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Evaluation of the Effect of Calcium Silicate Phosphate, Osteon, and Bio-Oss on Cell Viability and Cell Morphology of Human SaOS-2

Sadatmansouri S[1], Ayubianmarkazi N[2], Aghajeri S[3], Delaram MR[3], Iranpour B[3]

[1]Associated professor, Department of periodontology and member of implant research center, Dental branch of Tehran , Islamic Azad University, Tehran, Iran.
[2]Associate professor, Department of periodontology, Dental branch of Tehran, Islamic Azad University, Tehran, Iran.
[3]Periodontist.

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ABSTRACT

Background and Aim: Several reports have been published on the successful application of various bone substitute materials (BSM). Appropriate physiologic and histologic characteristics and reactions of these materials against host cells are critically important. In this study, the biocompatibility of a new bone substitute material has been evaluated.

Methods and Materials: In this experimental In vitro study, the biocompatibility of silicate calcium phosphate, Bio-Oss and Osteon were compared by evaluation of cell viability and differentiation rate of human osteoblast-like cell line (SaOS-2). No graft material was used in the control group. Cell viability rate was evaluated by MTT test after 1, 3 and 14 days of incubation. Inverted Light Microscope and SEM were utilized for evaluation of cell morphology. MTT and cell morphology were analyzed by ANOVA test in all groups.

Results: Cell viability of the control group equaled 0.453±0.016, while in the test groups it equaled 0.453±0.016 for Bio-Oss, 0.439±0.011 for Osteon and 0.425±0.026 for silicate calcium phosphate. There was no significant difference between the control and the test groups. Spindle form was the dominant SaOS-2 cell morphology in all groups.

Conclusion: This study showed that calcium silicate phosphate has appropriate biocompatibility comparable with that of Bio-Oss and Osteon.

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***Corresponding author:**
Nader Ayubianmarkazi
Email: naderayubian@yahoo.com
Tel: 9121548739

Introduction:

Bone substitute materials have been successfully used for bone replacement. [1] One of the most important roles of bone substitute materials is to imitate the physiologic and histologic properties of the deteriorated bone. They also interact with host osteogenic cells. A proper bone substitute material should have characteristics such as biocompatibility, the ability to be sterilized, and act as an osteoconductive agent.[2, 3] A successful bone replacement depends on survival and morphology of the existing cells which are responsible for bone material reproduction.[4, 5]

There are several sources of bone substitute materials such as auto graft, xenograft, and allograft and alloplastic materials. Auto graft bone substitute materials are considered the gold standard for grafting, but additional surgery is required in the donor site to gain bone graft material and also there is not enough substance available. [6,7,8] Moreover, there are worries about the spread of infectious disease around xenografts and allografts. Therefore, alloplastic materials have gained popularity in recent years. [9, 10] Zhau et al have introduced a new alloplastic material which is basically calcium phosphate silicate. Silicate affects the formation and repair of destructed bone. Silicate has some significant effects on bone formation especially in the presence of calcium. Some of these effects are elevation of collagen I synthesis, progression of osteoinductive gene expression, and elevation of osteogenic cells reproduction.[11, 12] In an animal study, Si-CaP was used as bone graft in sheep, and the results showed that the bone formation after bone grafting was histologically and biomechanically identical to that after the application of autogenous bone graft. [10] Since there is not enough information about the effect of Si-CaP (silicate calcium phosphate) on viability and morphology of human osteoblast cells, the aim of this study was to compare the effect of Si-CaP with that of Osteon and Bio-Oss on viability and morphology of cells in Pasture institute of Tehran, Iran.

Materials and Methods:

Cell culture

In this experimental In vitro study, human osteoblast-like cell line (Pasture institute, Tehran, Iran) was gathered for morphologic and survival rate evaluation. 84 wells of cell culture plates filled with cell solution were evaluated in 4 groups: the control group which included SaOS-2 (cells with osteoblastic phenotype for evaluating the biological compatibility of grafting materials) was added to polystyrene plates without any bone graft. The groups with Osteon (Dentium, Seoul, South Korea), Bio-Oss® (Geistlich Bio-materials, Switzerland), and silicate calcium phosphate (Actifuse Synthetic bone Graft, Apatech Limited, London, UK) were added to human osteogenic cells. SaOS-2 cells were incubated in a broth of 100 U/ml penicillin, 100 µg/ml streptomycin and 10% Dulbecco Modified Eagle Medium (DMEM) solved in 10% Fetal Bovine Serum (Gibco®, InvitrogenTM GmbH, Karlsruhe, Germany). All cells (SaOS-2 cells) were incubated with 5% CO2 at 37°C. Before the experiment, the cells were washed with Phosphate Buffer Solution (PBS) and were detached with trypsin/ Ethylenediaminetetraacetic acid (EDTA). Cell concentration in the solution equaled 2×104.[8]

