

# Evaluation of the Alkaline Phosphatase Level After Subcutaneous Implantation of Three Biomaterials Used in Endodontic Treatment in Prosthetics Purpose

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*During endodontic therapy, an important effect of the reparative materials used is to induce periapical recovery and stimulation of osteogenesis. The study aimed to assess the dynamics of alkaline phosphatase levels measured at different time intervals and was conducted by implanting three biomaterials in the rabbit subcutaneous connective tissue, next to the bone. The biomaterials used were: MTA (Mineral Trioxide Aggregate, Dentsply, Tulsa Dental, U.S.A.), Sealapex (Kerr, Switzerland), and DiaRoot Bioaggregate (Inovatore BioCaramixInc, Vancouver, BC, Canada). This study was focused on a quantitative analysis, based on biochemical examination.*

*Keywords: alkaline phosphatase, osteogenesis, biomaterials, endodontic treatment, stomatognathic system homeostatis*

An important effect in the endodontic therapy is to induce periapical repair and to stimulate osteogenesis in the periradicular bone [1, 2]. In this respect, the biomaterials are placed in close contact with both soft and hard periodontal tissues [3, 4]. The biomaterial may cause local or systemic side effects due to direct contact or through microleakage of the substances released from the implanted material in the periodontal tissue or alveolar bone [5, 6].

The biological compatibility of the root canal sealers is highly important, given that in clinical conditions these biomaterials are placed in direct contact with living tissues [7-9], and the tissue response to these materials may influence the final result of the endodontic treatment [10, 11].

## Experimental part

### Materials, method and equipment

Considering the above mentioned aspects, the ideal endodontic repair and sealing materials should combine several proprieties: to adhere to the root canal or cavity, forming a tight seal in the root canal system [12], to be nontoxic, well tolerated by the periradicular tissue [13, 14], to promote healing to be radiopaque, nonresorbable, not to be affected by the presence of moisture [15, 16], to be an electron-chemical indicative and not to stain the surrounding tissues [17]. The used materials were:

- MTA (Mineral Trioxide Aggregate, Dentsply, Tulsa Dental, U.S.A.) is a material with a highly efficient antibacterial effect, alkaline, made of calcium hydroxide -  $\text{Ca}(\text{OH})_2$ , bismuth oxide -  $\text{Bi}_2\text{O}_3$ , calcium sulfate  $\text{CaSO}_4$ , tricalcium silicate -  $(\text{CaO})_3\text{SiO}_2$ , dicalcium silicate -  $(\text{CaO})_2\text{SiO}_2$ , tricalcium aluminate  $(\text{CaO})_3\text{Al}_2\text{O}_3$ .

- SealApex (Kerr, Switzerland) - used for root canal sealing - has the following chemical composition: barium

sulfate -  $\text{BaSO}_4$ , titanium dioxide -  $\text{TiO}_2$ , zinc oxide -  $\text{ZnO}$ , calcium hydroxide -  $\text{Ca}(\text{OH})_2$ , butilbenzen -  $\text{C}_{10}\text{H}_{14}$ , sulfonamide -  $\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{SO}_2\text{R}$ , zinc stearate -  $\text{C}_{36}\text{H}_{70}\text{O}_4\text{Zn}$ .  
- DiaRoot Bio Aggregate (Inovatore BioCaramixInc, Vancouver, BC, Canada) - material similar in structure with MTA that additionally contains ceramic nanoparticles. It has proven antiseptic proprieties and at the same time it stimulates cementogenesis. The chemical composition is: calcium silicate, calcium hydroxide, hydroxyapatite, tantalum oxygen -  $\text{Ta}_2\text{O}_5$ .

The endodontic materials were prepared according to the instructions of the producer and then were introduced in polyethylene tubes, 10mm length and 1.5mm diameter.

### In vivo experiment

In order to assess the comparative biochemical response of living tissues to the materials used, twenty-one Belgian Giant rabbits, aged 4 months and weighing 3.5kg ( $\pm 50\text{g}$ ), raised and fed in identical conditions (food and water *ad libitum*) were used. The rabbits were divided into 4 groups:

- Group A - 6 rabbits - receiving MTA implants
- Group B - 6 rabbits - receiving SealApex implants
- Group C - 6 rabbits - receiving DiaRoot implants
- Group D (control) - 3 rabbits - receiving empty polyethylene tube implants

The experimental period lasted 60 days, while the rabbits were kept in similar conditions and were fed identical food, except 24 h before dental implant surgery when they got water but no food. All rabbits were fed a normal diet until the end of the study.

