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Original article

INFLUENCE OF STORAGE MEDIA ON THE VITALITY OF STEM CELLS FROM APICAL PAPILLA

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ABSTRACT

Purpose: Immediate replantation is the treatment of choice for avulsed permanent teeth. Transportation of avulsed teeth to the dentist office requires storage in suitable media, thus keeping periodontal and periapical tissues viable. Effect of different storage media on periodontal cells vitality has been thoroughly researched, but there is no available data concerning SCAP. The purpose of this study is to evaluate the storage media effect on SCAP vitality.

Materials and methods: Our study includes 10 third molars of 14-17 years old patients, extracted due to orthodontic reasons. The apical papilla of those teeth is handled according to protocol, and the cell growth is maintained in DMEM. Cells are divided into 4 groups: 1-positive control group, 2- SOS Dentobox, 3 - (HBSS), 4 - negative control group. A colourimetric assay was used to determine the vital cell count in each group.

Results: Signal strength of the cells, stored in SOS Dentobox, is on average 30% weaker than the positive control group cells' signal, while the signal strength of the cells, stored in HBSS, is 65,2% weaker.

Conclusion: SOS Dentobox provides better SCAP cell vitality than HBSS.

Keywords: stem cells from apical papilla, storage media, avulsion,

INTRODUCTION:

Root development of permanent teeth takes 3 years after the eruption. During this time different factors can affect the developing tooth and can cause pulp degeneration and periapical infection. Among these factors, avulsion is associated with the highest risk for apical papilla mortality. Avulsion affects the pulp, the apical papilla, PDL and alveolar bone and it constitutes 1%- 16% of all traumatic injuries affecting permanent teeth [1]. Treatment of choice for avulsed teeth is immediate replantation, minimizing the risk of resorption after the replantation [2]. Different obstacles like the neurological status of the patient or lack of competent first aid at the place of the accident may prevent the immediate replantation. Thus, avulsed teeth are often stored extra orally for different periods of time before treatment in the dental office. This causes drying of the root surface, increasing the risk of PDL, pulp and apical papilla cell death [2].

Different factors and their interaction like patient's age, stage of root development, the diameter of the apical foramen, mechanical injuries caused by the trauma or the replantation, the type of the splint, antibiotics, contamination, extraoral time without proper storage, time to the replantation, storage media, are important and can affect clinical success [2, 3, 4, 5]. From these factors, the most important are the extraoral time, the type of storage media and the stage of root development [6].

Effect of different storage media like HBSS, coconut water, soy milk, propolis, egg white, tap water, on periodontal cell vitality is widely researched [6, 7, 8, 9]. Their effect on the cells of the apical papilla of immature teeth is unknown.

Aim

The aim of our study is to evaluate the effect of two storage media: the commercially available SOS Dentobox and HBSS, on the vitality of SCAP.

The working hypotheses are: 1) these storage media do not have a negative influence on SCAP 's vitality, 2) there is no significant difference in the count of vital cells stored in the two media.

MATERIALS AND METHODS:

Our study includes 10 third molars of 14 - 17 years old patients, extracted due to orthodontic reasons. Informed consent was signed by the patients' parents before the use of the teeth. After the extraction, the teeth were placed in Dulbecco's Modified Eagles's Medium, and they were transported within 60 minutes to the laboratory. The SCAPs were isolated by enzyme digestion

method in a solution of 3 mg/ml collagenase type I and 4 mg/ml dispase. The cells were cultured with Dulbecco's Modified Eagle's Medium, supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37 ! in 5% CO2. When the cells reached 80 % of confluence, they were subcultured, using 0.05% of trypsin. For the purposes of this study, cells from passages 3 to 5 were used. A flow cytometric identification of cell surface markers STRO-1, CD – 146 and CD – 34 was used to demonstrate the stem cell nature of these cells.

In a 96-well plate, 10 000 SCAP were cultivated for a day. The cells were divided into 4 groups based on the used storage media: group 1 – positive control group (DMEM); group 2 - SOS Dentobox; group 3 - HBSS; group 4 - negative control group.

Cells of group 2 and 3 were left dry for 15 minutes at room temperature – this is the supposed time from the avulsion accident until gaining access to storage media. After 15 minutes SOS Dentobox was added for 45 minutes to the cells of the second group and HBSS to the cells of the third group. The cells of the fourth group were left dry for an hour. After this time, the vital cell count was evaluated with the use of Cell counting kit - 8 (Sigma - Aldrich) colourimetric assay. The experiment was repeated 3 times, and the data were obtained using microplate reader Varioskan (ThermoFisher Scientific).

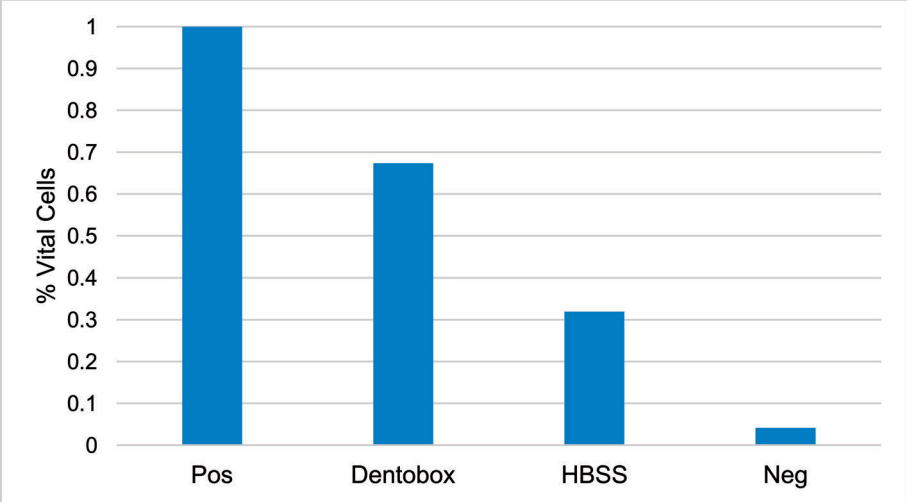
Data Analysis:

Data were analyzed with Parameter t-test and non - parametric test (Wilcoxon test) for the difference in mean values at 95% significance level, using statistical software SPSS – 17.

