

Is there a connection between synthetic bone grafts and sisters chromatide exchange?

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ABSTRACT

Background: In oral and maxillofacial surgery, synthetic bone grafts are most widely used as bone substitutes, due to the limited sources of autologous bone. The aim of this study was to examine the influence of three different synthetic bone grafts (Cerasorb, Fortoss and Perioglass) on sisters chromatide exchanges (SCEs) in peripheral lymphocytes. **Materials and Methods:** Peripheral blood samples taken from 68 patients (45 females and 23 males), who underwent oral surgery procedures, such as apical resection, cyst enucleation or periodontal curettage, were obtained for SCE a day before and two months after the surgeries. A control group included 30 patients, while the study group was made of the patients who underwent bone grafting with Cerasorb[®] (11 patients), Fortoss[®] VITAL (10 patients) or Perioglass[®] (17 patients). **Results:** Comparing with the results of the study group before and after the treatment, it was concluded that the results were statistically significant ($p = 0.001$). In the Perioglass[®] subgroup, a greater statistical significance ($p = 0.003$) was noted, than that in either the Cerasorb[®] ($p = 0.620$) or Fortoss[®] ($p = 0.210$) subgroups, in which there was no statistical significance. **Conclusions:** Although further investigations may be necessary, our results suggest that the synthetic bone grafts might have an influence on SCE in peripheral lymphocytes.

Keywords: Bone Graft; Sisters Chromatide Exchange; Genotoxic; Bone Defect

1. INTRODUCTION

Bone grafts present different spectrum of organic and

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synthetic materials that are necessary to provide structural stability and linkage by stimulating osteogenesis, which leads to biological repair of skeletal defects.

In oral and maxillofacial surgery, the use of bone grafts is limited to reconstruction of bone defects after trauma, cyst or tumor removals in implant and periodontal surgery. The gold standard of bone grafts is considered to be an autologous bone (bone harvested from the patient's own body), due to the retaining of cell viability [1,2]. However, limited autograft quantities and biologic performances, extra donor site surgery including morbidity, as well as infection risk and other complaints, have brought some other materials in use such as alloplasts [1,3-5]. These synthetic materials are biocompatible, resorbable, easily and rapidly replaceable by authentic bone tissue [1,6,7]. The resorption rate is synchronous with bone remodelling [1]. Some of these materials are bioactive glasses and calcium phosphates which can be effective for reparation of bone defects in orthopedic and maxillofacial surgery.

Bioactive glasses are composed of SiO_2 , CaO , Na_2O , P_2O_5 and bond to bone through the development of a surface layer of carbonated hydroxylapatite (HA) [3,8,9]. Some other authors suggest that the mechanism of activity of Bioactive glasses have an effect of cortical concentrations of soluble Si, Ca, P and Na ions, inducing favorable intracellular and extracellular responses, leading to rapid bone formation [8]. One of representatives of these materials is PerioGlass[®] or Bioglass, which has proven to be an effective graft material owing to the apatite layer on the surface of the particles, which attracts osteoprogenitor cells and osteoblasts, thus stimulating bone formation [3,10]. This material is not only a osteoconductive scaffold for new bone formation, it also may act as a biological membrane retarding epithelial downgrowth [11].

Calcium phosphates are synthetic materials in the form of isolated alpha or beta-tri calcium phosphate (beta-TCP) (Cerasorb[®]) or in combination with Hydroxyl sulphate matrix (Fortoss[®] VITAL). The TCP mostly behaves as osteoconductive material, which allows bone osteoprogenitor cells to grow on its surface or in its pores, channels or pipes, and to differentiate into osteoblast, thus bringing bone deposition [12]. This phenomenon might also be called “osteostimulation”, which implies upregulation of osteoblast precursors [13]. However, due to the fact that this differentiation does not take place in an ectopic site, the phenomenon does not meet the strict standard of osteoinduction [14].

Synthetic bone grafts were a subject of various studies in which their biocompatibility and osteoinductive and osteoconductive qualities were examined [1,3,8,10,15]. So far, potential genotoxic effects caused by synthetic bone grafts are almost unknown.

Sisters chromatid exchange (SCE) is one of the methods used for examination of chromosomes and genetic damages, caused by exposure to potential environmental mutagens and carcinogens [16-19]. It represents the interchange of DNA replication at apparently homologous chromosomal loci, involving DNA breakage and reunion [18].

The aim of this study was to compare *in vivo* genotoxic effects of three commercially available synthetic bone graft materials: beta-tri calcium phosphate (Cerasorb[®]), beta-tricalcium phosphate in a hydroxyl sulphate matrix (Fortoss[®] VITAL) and bioactive glass ceramic (Perioglass[®]) by analyzing the frequency of sisters chromatid exchange of peripheral lymphocytes.

2. MATERIAL AND METHODS

The study was approved by the Istanbul University Ethics Committee for Scientific Research in Humans (NO: 2006/507). Written and oral consents were obtained from all patients.

