Comparative Biochemical Evaluation of Ca, P and Mg, after Subcutaneous Implantation of Some Biomaterials Used in Endodontic Treatment

LIANA AMINOV[1], MARIA VATAMAN[1], DANA CRISTIANA MAXIM[2*], MIHAELA SALCEANU[1], PETRA SURLIN[3*], LAURA ELISABETA CHECHERITA[1*]

[1] University of Medicine and Pharmacy „Gr. T. Popa" Iasi, Department of Odontology-Periodontology and Fixed Prosthesis, 16 Universitatii Str., 700115, Iasi, Romania

[2] University of Medicine and Pharmacy „Gr. T. Popa" Iasi, Department of Surgical Sciences, 16 Universitatii Str., 700115, Iasi, Romania

[3] University of Medicine and Pharmacy Craiova, Department of Othodontics, 2-4 Petru Rareş Str., 200349, Craoiva, Romania

An important desirable effect of endodontic therapy is to induce the repair of periradicular tissues and to stimulate the cementogenesis. Endodontic perforations interfere with these goals, therefore, the discovery of an ideal material for perforations repair, with high biocompatibility, has become a necessity. The study is based on the comparative analysis of the biochemical profile of the animals implanted with three materials used in endodontics, at 7, 30 and 60 days after intervention, for Ca, P and Mg evaluation.

Keywords: stomatognathic system homeostasis, biocompatibility, biochemical profile, Ca, P and Mg evaluation, biomaterials.

After sealing the endodontic space, the filling material comes into direct contact with the periapical connective tissue. The chemical composition of the material used for the filling can positively or negatively influence the outcome of the root canal treatment [1-5]. Therefore, the material used must be inert, non-irritating and as compatible as possible with the periradicular tissues [6-9].

Endodontic perforations interfere with these goals due to the damage caused for the periodontal ligament and for the whole dental support system, by subsequent bacterial proliferation [10-16]. The discovery of an ideal material for perforations repair that meets as many qualities, has become a challenge that must be based on real research, oriented on motivation and scientifically based arguments [17-21].

The biological compatibility of the root sealant is of key importance, because in clinical conditions such materials are placed in contact with vital tissues and tissue response to these materials can influence the outcome of the endodontic treatment [22].

In this context, specific stomatognathic system homeostasis is achieved by the morphological, biochemichal and functional equilibrium between its components [23], due to the specific mechanisms of reaction and adaptation [24, 25].

The specific objectives of this research were to analyze the biochemical profile of the animals implanted with three materials used in endodontics, at 7, 30 and 60 days after intervention, for Ca, P and Mg, reported at surgery, initial biochemical parameters in all analyzed cases corresponding to these materials [26-27].

Experimental part

The experimental procedures performed in this study were performed according to the protocol reviewed and approved by the Ethical Committee on Animal Research of the Faculty of Veterinary Medicine "Ion Ionescu de la Brad" Iasi, in accordance with international principles of biomedical research on experimental animals and with the Ministry of Education, Research and Innovation.

The biomaterials used in the study were:

- **MTA** (Mineral Trioxide Aggregate, Dentsply, Tulsa Dental), highly effective antibacterial material, alkaline, consisting of calcium hydroxide - $Ca(OH)_2$, bismuth oxide - Bi_2O_3, calcium sulfate - $CaSO_4$, tricalcium silicate - $(CaO)_3 \cdot SiO_2$, dicalcium silicate - $(CaO)_2 \cdot SiO_2$, tricalcium aluminate $(CaO)_3 \cdot Al_2O_3$;
- **Sealapex** (Kerr, Switzerland) - used to seal the endodontic space, with the following chemical composition: barium sulfate - $BaSO_4$, titanium dioxide, TiO_2, zinc oxide- ZnO, calcium hydroxide - $Ca(OH)_2$, butylbenzene - $C_{10}H_{14}$, sulfonamide - $C_6H_8N_2O_2S$, zinc stearate - $ZnC_{36}H_{70}O_4$;
- **DiaRoot BioAggregate** (Innovative BioCaramix Inc., Vancouver, BC, Canada) - material similar in structure to MTA further comprising ceramic nanoparticles. It has proven antiseptic proprieties, simultaneously stimulating the cementogenesis. The chemical composition is as follows: calcium silicate - $CaSiO_3$, calcium hydroxide - $Ca(OH)_2$, hydroxyapatite -$Ca_5(OH)(PO_4)_3$, tantalum oxide - Ta_2O_5.