Cell proliferation and viability

Cell proliferation and cell viability were evaluated by MTT assay (Methyl Thizole Tetrazolium). This test is based on evaluating the cell proliferation by reduction and breaking of Formazan blue crystals. Examination was conducted on days 1, 3 and 14. First, cells were washed with PBS and then the naked cells were immersed in MTT solution (5 mg/ml MTT). Then, the culture plates were incubated for 4 hours, and afterwards the reduction process initiated in the incubation tank. After 4 hours of incubation, the wells were emptied from MTT solution, and 1 ml of isopropile acid was added to the wells. The plates were kept at room temperature to incubate for 10 minutes. In this process, alcohol defragmented the cytoplasmic part of cells. Formazan solution released in the media produced blue color at wavelength of 630 nm. Elisa Reader (Anthos2020 Ver1.8, Antous Lab Tec Instrument, Austria) was

used to detect the wavelength radiated from each well.

Cell morphology

The cell growth and proliferation patterns were investigated by scanning electron microscopy at 1, 3, and 14 days after incubation. The cells were fixed with 2.5% glutaraldehyde for two hours and were washed three times with PBS. One percent osmium tetroxide was used for secondary fixation .After washing, dehydration of the samples was performed for 30 minutes. Subsequently, the samples were subjected to sputter-coating with gold/palladium and were examined using a Vega-TEScan (Tescan USA Inc., USA) at 20KV. [8]

Statistical analysis

The results of MTT assay were analyzed by ANOVA test in all groups. Digital counting of cells was done and cell morphology was analyzed by K2 test

Result:

This study was performed on 84 cell specimens of SaOS-2. As it is summarized in Table 1, the highest rate of cell viability after 24 hours was detected in the control group (0.453±0.016). Although the lowest amount of cell viability belonged to Silicate calcium phosphate (0.425±0.026), there was no significant difference between the experimental groups (p=0.4). The results after 72 hours were similar with the above mentioned results and there was no significant difference between the groups (p=0.4). Evaluation of the specimens after 14 days showed that higher MTT rate belonged to the control group and lower rate belonged to Osteon® but still no significant difference was detected between the groups (p=0.3).

The results showed that survival rate of all groups elevated over time, except for the Osteon group.

Table 1- Cell viability rate in the study groups according to follow-up time

Time/groups	N	After 24 hours	After 72 hours	After 14 days	RESULTS(ANOVA)
Control	15	0.453±0.016	0.485±0.015	0.468±0.030	P=0.2
Bio-Oss®	15	0.432±0.020	0436±0.013	0.463±0.025	P=0.2
Osteon®	15	0.439±0.011	0.432±0.0152	0.431±0.027	P=0.2
Silicate calcium phosphate	15	0.425±0.026	0.432±0.035	0.456±0.020	P=0.2
RESULT (ANOVA)	-	P=0.4	P=0.3	P=0.3	

Table 2- Distribution of cellular morphology in the study groups according to follow-up time.

Time	After 24 hours		After 72 hours		After 14 days	
morphology	Spherical %	spindle %	Spherical %	Spindle %	spherical %	Spindle %
Control	--	100	--	100	--	100
Bio-Oss	4.81	95.19	4.68	95.32	0.98	99.02
Osteon	4.72	95.28	5.64	94.36	7.91	92.09
Calcium phosphate silicate	6.18	93.82	5.59	94.41	2.57	97.43
K2 test	P=0.4		P=0.2		P=0.2	

According to Table 2, morphological cell evaluation shows that the majority of cells in all groups were spindle SaOs-2 cells. Scanning Electron Microscope (SEM) images of biomaterials seeded with SaOs-2 are shown in figures 1 to 6.