2.1. Patient Selection

Sixty eight patients (45 females and 23 males), age range from 24 to 58 years, who were referred to the Istanbul University, School of dentistry, Department of Oral and Maxillofacial Surgery, for oral surgery procedures, such as apical resection, cyst enucleation or periododontal curettage were included into a study. All participants in the study were medically healthy, were nonsmokers, and were not taking any drugs for at least 2 months before surgery. Work histories excluded relation to chemical mutagens and/or exposure to ionizing radiation. The patients were randomly separated into a two groups. The first group was control group and included 30 patients (16 females and 14 males). The second group, subdivided into three subgroups, included 38 patients who

underwent oral surgery procedures in combination with one of the three synthetic bone grafts; Beta-tricalcium phosphate (Cerasorb[®]), Beta-tricalcium phosphate in a hydroxyl sulphate matrix (Fortoss[®] VITAL) or bioactive glass ceramic (Perioglass[®]). The Cerasorb subgroup included eleven patients (7 females and 4 males), the Fortoss[®] subgroup comprised ten patients (7 females and 3 males), while 17 patients (15 females and 2 males) were included into the Perioglass[®] subgroup. The quantity of the graft materials, used in all of the patients from the second group was 2 gr.

Following surgical procedures, the patients were under antibiotic (Amoxicillin 2000 mg/day for 7 days) and analgesic (Naproxen sodium 1100 mg/day, as needed) therapy.

2.2. Sisters Chromatid Exchange (SCE) Analysis Procedure

Sisters Chromatid Exchange (SCE) analysis was performed in the cytogenetic laboratory of the Istanbul University, School of Medicine, Department of Internal Medicine, Division of Medical Genetics.

Heparinized blood samples (3 ml) taken from all of the patients one day before and two months after the oral surgery procedures, were cultured and examined for sisters chromatid exchanges. The peripheral lymphocytes were cultured in medium containing RPMI 1640 (Biocrom KG, Berlin, Germany) supplemented with 1% phytohaemagglutinin-M (Biological Industries, Kibbutz-Beit Haemek, Israel), 20% fetal calf serum, 1% penicillin-streptomycin and 1% L-glutamine (PAA Laboratories GmbH, Pasching, Austria). At 24 hours 0.5 µg/ml 5'-bromodeoxyuridine (BrdU, Sigma Chemical Company, USA) was added to the medium and it was further incubated in the dark. Later at the 70th hours a Colchicine (0.2 µ/ml) (Colchicine powder, Sigma Chemical Co, USA) was added. Two hours later the cells were collected and treated 10 minutes with 0.075 mol/L KCl at 37°C, then fixed with methanol-acetic acid (Merck, Darmstadt, Germany) and standard harvesting procedure was performed. Slides were stained by fluorescein plus Giemsa technique (FPG). The slides were examined by a Leitz-Ortoplan microscope (100×) with respect to SCE frequency per metaphases. 30 metaphases were analyzed for each patient before and after treatment.

2.3. Statistical Analyses

Analyses in this study were performed by NCSS 2007 packet program.

Kruskal-Wallis test was used for group comparison, while Dunn's multiple comparison test was performed for subgroups comparison. For repeated-measures Wilcoxon test was used. The results were evaluated in a con-

fidence interval of 95% and $p < 0.05$ was considered statistically significant.

3. RESULTS

A blood samples from 68 patients (separated into control and experimental groups), who underwent one of oral surgery procedures in combination with synthetic bone grafts or without, were evaluated for sisters chromatid exchange a day before and two months after the surgery. Evaluation of results in control and working groups, before and after the surgeries have showed no statistical significance ($p = 0.501$ and $p = 0.209$). The mean SCE frequencies before the surgeries, in patients from control group, Cerasorb[®], Fortoss[®] and Perioglass[®] subgroups were 7.06 ± 1.54 , 7.41 ± 0.62 , 6.95 ± 0.87 and 7.47 ± 0.78 respectively, while these values after the surgery were 7.06 ± 1.54 , 7.32 ± 0.72 , 7.08 ± 0.71 and 7.78 ± 0.83 , respectively.

The results from the Cerasorb[®] ($p = 0.620$) and Fortoss[®] ($p = 0.210$) subgroups were not statistically significant. However, the results from the Perioglass[®] subgroup have shown a difference between blood samples taken before and after the surgeries, which were statistically significant $p = 0.003$ (Table 1). The statistical significance of $p = 0.001$ in SCE frequency before and after the surgeries, between subgroups appears in Table 2. While the comparison of the Cerasorb[®] versus Fortoss[®] and Fortoss[®] versus Perioglass[®] subgroups did not show any statistical significance ($p = 0.245$ and $p = 0.669$ re-

spectively), the Cerasorb[®] versus Perioglass[®] subgroup showed a meaningful low value of $p = 0.018$ (Table 3).

4. DISCUSSION

Sisters chromatid exchange (SCE) of peripheral lymphocytes presents one of cytogenetic methods for detecting DNA damage [16]. It is well known that different medications, chemicals, viral infections, malignant diseases, ultraviolet light and smoking are the most common factors inducing SCE [17-19]. Because of the mentioned, in our study the selection of the patients was limited to those individuals, who'se medical histories excluded these factors.