### Surgical and postoperative protocol

The surgical interventions were made under general anesthesia and aseptic conditions. Prior to anesthesia,

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**Table 1**  
VALUES OF ALKALINE PHOSPHATASE AT 7 DAYS POSTOPERATIVELY (iu/L)

		Sealapex	MTA	BioAggregate
1	163.6	164.3	176.5	182.4
2		168.8	178.4	180.1
3		165.6	181.3	179.8
4		167.4	179.5	182.3
5		169.1	180.2	183.2
6		168.3	177.8	183.8

**Table 2**  
VALUES OF ALKALINE PHOSPHATASE AT 30 DAYS POSTOPERATIVELY (iu/L)

		Sealapex	MTA	BioAggregate
1	163.6	181.3	196.5	199.4
2		173.9	198.4	193.1
3		180.6	189.8	196.8
4		188.9	191.5	195.3
5		178.2	188.9	194.5
6		179.7	187.6	197.3

**Table 3**  
VALUES OF ALKALINE PHOSPHATASE AT 60 DAYS POSTOPERATIVELY (iu/L)

		Sealapex	MTA	BioAggregate
1	163.6	162.8	164.2	164.8
2		163.4	165.4	164.1
3		160.2	164.6	166.3
4		162.1	165.7	163.2
5		163.4	166.9	165.9
6		161.7	166.7	165.7

rabbits received atropine premedication (0.02 mg/Kg; Atropina, Pasteur Institute, Romania). Anesthesia was induced with xylazine (0.1mg/kg i.m, XylazineBio 2%, Bioveta, Czech Republic) and ketamine (10mg/kg i.m., Ketaminol® 10, Intervet International GmbH, Germany). Preoperatively, the lateral thoracic regions were shaved and disinfected with antiseptic solution 96% (Videne; Adams Healthcare Ltd., UK).

The rabbits were first placed in the left and then in the right lateral decubitus position.

Skin incisions were made for implanting the tubes, which were inserted as deep as the created tissue pocket allowed, next to the scratched bone. In the end the surgical wound was sutured with non-absorbable suture thread. For 3 postoperative days, each rabbit was given an analgesic (carprofen 4mg×kg<sup>-1</sup> i.sc; Rimadyl®, Pfizer, UK) and prophylactic antibiotic (7.5 mg×kg<sup>-1</sup> amoxicillin; VEYX® YL LA 200, Veyx-Pharma GmbH, Germany). The photographs were done with Leica Application Suit Software (LAS) version 4.2.

Preoperative blood samples were taken, aiming to assess that dynamic processes of bone formation parameters for each animal. The values for alkaline phosphatase were then evaluated.

### Results and discussions

The following analysis presents the biochemical profile of implanted animals at 7 days (fig. 1) postoperatively for alkaline phosphatase, reported at surgery with initial biochemical parameters in all analyzed cases, corresponding to the three materials (table 1, 2 and 3). The baseline value in control rabbits = 163.6iu/L. Below is presented the detailed evolution of alkaline phosphatase for each biomaterial used in this study, in view to highlight the changes in their dynamic values (figs. 2-4)

Mineral Trioxide Aggregate (MTA) has been recommended as a repair material for root perforations long time ago, being considered the material of choice for the repair of root perforation, because its high biocompatibility with the periradicular tissue [19]. Sealapex is a calcium hydroxide-based sealer commonly used in endodontics, both its biostimulating properties and cytotoxicity in contact with the periradicular tissues being extensively discussed in the literature [15]. The cytotoxicity of bioaggregate (BA), the third material used in the study, showed no statistically significant difference compared to MTA in all experimental time periods ( $p > 0.05$ ) [18, 19].

After analyzing the biochemical profile of implanted animals at intervals set at 7 days, 30 days and 60 days after surgery, the results showed increases in alkaline phosphatase values reported within 7 days at operation, growth that is amplified up to 30 days, thus reaching significantly higher levels than the control group. After 30 days postoperatively, alkaline phosphatase values decreased significantly, the results showing the effectiveness of treatment in all three cases studied (fig. 1 and table 5). The baseline value in control rabbits = 163.6iu/L.