In order to assess the comparative response of living tissues to these biomaterials, we used 19 rabbits bred Belgian giant 4 months of age who were implanted into the subcutaneous connective tissue, close to the bone, polyethylene tubes of the same diameter and length, containing biomaterials used. They formed three groups of 6 rabbits each, to test each biomaterial, a rabbit was used as a negative control.

Preoperative blood samples were taken, aiming to assess the dynamic variation of bone formation parameters for each animal. The values for Ca, P and Mg were measured and a comparative analysis was done, based on the biochemical profile of implanted animals at 7, 30 and 60 days after surgery.

* email: p_surlin@hotmail.com; danamaxim@yahoo.com; checherita.laura@gmail.com

Rabbit No.	Control rabbit			Implanted material								
	Ca	P	Mg	Sealapex			MTA			BioAggregate		
				Ca	P	Mg	Ca	P	Mg	Ca	P	Mg
1	4.69	5.35	2.81	4.75	5.33	2.88	4.78	5.38	2.79	4.76	5.64	2.67
2				4.20	5.28	2.77	4.70	5.24	2.65	4.89	5.71	2.81
3				4.35	5.19	2.83	4.82	5.67	2.45	4.78	5.83	2.78
4				4.28	5.32	2.85	4.95	5.63	2.86	4.98	5.32	2.67
5				4.34	5.17	2.90	4.84	5.67	2.55	4.86	5.53	2.74
6				4.20	5.30	2.87	4.90	5.31	2.42	4.95	5.45	2.69

Table 1
VALUES OF **Ca, P, Mg**, 7 DAYS AFTER SURGERY (mg/dL)

Rabbit No.	Control rabbit			Implanted material								
	Ca	P	Mg	Sealapex			MTA			BioAggregate		
				Ca	P	Mg	Ca	P	Mg	Ca	P	Mg
1	4.69	5.35	2.81	4.98	5.54	2.90	5.24	6.03	3.24	5.43	5.98	2.97
2				4.67	5.65	2.97	5.12	6.12	3.19	5.24	6.03	2.89
3				4.49	5.67	3.01	5.30	5.98	3.29	5.38	6.12	2.92
4				4.68	5.61	3.17	5.35	6.19	3.08	5.34	5.99	2.96
5				4.78	4.96	2.97	5.38	5.96	3.05	4.96	6.07	2.75
6				4.56	5.02	3.12	5.10	6.17	3.15	5.11	5.97	2.79

Table 2
VALUES OF **Ca, P, Mg**, 30 DAYS AFTER SURGERY (mg/dL)

Rabbit nr.	Control rabbit			Implanted material								
	Ca	P	Mg	Sealapex			MTA			BioAggregate		
				Ca	P	Mg	Ca	P	Mg	Ca	P	Mg
1	4.69	5.35	2.81	4.72	5.33	2.88	4.78	5.37	2.90	4.70	5.29	2.79
2				4.20	5.28	2.77	4.70	5.29	2.85	4.72	5.32	2.84
3				4.36	5.24	2.75	4.88	5.65	2.50	4.75	5.38	2.70
4				4.31	5.29	2.99	4.92	5.61	2.83	4.68	5.36	2.65
5				4.55	5.31	2.89	4.83	5.41	2.78	4.71	5.49	2.88
6				4.62	5.33	2.87	4.75	5.39	2.68	4.72	5.37	2.79

Table 3
VALUES OF **Ca, P, Mg**, 60 DAYS AFTER SURGERY (mg/dL)