Using the sisters chromatid exchange method, mutagenetic and carcinogenic potentials of different substances were examined [16,17,20-25]. Antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) were just some of the most investigated subjects. Various studies in vitro and vivo deal with different results and conclusions. Xie *et al.* [21] suggest that Tetracycline induce significant increases in SCE even at the lowest concentrations, while, according to Istifli & Topaktaş [20] and Jaju *et al.* [22], Amoxicillin does not behave genotoxic, with recommendation for safe usage in cases of bacterial infections. Also the metabolites of Amoxicillin do not have a significant effect on cell proliferation [20]. Benzathine penicillin G (BPG) might also be considered as the "safe" antibiotic for a short-term exposure [23], although in cases of a long-time exposure, SCE

Table 1. Results in controle and working groups, before and after the surgeries.

	Cerasorb [®] subgroup	Fortoss [®] subgroup	Perioglass [®] subgroup	Control group	KW	P
SCE before procedure	7.41 ± 0.62	6.95 ± 0.87	7.47 ± 0.78	7.06 ± 1.54	2.36	0.501
SCE two months later	7.32 ± 0.72	7.08 ± 0.71	7.78 ± 0.83	7.06 ± 1.54	4.63	0.209
Z	-0.48	-1.24	-3,01	-		
p	0.620	0.210	0,003	-		

Table 2. Results show statistically significance in SCE frequency before and after the surgeries.

	Cerasorb [®] subgroup	Fortoss [®] subgroup	Perioglass [®] subgroup	KW	p
SCE Difference	-0.09 ± 0.49	0.13 ± 0.32	0.31 ± 0.34	16.5	0.001
SCE % Changes	-1.51 ± 6.56	2.02 ± 5.01	3.86 ± 4.2	16.7	0.001

Table 3. Results obtained by Dunn's multiple comparison test.

Dunn's multiple comparison test	Difference	% Changes
Cerasorb[®]/Fortoss[®]	0.378	0.245
Cerasorb[®]/Perioglass[®]	0.018	0.018
Fortoss[®]/erioglass[®]	0.496	0.669

frequency changes might be expected [24].

Non-steroidal anti-inflammatory drugs (NSAIDs) include variety of chemicals with analgesic, antipyretic and anti-inflammatory effects [16]. In one of our previous studies, we examined genotoxic effects of the etodolac, nimesulid and naproxen sodium, and concluded that short term use of selective and non-selective NSAIDs was not associated with a significant genotoxic effect that could be detected using the SCE method in peripheral lymphocytes [16]. However, findings from Özkul *et al.* [25], although statistically insignificant were opposite to ours in case of naproxen sodium.

The patients included in our study were under antibiotic and analgesic therapy after the surgeries. Following the results of previously mentioned studies, Amoxicillin 2 gr/day (for 7 days) and Naproxen sodium 1100 mg/day (as needed) were prescribed. Due to the fact that all of the patients were under the same therapy, with changes of SCE frequency in working subgroups, the results of the study suggests that synthetic bone graft materials might be genotoxic.

The results before and after the treatment have shown the statistical significance ($p = 0.003$) in the Perioglass[®] subgroup. Also, multiple subgroup comparison tests were statistically significant $p = 0.001$, while comparison between subgroups have shown that a meaningful low value was in the Cerasorb[®] versus Perioglass[®] subgroup ($p = 0.018$). This finding suggests, that the bone augmentation with synthetic bone grafts might have an influence on the sisters chromatid exchange frequency.

Although studies of bone grafts are limited to examinations of their biocompatibility, osteoinductive and osteoconductive qualities by radiographic, histologic or histomorphometric analysis, a review of the English literature suggests that this could be the first study investigating a genotoxic influence of the synthetic bone grafts by sisters chromatide exchange method. Due to the fact that the examined materials in the previous studies have shown good results as the bone substitutes [26,27], we wonder whether the resorption rate of the materials play a role in their genotoxicity. A TCPs show bioresorbability during bone regeneration and completely substitute for a bone tissue after bone stimulation, while hydroxyl apatite (HA) remains in the body for a long time after replantation, due to a low grade of bioresorbability [26]. Low grade of resorption is also characteristic of bioactive glasses, where radiographic density and volume are more expressed in bone sites treated by these materials [27]. These senses might explain a meaningful low values ($p = 0.018$) in the Cerasorb[®] (TCP) versus Perioglass[®] (bioactive glass) and Cerasorb[®] versus Fortoss[®] (TCP + HA) ($p = 0.245$), as compared with the results with the Fortoss[®] versus Perioglass[®] subgroups ($p = 0.669$).

Although this is just hypothesis, because we did not look at the resorption rate of the materials, we are of the opinion that the bioresorption of the synthetic bone substitutes did not have any influence on sisters chromatide exchange frequency, due to the fact that the blood samples were taken two months after the bone augmentation, which is not long enough a period for the graft replacement with the authentic bone tissue.

We hope that with these findings, we will open a gate to some other researchers and studies that will examine the genotoxic influence of other bone synthetic substitutes.

5. CONCLUSION

Although further investigations may be necessary, our results suggests that some alloplasts such as bioactive glass ceramic might have an influence on SCE in peripheral lymphocytes.

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