Aspects of *Candida albicans* Colonization for a New Polymer Used in Complete Dentures Fabrication (II)

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The present paper aims to assess the quality of materials used in the field of removable dentures namely classic acrylate and crosslinked acrylate with improved polymerization using as template vitamin B12 (cyanocobalamin). A major task is the trajectory of Candida albicans biofilm formation and their adherence to the surface of these materials.

Keywords: Candida albicans, complete dentures, acrylate, crosslinked, vitamin B12

The ability to form biofilms, recently considered the prerogative of only a few species is today seen as an attribute almost all microorganisms. It also became known that the bacteria are building ways in which biofilms are extremely ranging from one species to another under the influence of various environmental conditions. Their growth in a biofilm may affect immune function and antibiotic therapy, thus complicating treatment of infectious diseases and especially chronic ones [1]. Candida albicans incidence is considered to be 45% in neonates [2], 45-65% in healthy children [3], 30-45% in healthy adults [4], 50-65% denture wearers [4], 65-88% in patients acute and chronic [2, 5], 90% in patients with acute leukemia [6] and 95% in HIV positive patients [7].

Experimental part

Materials and methods

The present paper is a continuation of a previous study published in Revista de Chimie as part I conducted on the samples for 24 h. The part II is a study conducted on the samples for 48 h.

The specimens used in this study were obtained by polymerization method called surface template polymerization, in which we used acrylate monomer and polymer with the following composition:polymethyl methacrylate powder, methylmethacrylate liquid, ethylenedimethacrylate liquid. As the template molecule, we used cyanocobalamin, which is the form of vitamin B12, with the wide spread use of clinically due to its availability and stability [8, 9]. Specimen dimensions were1cm²/1cm² (fig.1) [10].

The culture media used was agar Sabouraud 2% glucose, Sabouraud broth 8% prepared from the broth Sabouraud 4% (Biokar, France) and glucose (Sigma, Germany) and

Fig. 1. Acrylates amples classic and with vitamin B12

Fig. 2. Agar Sabouraud growth environment

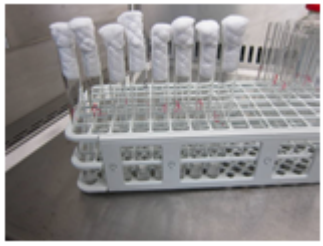

Fig. 3. Standard tubes McFarland

McFarland standard tubes (BioMeriuexFrance) (figs. 2 and 3).

As a test strain used was a strain of Candida albicans isolated from stomatitis, denture lesion, then purified and identified by common laboratory techniques [11].

The numbered 1to 15 have been obtained by conventional polymerization method,with the vitamin B12and the 1B-15B were obtained by template polymerization using as atemplate B12, polymerized by mixing it with the monomer [12, 13]. Ratio polymer: monomer was kept constant, following the classic recipe polymerization indicated by the manufacturer.

The samples were processed and then polish with paste gloss Bimsstein Abraso Starglanz from Bredent Company. Polishing brushes were used with 2 x 2 inserts; special fabric Abraso Scwabbel Acrylic with 8 mm diameter from the same company and the gloss was obtained with Acrylic Brush diameter 10 to 35 mm.

From a stock culture preserved at -80°C was inoculated plate with agar Sabourand which was then incubated 24 h at T = 36°C ± 1 of the culture obtained was prepared, a slurry with a density of 5 McFarland (~ 107CFU/mL) in distilled water (fig. 4).

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Fig. 4. Candida culture

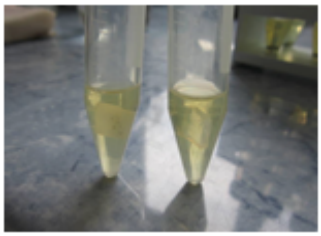

Fig. 5. Tubes test specimens

Fig.6. Electronic microscope Quanta 2003 DDualBeam

The suspension was distributed in sterile tubes each containing a test specimen (1 mL suspension/tube). The tubes were then maintained for 90 min at 36°C ± 1. Necessary for access into the surface of yeast cells tested materials (fig. 5).

After this time the samples were placed in tubes containing 3mL broth-Sabourand 8% glucose and incubated under the same conditions of T°, time (48h, during the occurrence and extension of the biofilm) [14]. At the expiration of the incubation periods the medium was removed, samples were washed in distilled water, allowed to stand 10 min on filter paper for air drying and were then examined under an electron microscope Quanta 2003DDualBeamisa microscope, in which are embedded two systems, namely, SEM electron microscope which provides a magnification of 000x100, and a high-resolution digital format which is a FIB ion beam system capable of fast and precise grinding of different geometries(of ìm) of the sample material, revealing sub-surface structure, obtaining sections deposition layers etc. Ionic system also provides a high resolution image (fig. 6).