Postoperative Ca values

Implanted material	Evaluation moment	Average Ca	Average -95%	Average +95%	Std dev.	Std er.	Min	Max	Median
Sealapex	7 days	4.4	4.1	4.6	0.2	0.1	4.2	4.8	4.3
Sealapex	30 days	4.7	4.4	5.0	0.2	0.1	4.5	5.0	4.7
Sealapex	60 days	4.5	1.0	8.0	0.4	0.3	4.2	4.8	4.5
MTA	7 days	4.8	4.7	4.9	0.1	0.0	4.7	5.0	4.8
MTA	30 days	5.3	5.2	5.5	0.1	0.0	5.2	5.4	5.4
MTA	60 days	4.7	4.2	5.2	0.1	0.0	4.7	4.8	4.7
BioAggregate	7 days	4.9	4.8	5.0	0.1	0.0	4.8	5.0	4.9
BioAggregate	30 days	5.3	5.1	5.4	0.1	0.0	5.1	5.4	5.3
BioAggregate	60 days	4.7	4.6	4.8	0.0	0.0	4.7	4.7	4.7
control		4.7			0.0		4.7	4.7	4.7

Table 4
STATISTICAL INDICATORS OF **Ca** IN THE STUDY GROUP BASED ON THE BIOMATERIAL USED AND THE TIME OF DETERMINATION

Table 5
TEST FOR **Ca** COMPARISON ACCORDING TO THE TIME OF ASSESSMENT AND MATERIAL USED

Ca	F *(95% interval of confidence)*	p
ANOVA Test	19.44374	0.000000

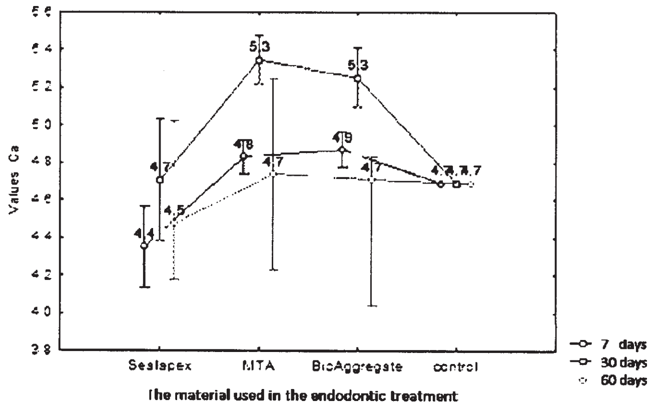

Fig. 1. Statistical indicators of Ca values depending on time of assessment and material used

Results and disscusions

The analysis shows the following biochemical profile of implanted animals 7, 30 and 60 days after the operation, for Ca, P and Mg, based on the starting time of operation and the biochemical parameters in all the cases analyzed, corresponding to the three materials (table 1, 2 and 3).

In analyzing the biochemical profile of implanted animals at intervals set at 7 days, 30 days and 60 days after surgery, it was found an increase in calcium values reported within 7 days after operation, values which gradually increased up to 30 days and reached significantly higher levels than the control group. After 30 days, postoperative calcium levels decreased significantly, reaching values comparable to those of the control group. The measurement results confirm the effectiveness of treatment in all the three materials used (fig. 1, table 5).

A detailed analysis of calcium values evolution for each biomaterial used in this study was made to highlight changes in the dynamic Ca values, for SEALAPEX (fig. 2, table 6), for MTA (fig. 3, table 7), for BioAggregate (fig. 4, table 8). In conclusion, it is noted that all materials used, after 30 days, decreased significantly, reaching values at 60 days without significant differences from control specimen (fig. 4, table 8).

The P values show significant differences depending both on the biomaterial used and on the time of evaluation. The highest values were recorded for BioAgreggate, followed in descending order by the P values obtained when using the MTA, and the lowest values were recorded for P when Seaplex was used. The same situation was also found for Ca values.

For MTA and BioAggregate , baseline P values are higher than the control group and than the group where Sealapex was used. These values are increasing significantly after 30 days, and then normalize, reaching levels comparable to those of the control group (fig. 5, table 10).