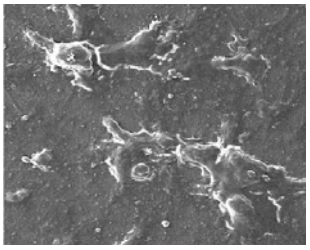

Figure-1: SEM of Bio-Oss on day 3

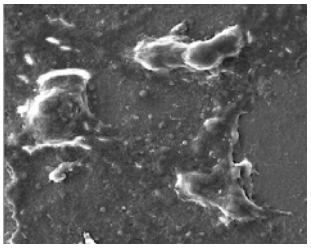

Figure-2: SEM of Bio-Oss on day 14

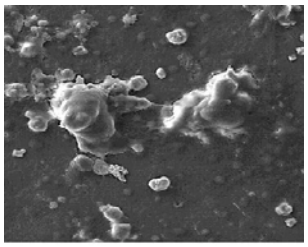

Figure-3: SEM of Osteon on day 3

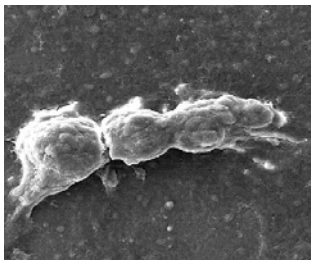

Figure-4: SEM of Osteon on day 14

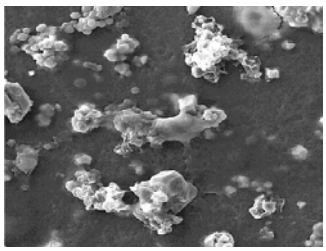

Figure-5: SEM of SCP on day 3

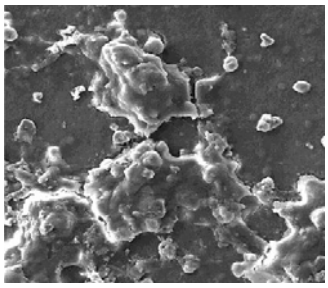

Figure-6: SEM of SCP on day 1

Discussion:

The purpose of this study was to evaluate the biocompatibility of three different alloplastic materials .SaOS-2 cell culture is mostly used for basic science investigations and clinical applied researches.[8]

The results of the present study suggest that although the highest level of cell viability at three different time intervals (1, 3, and 14 days of incubation) belonged to the control group, there was no significant difference between the control and the test groups. It can be concluded that Si-CaP can be used as an appropriate and biocompatible material like Osteon and Bio-Oss.

There are several different bone graft materials of different sources. After evaluation of cell survival rate, Trentz et al reported that there was no difference between allogeneic and xenogeneic bone graft materials. [3] These results are consistent with the findings of our study and show that there is no significant difference in cell viability of human osteoblastic cells adjacent to graft materials. However, there are limitations to the use of allograft and xenograft materials. One of the most important factors that sets limitations to the use of these sources of bone graft is the spread of infectious diseases.[3] In another study, Alcaide et al reported that the presence of HA/B-Tcp in cell culture media decreased the survival rate of osteoblast-like cells but the difference was not significant and HA/B-Tcp was shown to be biocompatible with osteoblast-like cells.[13] Saldana et al evaluated the effects of Biphasic calcium phosphate (BCP) on human mesenchymal stem cells and reported that cell viability was not affected by BCP during the study (1 to 4 days) [14]

Zhou et al evaluated the effects of synthetic silicate calcium phosphate on gingival fibroblasts in vitro and showed that this cement induced the formation of Hydroxyapatite crystals in Stimulated Body Fluid (SBF) with no toxicity, and that Si-cap had high biocompatibility with gingival fibroblasts.[12]

Ayubian et al investigated the cell viability of Osteon, Bio-Oss, Tutudent and Cerasorb as bone graft materials using MTT assay. The results showed that the highest level of cell viability was related to Tutudent followed by Osteon. [8]

Evaluation of cell morphology is important because it can demonstrate cellular tendency to join

surface. It seems that cells with elongated spindle shaped morphology and pseudo foot attach firmly to the surface in comparison with the spherical morphology .Flat cells with extended cytoplasmic projection promote bone formation adjacent to connective tissue.[8]

Ayubian et al evaluated cell morphology with SEM, and reported that in Osteon group the majority of cell population after proliferation were spindle shaped while in Bio-Oss group the majority of cells were spherical.[8]

Kubler et al reported that cells in Bio-Oss grafts were weak and unable to attach to the graft material. They believe that the plane surface of Bio-Oss Granules was incapable to attach to the cells .[15]

Schmitt et al evaluated cell morphology with SEM, and reported that in Bio-Oss group, SaOs-2 cells were flat and diffuse.[16]

In using bone graft materials, it should be considered that bone regeneration depends highly on the attachment and proliferation of osteoblasts, and also on the roughness, amount and size of porosities in the material. Another reason for the differences between the results of different studies is the pH of the surrounding environment. It seems that the release of phosphate from the graft material can inhibit cell proliferation.[8]

The results of the present study showed that Silicate calcium phosphate cement has effects on cell viability similar to those of Bio-Oss and Osteon and has acceptable biocompatibility as a graft material.

After contacting tissue fluids, Silicate calcium phosphate cement produces a two-layered silicate and phosphate gel on the surface. These layers can attract certain proteins and build a matrix for bone formation. Silicate can also generate osteoinductive gene expression in the presence of Calcium ions and can elevate collagen synthesis .[10]

Conclusion:

The results of this study showed that calcium silicate phosphate has appropriate biocompatibility comparable with that of Bio-Oss and Osteon. However, further animal studies and clinical investigations seem necessary to provide stronger evidence.

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