RESULTS:

Colorimetric assay CCK-8 was used to evaluate SCAP's vitality after incubation in transporting media. Test results show that signal strength of group 2 cells, stored in SOS Dentobox, is on average 30% weaker than the positive control group cells' signal, while the signal strength of group 3 cells, stored in HBSS, is on average 65,2% weaker (diagram 1). SOS Dentobox provides much better cell survival compared to HBSS.

Diagram 1. SCAP vitality, evaluated with CCK-8 after incubation in different storage media (HBSS, Pos - positive control group, Neg – negative control group)



The mean values of the vitality of SCAP in different groups are presented in Table 1.

Table 1. Mean values of the vitality of SCAP incubated in different storage media.

Group	Mean	Standard Deviation	Minimum	Maximum
Group 1	18361.230	358.018	13023.158	19600.983
Group 2	12365.661	1314,740	10337.686	14248.876
Group 3	5868.296	2465,848	2385.872	8216.76
Group 4	763.174	148,880	491.407	896.994

SCAP highest survival rate is observed in group 1 (positive control group m=18361.230), followed by group 2 (SOS Dentobox – m=12365.661, table 1). The average value of group 3 (HBSS) is m=5868.296 table 1. Lower vitality rate is registered in group 4 (negative control group m=763.174, table 1).

The mean values of SCAP vitality are compared in Table 2.

Table 2. Comparison of the mean values of SCAP vitality in different groups.

Group	Compared to group	Mean	Standard deviation	Standard Error Mean	95% Confidence Interval of the Difference		T	p*
					Lower	Upper		
Group 1	Group 2	5040.051	6533.117962	1308.624	2343.312	7736.789	3.857	.001
Group 1	Group 3	10126.89	2380.565843	476.1132	9144.241	11109.54	21.270	.000
Group 1	Group 4	12687.80	6022.138947	1204.428	10201.99	15173.62	10.534	.000
Group 2	Group 3	5086.839	5061.173158	1012.235	2997.690	7175.989	5.025	.000
Group 2	Group 4	7647.752	4746.930185	949.3860	5688.316	9607.189	8.055	.000
Group 3	Group 4	2560.913	4315.126621	863.0253	79.71651	4342.110	2.967	.007

**p – empirical level of statistical significance*

There is a statistically significant difference between all pairs of the four examined groups (p<0.5). This difference is most evident between group 1 and groups 3 and 4 (ð<0.000, tabl. 2) and between group 2 and group 3 and 4 (ð<0.000, Tabl. 2)

DISCUSSION

Different transport media are most often evaluated based on their capability to maintain periodontal ligament cells vitality for the maximum time, as well as their availability [10, 11, 12]. To our best knowledge, we did not find any publication depicting transport media ability to affect SCAPs vitality.

It is known that immature teeth have better chances for revascularization after avulsion accident if their apical foramen is 1 mm or more in diameter [4]. Complete revascularization is achieved in 18 % of avulsed immature teeth [13]. Mature teeth or teeth with apical foramen less than 1 mm in diameter have minimal or no chance for revascularization [14].

American Academy of Pediatric Dentistry and American Association of Endodontists recommend the use of the following transport media: Viaspan™, Hank's Balanced Salt Solution (HBSS) or cold milk [15, 16, 17]. HBSS is considered to be the golden standard for transport media for its capability to maintain periodontal ligament cells vital for up to 48 hours [10, 18, 19]. SOS Dentobox (Miradent, Germany) contains Special Cell Culture Medium (SCCM), which is a combination of amino acids, vitamins, and glucose [20]. This medium is popular in Austria, Germany and Switzerland. It has been proven in in vitro studies that this media maintains periodontal cells vital for 48 hours at room temperature [21]. This product has long shelf life: up to 3 years if it is unopened and stored at room temperature.

In specialized literature, storage media have been evaluated for their ability to maintain periodontal cells vital, and their benefit has been proven [11]. The success of replantation procedures for avulsed immature teeth depends on saving the biological potential of apical papilla cells [22]. They are a supposed source of primary odontoblasts, capable of producing root dentin [22]. High mobility, high proliferative potential and odontogenic capacity characterize them [23]. Survival of these cells during treatment of immature teeth, allows root formation to continue until full completion and closure of the apical foramen - apexgenesis [22].

Our results show that the storage media do not have a negative influence on SCAP's vitality, which proves our first hypothesis (diagram 1). This makes them appropriate and usable in case of permanent tooth avulsion.

Test results show that signal strength of group 2 cells, stored in SOS Dentobox, is on average 30% weaker than the positive control group cells' signal, while the signal strength of group 3 cells, stored in HBSS is on average 65,2% weaker (diagram 1). This disproves our second hypothesis which stated that there should be no difference in vital cells' count between materials stored in the two media. Thus, SOS Dentobox provides much higher SCAP survival rates than HBSS and this difference is statistically significant (Diagram 1, Table 2).

CONCLUSION

The storage media for avulsed immature permanent teeth should preserve the vitality of both periodontal and stem cells from the apical papilla. Our study concluded that SOS Dentobox storage media preserves SCAP's vitality better than HBSS, recommended by some guidelines. It is also relevant that this medium is more easily accessible and with a longer shelf life.

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