The above analysis clearly highlights that the largest increases recorded for alkaline phosphatase (AP) were found in the cases with BioAggregate but even in this case, after 60 days the values normalize, reaching values comparable to those recorded in the control group. For the MTA increases are slightly lower than those reported for BioAggregate but higher compared with the corresponding Sealapex, the latter showing the lowest values in the three studied materials. Homeostasis specific stomatognathic system is achieved by its morphological and functional balance between the components through specific mechanisms [14, 16].

Implanted material	Evaluation moment	Average AP	Average		Std dev.	Std Er.	Min	Max	Med
			-95%	+95%					
Sealapex	7 days	167.25	165.24	169.26	1.92	0.78	164.30	169.10	167.85
Sealapex	30 days	181.18	171.41	190.94	6.14	3.07	173.90	188.90	180.95
Sealapex	60 days	163.10	159.29	166.91	0.42	0.30	162.80	163.40	163.10
MTA	7 days	178.95	177.13	180.77	1.73	0.71	176.50	181.30	178.95
MTA	30 days	193.80	186.97	200.63	4.29	2.15	189.80	198.40	193.50
MTA	60 days	164.80	157.18	172.42	0.85	0.60	164.20	165.40	164.80
BioAgg.	7 days	181.93	180.22	183.65	1.63	0.67	179.80	183.80	182.35
BioAgg.	30 days	196.15	191.94	200.36	2.65	1.32	193.10	199.40	196.05
BioAgg.	60 days	164.45	160.00	168.90	0.49	0.35	164.10	164.80	164.45
Control		163.60					163.60	163.60	163.60

**Table 4**  
STATISTICAL INDICATORS OF ALKALINE PHOSPHATASE (AP) IN THE STUDY GROUP ACCORDING TO THE BIOMATERIAL USED AND THE DETERMINATION MOMENT

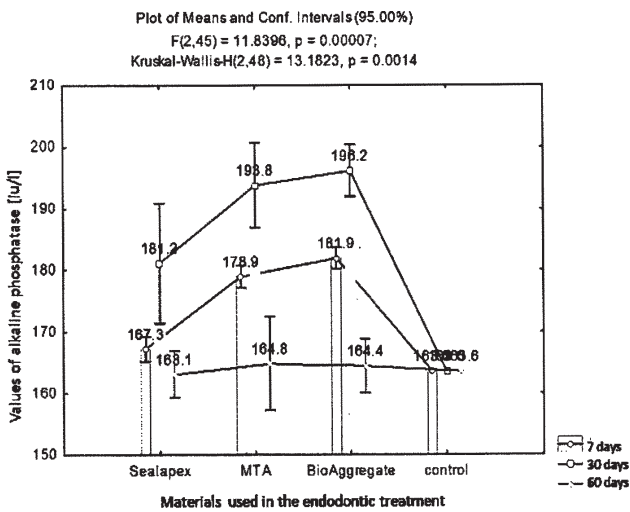


Fig. 1. Statistical indicators of alkaline phosphatase according to the assessing moment and material used

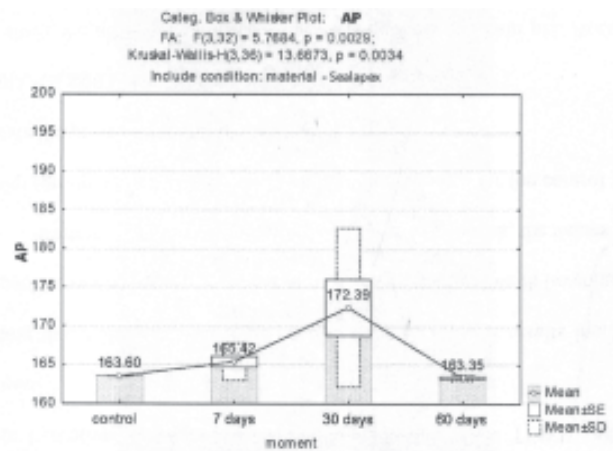


Fig. 2. Statistical indicators of alkaline phosphatase (AP) according to the assessing moment for Sealapex