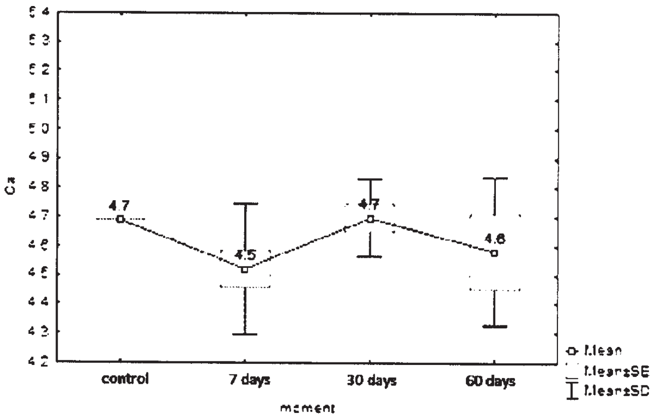

Fig. 2. Statistical indicators of Ca values depending on time of assessment for SEALAPEX

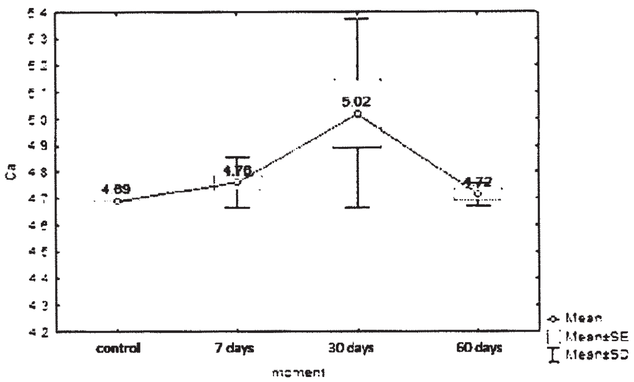

Fig. 3. Statistical indicators of Ca values depending on time of assessment for MTA

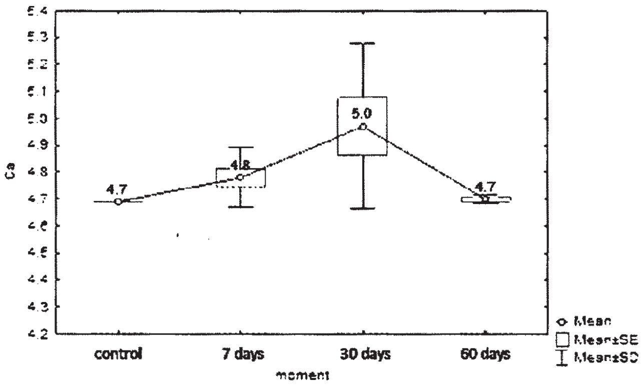

Fig. 4. Statistical indicators based on time of assessment for BioAggregate

Table 6

TEST FOR **Ca** COMPARISON ACCORDING TO THE TIME OF THE ASSESSMENT, FOR SEALAPEX

Ca - *SEALAPEX*	F *(95% interval of confidence)*	p
ANOVA Test	2.790773	0.056299

Table 7

TEST FOR **Ca** COMPARISON ACCORDING TO THE TIME OF THE ASSESSMEN, T FOR MTA

Ca - *MTA*	F *(95% interval of confidence)*	p
Testul ANOVA	6.162503	0.001988

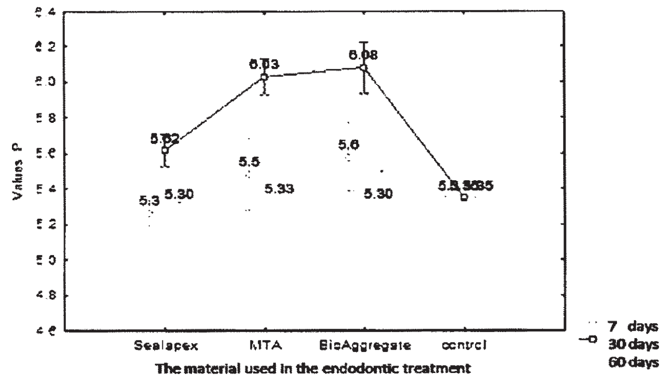

Fig. 5. Statistical indicators of P according to time of the assessment and material used

