainly caused by the acetic acid. Acetic acid in a concentration of 0.2% was still toxic to BJ cells

4. Conclusion

Based on the research that has been done we concluded that chitosan is cytotoxic and unsuitable to be used as removable denture cleanser solution A further research is needed using other solvents with different concentrations for chitosan besides acetic acid.

Acknowledgments

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