Akalkine phosphatase	F (95% confidence interval)	p
ANOVA Test	87.91336	0.000000

**Table 5**

TEST FOR THE ALKALINE PHOSPHATASE COMPARISON ACCORDING TO THE ASSESSING MOMENT AND MATERIAL USED

Alkaline phosphatase SEALAPEX	F (95% interval of confidence)	p
ANOVA Test	5.768362	0.002853

**Table 6**

TEST FOR THE ALKALINE PHOSPHATASE COMPARISON ACCORDING TO THE ASSESSING MOMENT FOR SEALAPEX

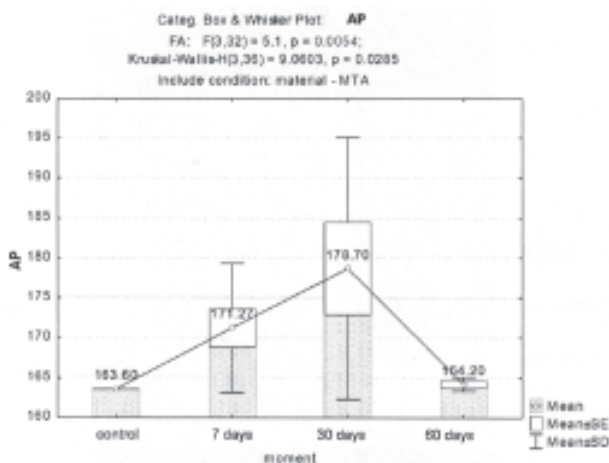


Fig. 3. Statistical indicators of alkaline phosphatase (AP) according to the assessing moment for MTA

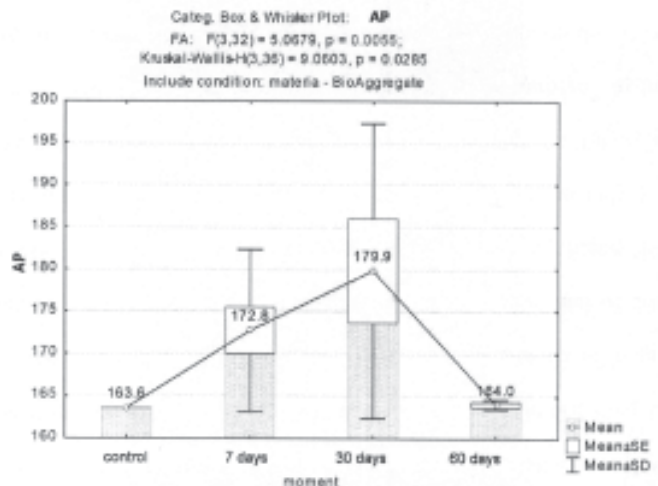


Fig. 4. Statistical indicators of alkaline phosphatase (AP) according to the assessing moment for BioAggregate

Alkaline phosphatase MTA	F (95% interval of confidence)	p
ANOVA Test	5.099973	0.005354

Alkaline phosphatase BioAggregate	F (95% interval of confidence)	p
ANOVA Test	5.067879	0.005522

**Table 7**  
TEST FOR THE ALKALINE PHOSPHATASE COMPARISON  
ACCORDING TO THE ASSESSING MOMENT FOR MTA

**Table 8**  
TEST FOR THE ALKALINE PHOSPHATASE COMPARISON  
ACCORDING TO THE ASSESSING MOMENT FOR BIOAGGREGATE

## Conclusions

In conclusion, it is noted that for all materials used, after 30 days the alkaline phosphatase values decreased significantly, reaching at 60 days no significant differences from the control group.

The fact that the absolute values of alkaline phosphatase show, in our study, an increased level in all implanted cases (more pronounced in the MTA and BioAggregate), shows that alkaline phosphatase was an effective indicator of bone formation which takes place after the material implantation next to it, with the highest values at 30 days postoperatively. This translates - in the absence of other existing associated pathologies - through an intense reparative and restorative bone activity in the body.