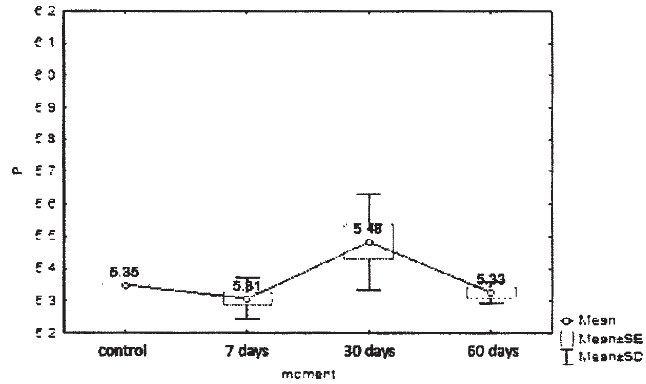

Fig. 6. Statistical indicators of P values depending on time of assessment for SEALAPEX

Table 8

TEST FOR Ca COMPARISON ACCORDING TO THE TIME OF ASSESSMENT, FOR BIOAGGREGATE

Ca – BioAggregate	F *(95% interval of confidence)*	p
Testul ANOVA	5.537506	0.003537

Postoperative P values (table 9)

Table 9

STATISTICAL INDICATORS OF **P** IN THE STUDY GROUP ACCORDING TO THE BIOMATERIAL USED AND THE TIME OF DETERMINATION

Implanted material	Evaluation moment	Average Ca	Average -95%	Average +95%	Std dev.	Std er.	Min	Max	Median
Sealapex	7 days	2.9	2.8	2.9	0.0	0.0	2.8	2.9	2.9
Sealapex	30 days	3.0	2.8	3.2	0.1	0.1	2.9	3.2	3.0
Sealapex	60 days	2.8	2.1	3.5	0.1	0.1	2.8	2.9	2.8
MTA	7 days	2.6	2.4	2.8	0.2	0.1	2.4	2.9	2.6
MTA	30 days	2.9	2.9	3.0	0.0	0.0	2.9	3.0	2.9
MTA	60 days	2.9	2.6	3.2	0.0	0.0	2.9	2.9	2.9
BioAggregate	7 days	2.7	2.7	2.8	0.1	0.0	2.7	2.8	2.7
BioAggregate	30 days	3.2	3.1	3.3	0.1	0.0	3.1	3.3	3.2
BioAggregate	60 days	2.8	2.5	3.1	0.0	0.0	2.8	2.8	2.8
control		2.8			0.0		2.8	2.8	2.8

Table 10

TEST FOR **P** VALUES COMPARISON ACCORDING TO THE TIME OF EVALUATION AND MATERIAL USED

P	F *(95% interval of confidence)*	p
Testul ANOVA	25.09959	0.000000

Table 11
TEST FOR **P** VALUES COMPARISON
DEPENDING ON THE TIME OF , FOR SEALAPEX

P - SEALAPEX	**F** *(95% interval of confidence)*	**p**
Testul ANOVA	8.514595	0.000267

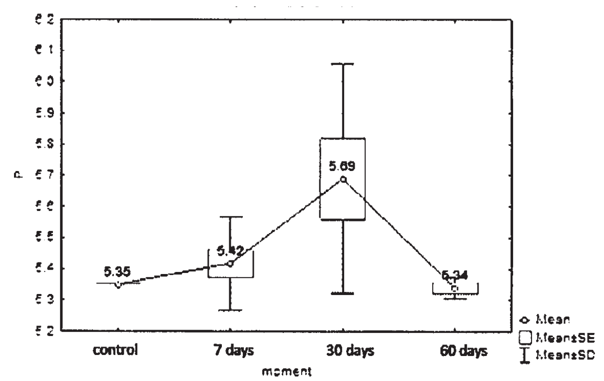

Fig. 7. Statistical indicators of P values depending on the time of evaluation for MTA

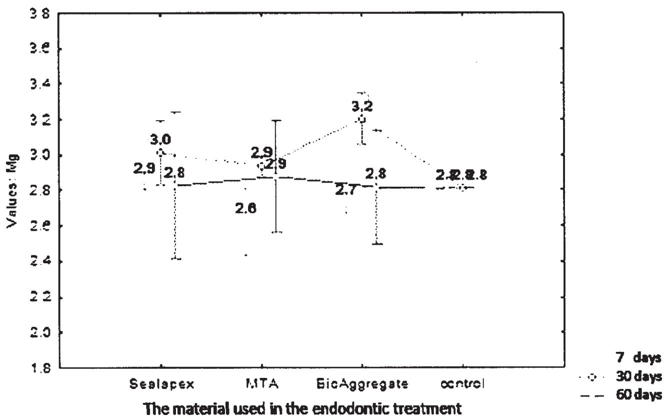

Fig. 9. Statistical indicators of Mg values depending on time of assessment and material used

