GENOTOXICITY OF ANTIBACTERIAL BIOGLASSES OBTAINED BY SOL-GEL METHOD FOR *SALMONELLA TYPHIMURIUM*

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Introduction

Periodontal disease causes problems in dentistry. Surgical intervention with appropriate biomaterials for tissue regeneration is necessary in advanced stages of the disease. Due to the risk of bacterial contamination during the regeneration of bone tissue in the oral cavity, studies are continually being undertaken with a view to creating new or modifying existing biomaterials and providing them with bactericidal properties [1,2].

Some of the materials that could potentially have medical uses contain substances exhibiting various types of toxicity. One of them is genotoxicity. It consists in causing the mutations – permanent, inheritable changes in hereditary substance (deoxyribonucleic acid - DNA). Some mutations can induce cancer, namely carcinogenesis. Therefore, before the introduction of new medical materials for widespread applications, they are examined to determine the genotoxic properties. The Ames test is one of the many bioassays for identifying the mutagenic activity of tested specimens [3,4].

Therefore, the aim of this study was to determine the mutagenicity of B-I calciumsilicate as well as Z-5 and Z-8 aluminosilicate bioglasses for *Salmonella typhimurium* in the Ames test.

Materials and Methods

The study involved bioglasses (TABLE 1) obtained by the sol-gel method using tetraethyl orthosilicate substrates as a silica precursor and aluminum isopropoxide, nitrate tetrahydrate calcium, triethyl phosphate and silver nitrate. The physicochemical properties e.g. grain morphology and the cytotoxicity and antibacterial potency of these bioglasses are known from earlier reports [5-7].

TABLE 1. The oxide compositions of tested bioglasses.

bioglass	content, wt %				
	SiO_2	Al_2O_3	CaO	P_2O_5	Ag_2O
Z-5	95,7	0,8	-	-	3,5
Z-8	89,0	7,5	-	-	3,5
B-I	60,0	-	37,0	2,0	1,0

Extracts of bioglasses were introduced into the test as solutions in DMSO. They were partially diluted to obtain B-I and Z-8 bioglass doses of (0,25, 0,5, 1, 2, 4, 8) mg/cm^3 of the mixture during exposure and a Z-5 bioglass dose of (0,125, 0,25, 0,5, 1, 2, 4, 8) mg/cm^3. The test bacteria was exposed to six dilutions of test samples for 90 minutes in a 24-well microplate in three replicates for each dilution.

The genotoxicity of bioglasses were provided by the Ames Xenometrix by Endotell microplate test using TA 98 and TA 100 *Salmonella typhimurium* strains (Ames MPF^{TM} 98/100). Tests were carried out with and without metabolic activation of 30% rat liver S9 microsomal fraction. Tests were performed according to the procedure described in the manufacturer's instructions; the result was positive when the number of holes containing revertants was at least three times greater than the negative control.

An Excel spreadsheet provided by the manufacturer of the test was used for statistical analysis. The statistical significance of the differences in the number of revertants between test samples and negative controls were studied in a unilateral t-test and were considered significant at $p \leq 0.05$. According to the procedure, the test results were considered reliable because the average number of positive holes (from the revertant) did not exceed 8 in the negative control for a TA 98 and 12 for the *Salmonella typhimurium* strain, and did not exceed 25 for the TA 100 *Salmonella typhimurium* strain in the positive control.

Results and Discussion

B-I calciumsilicate bioglass showed mutagenic activity against the TA100 strain with metabolic activation of the S9 fraction, and did not demonstrate mutagenic activity against the TA100 strain without metabolic activation or the TA98 strain with and without metabolic activation. They did not include direct mutagens causing base-substitution mutations and direct and indirect mutagens caused frame-shift mutations, the detection of which allows the TA98 strain. In the B-I bioglass could remain traces of substrates used to make it. Some of them cause the formation of different mutations [8-12]. The collected literature data indicate that reversion of mutations in the TA 100 strain in the presence of S9 caused by B-I bioglass could be a consequence of the combined effect of its components and the substrates remains.

Z-5 and Z-8 aluminosilicate bioglasses did not exhibit mutagenic activity applied to the *Salmonella typhimurium* strains tests with and without metabolic activation of S9 fraction in the tested concentrations.

Conclusions

The results indicated that the presence of intermediate mutagens in the B-I calciumsilicate bioglass cause base-substitution mutations.

The Z-5 and Z-8 aluminosilicate bioglasses did not exhibit mutagenic activity against TA 98 and TA 100 *Salmonella typhimurium* in the Ames test, with and without metabolic activation. This means that they do not cause frame-shift and base substitution mutations, which the applied strain allows for detection. Thus, there should be further study of Z-5 and Z-8 bioglasses prior to their clinical application.

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