## References

- BELTES, P., KOULAOUZIDOU, E., KOTOULA, V., KORTSARIS, A.H., *Endod. Dent. Traumatol.*, **11**, no. 5, 1995, p. 245.
- BARRETO, H., FALCÃO-FILHO, L., ISSA, J.P.M., DO NASCIMENTO, C., IYOMASA, M., REGALO, S.C.H., SIÉSSERE, S., PEDRAZZI, V., PITOL, D.L., *Int. J. Morphol.*, **25**, no. 4, 2007, p. 789.
- MAGGI MAIA SILVEIRA, C., SOUZA PINTO, S.C., DE ARRUDA MOURA ZEDEBSKI, R., SANTOS, F.A., PILATTI, G.L., *Braz. Dent. J.*, **22**, no. 1, 2011, p. 14.
- BADILA, A., RADULESCU, R., NINULESCU, C., BOLOCAN, A., CONSTANTINESCU, S., PADURARU, D.N., *Mat. Plast.*, **49**, no. 2, 2012, p. 81.
- CAMILLERI, J., MONTESIN, F.E., PAPAIOANNU, S., McDONALD, F., PITT FORD, T.R., *Int. Endod. J.*, **37**, 2004, p. 699.
- STOLERIU, S., IOVAN, G., PANCU, G., GEORGESCU, A., SANDU, A.V., ANDRIAN, S., *Rev. Chim.(Bucharest)*, **63**, no. 11, 2012, p. 1120.
- CINTRA, L.T.A., de MORAES, I.G., BERNABÉ, P.F.E., GOMES-FILHO, J.E., BRAMANTE, C.M., GARCIA, R.B., BERNARDINELLI, N., *J. Endod.*, **32**, 2006, p. 556.
- PETRESCU, H.P., BERCEANU-VADUVA, M., DINU, G., BRATU, D.C., BERCEANU-VADUVA, D., *Mat. Plast.*, **50**, no. 1, 2013, p. 53.
- CERNAT, R.I., MOCANU, R.D., POPA, E., SANDU, I., OLARIU, R.I., ARSENE, C., *Rev. Chim. (Bucharest)*, **61**, no. 11, 2010, p. 1130.
- ECONOMIDES, N., KOTSAKI-KOVATSI, V.P., POULOPOULOS, A., KOLOKURIS, I., ROZOS, G., SHORE, R., *J. Endod.*, **21**, 1995, p. 122.
- CHECHERITA, L., FORNA, N.C., MACOVEI SURDU, A., RACOVITA, S., FILIP, F., CHIRIAC, A., *Rev. Chim. (Bucharest)*, **64**, no. 11, 2013, p. 1312.
- GENCOGLU, N., SENER, G., OMURTAG, G.Z., TOZAN, A., USLU, B., ARBAK, S., HELVACIOGLU, D., *Acta Histochem.*, **112**, 2010, p. 567.
- da SILVA, P.T., PAPPEN, F.G., SOUZA, E.M., DIAS, J.E., BONETTI FILHO, I., CARLOS, I.Z., LEONARDO, R.T., *Braz. Dent. J.*, **19**, 2008, p. 228.
- CHECHERITA, L., BELDIMAN, M.A., STAMATIN, O., FOIA, L., FORNA, N.C., *Re. Chim. (Bucharest)*, **64**, no. 8, 2013, p. 864.
- HANGANU, S.C., ARMENCIA, A.O., MURARIU, A.M., GRIGORAS, S., HANGANU, L.C., *Mat. Plast.*, **50**, no. 2, 2013, p. 119.
- CHECHERITA, L., FORNA, N.C., STAMATIN, O., COBZARU, R., LEON, M.M., CIOLOCA, D., *Rev. Chim. (Bucharest)*, **64**, no. 10, 2013, p. 1172.
- SARKAR, N.K., CAICEDO, R., RITWIK, P., MOISEYEVA, R., KAWASHIMA, I., *J. Endod.* **31**, 2005, p. 97.
- JUMANCA, D., GALUSCAN, A., PODARIU, A.C., ARDELEAN, L., RUSU, L.C., *Rev. Chim. (Bucharest)*, **63**, no. 8, 2012, p. 783.
- ROMINU, R.O., NEGRUTIU, M., RUSU, L.C., ARDELEAN, L., TUDOR, A., *Rev. Chim. (Bucharest)*, **63**, no. 3, 2012, p. 265

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