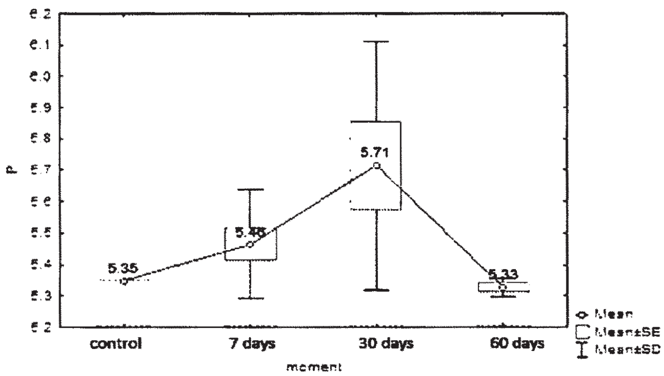

Fig.8. Statistical indicators of P values depending on the time of evaluation for BioAggregate

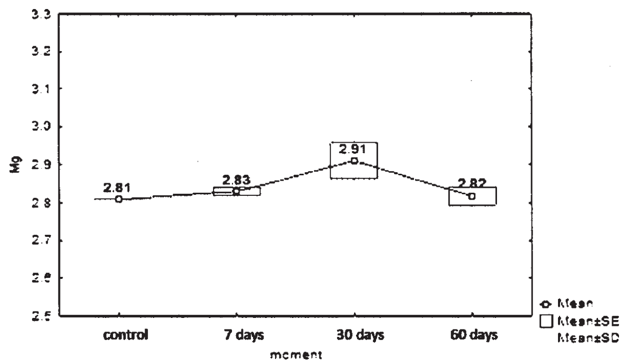

Fig.10. Statistical indicators of Mg values depending on time of assessment for SEALAPEX

Table 12
TEST FOR **P** VALUES COMPARISON
DEPENDING ON THE TIME OF ASSESSMENT, FOR MTA

P - MTA	**F** *(95% interval of confidence)*	**p**
Testul ANOVA	5.779778	0.002823

Table 13
TEST FOR **P** VALUES COMPARISON
DEPENDING ON THE TIME OF ASSESSMENT, FOR BIOAGGREGATE

P - BioAggregate	**F** *(95% interval of confidence)*	**p**
Testul ANOVA	5.502730	0.003654

Postoperative Mg values

Table 14
STATISTICAL INDICATORS OF Mg IN THE STUDY GROUP ACCORDING TO THE BIOMATERIAL USED AND THE TIME OF DETERMINATION

Implanted material	Evaluation moment	Average Ca	Average -95%	Average +95%	Std dev.	Std er.	Min	Max	Median
Sealapex	7 days	2.9	2.8	2.9	0.0	0.0	2.8	2.9	2.9
Sealapex	30 days	3.0	2.8	3.2	0.1	0.1	2.9	3.2	3.0
Sealapex	60 days	2.8	2.1	3.5	0.1	0.1	2.8	2.9	2.8
MTA	7 days	2.6	2.4	2.8	0.2	0.1	2.4	2.9	2.6
MTA	30 days	2.9	2.9	3.0	0.0	0.0	2.9	3.0	2.9
MTA	60 days	2.9	2.6	3.2	0.0	0.0	2.9	2.9	2.9
BioAggregate	7 days	2.7	2.7	2.8	0.1	0.0	2.7	2.8	2.7
BioAggregate	30 days	3.2	3.1	3.3	0.1	0.0	3.1	3.3	3.2
BioAggregate	60 days	2.8	2.5	3.1	0.0	0.0	2.8	2.8	2.8
control		2.8			0.0		2.8	2.8	2.8

Table 15
TEST FOR **Mg** VALUES COMPARISON ACCORDING TO THE TIME OF EVALUATION AND MATERIAL USED

Mg	**F** *(95% interval of confidence)*	**p**
Testul ANOVA	13.00560	0.000000

Table 16
TEST FOR **Mg** VALUES COMPARISON
DEPENDING ON THE TIME OF , FOR SEALAPEX

Mg - SEALAPEX	**F** *(95% interval of confidenţce)*	**p**
Testul ANOVA	4.076594	0.014676

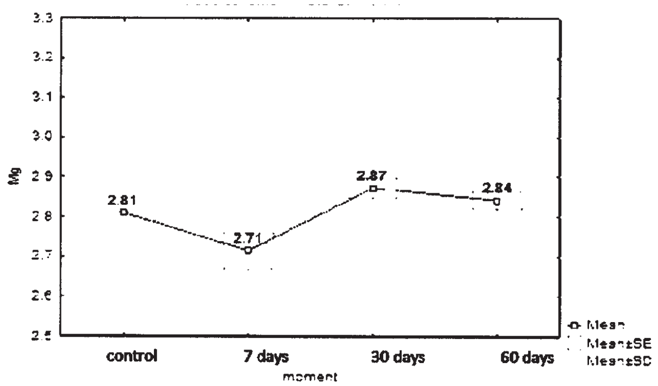

Fig. 11. Statistical indicators of Mg values depending on the time of evaluation for MTA

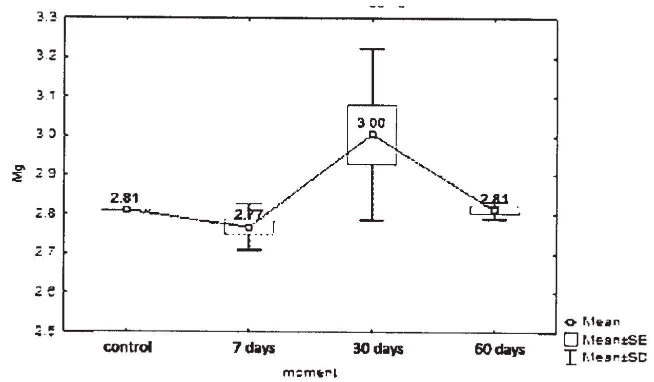

Fig.12. Statistical indicators of Mg values depending on the time of evaluation for BioAggregate

Table 17
TEST FOR **Mg** VALUES COMPARISON DEPENDING ON THE TIME OF ASSESSMENT, FOR MTA

Mg - MTA	**F** *(95% interval of confidence)*	**p**
Testul ANOVA	4.698574	0.007939

Table 18
TEST FOR **Mg** VALUES COMPARISON DEPENDING ON THE TIME OF ASSESSMENT, FOR BIOAGGREGATE

Mg - BioAggregate	**F** *(95% interval of confidence)*	**p**
Testul ANOVA	8.494583	0.000271

Below is a detailed analysis of P values evolution for each biomaterial used in this study, in order to highlight the dynamic changes of these values for SEALAPEX (fig. 6, table 11), for MTA (fig. 7, table 12), for BioAggregate (fig. 8, table 13).

In conclusion, it is noted that for all materials used, after 30 days, P values decreased significantly, reaching 60 days without significant differences from the control group (fig. 8, table 13).

The mean values of Mg between the three materials used are not significantly different (F = 1.995, p = 0.1285, 95% CI), but in dynamics they vary significantly, after 60 days reaching values comparable to the control group (fig. 9, table 15).

Further, a detailed analysis of magnesium levels evolution for each biomaterial used in this study was made to highlight the changes in dynamics for SEALAPEX (fig. 10, table 16), for MTA (fig. 11, table 17), for Bio Aggregate (fig. 12, table 17) ..

Conclusions

After analyzing all these results, we can state that for all the materials used, after 30 days, the magnesium levels significantly decreased, reaching 60 days without significant differences from the control specimen.

A different aspect than the previously analyzed parameters is the decrease in values of Mg levels after 7 days compared with control specimen, when using MTA or BioAggregate, whereas at 7 days after SEALAPEX the values remain approximately equal. The same behaviour is also found in the case of calcium values after 7 days of treatment, the comparison being made in this case as compared with the control group [28-32].

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