Under the microscope, the following parameters were followed at 48h, parameters on which to set a score for samples of acrylic simple and one for vitamin B12 incorporated:

-presence/absence of biofilm (presence noted by + and absence by -);

-the extension of the biofilm [15]: 0 - rare cell clusters; 1- biofilm island; 2 - compact biofilm;

-the thickness: 0 = 0-5μm,1 = 5-10 μm, 2 = 10-15μm, 3=15-20 μm, 4 = 20-25 μm, 5 = 25-30 μm, 6 > 30μm.

In this paper, part II we will analyzed only the first parameters after 48 h.

Results and discussions

The images captured by scanning electron microscope revealed the following present in samples obtained by polymerization of acrylate classic without template molecule B12 after 48 h (fig.7a, b and c).

Of the 15 samples acrylate classic analyzed after 48 h, all had their biofilm formed on the surface, the degree of extension of the biofilm from the value of 1 for three of the samples and the amount of two to twelve samples. The thickness was also different values between 20-25 μm for five of the samples, between 25-30 μm for six samples and >30 μm for 4 samples. Therefore the final score of the samples had value 5 for two of the samples, the value 6 for three samples ,a value of 7 for seven samples, a value of 8 for three samples, as also have been the data summarized in the table below (table 1).

Tabel 1
EVIDENCE OF ACRYLATE SCORE COLONIZED WITH CANDIDA ALBICANS ACRYLIC AFTER 48H

SAMPLES	Biofilm present	Extension biofilm	Width	Final score
1	+	1	4	5
2	+	2	5	7
3	+	2	5	7
4	+	2	6	8
5	+	2	5	7
6	+	2	6	8
7	+	1	4	5
8	+	2	5	7
9	+	2	6	8
10	+	2	4	6
11	+	2	5	7
12	+	2	4	6
13	+	2	4	6
14	+	1	6	7
15	+	2	5	7

Of the 15 samples enriched with vitamins B12 acrylate, crosslinked by the polymerization recipe, biofilm formation was observed on all specimens, (fig. 8a, b and c) with its extension values ranging from 0 to 2, 0 value for two sample, 1 value for nine samples, 2 value for four samples, and biofilm thickness of 2 for four samples, 3 for one sample, 4 for six samples and 5 for four samples. So finally score value was 2 for two samples, 3 for two sample, 5 for five test samples, 6 for five samples, 7 for one sample and were also synthesized as the data in the table 2.

We are interested in whether, after 48 h, between the two groups-classic acrylate and acrylate with B12 were differences statistically significant. For statistical analysis we used SPSSv.17and Microsoft Excel 2007.

From the table 3 we find that the average scores descriptive statistics for the classic acrylate (6.73) is higher than the average scores of the acrylate B12 (4.80). To determine if this difference is statistically significant will have to use statistical tests, not before checking the

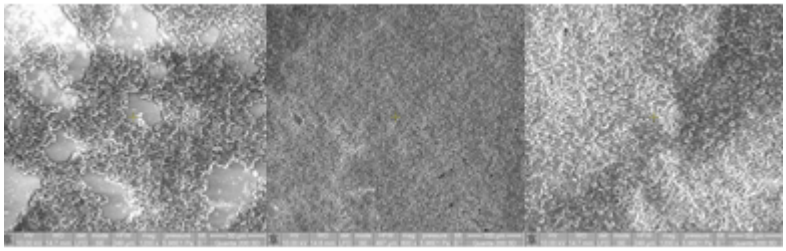

Fig.7. Sample acrylate colonized with Candida albicans after 48 h, colony appearance: a. island, b. compact, c. compact

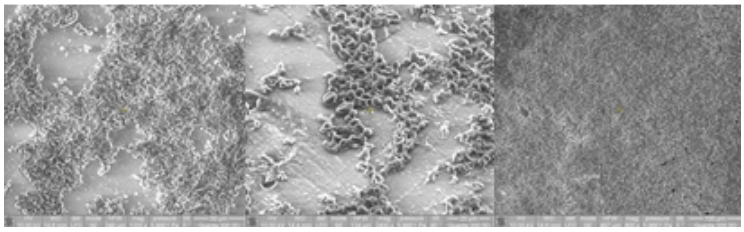

Fig. 8. Sample acrylate with vit. B12 colonized with Candida albicans after 48 h, colony appearance: a. island, b. compact, c. compact

Tabel 2
EVIDENCE OF ACRYLATE WITH VIT. B12 SCORE COLONIZED WITH CANDIDA ALBICANS AFTER 48h

SAMPLES	Biofilm	Biofilm extension	Width	Final score
1B	+	1	2	3
2B	+	1	4	5
3B	+	1	5	6
4B	+	1	4	5
5B	+	1	4	5
6B	+	1	5	6
7B	+	2	3	5
8B	+	1	4	5
9B	+	0	2	2
10B	+	1	5	6
11B	+	1	2	3
12B	+	0	2	2
13B	+	2	5	7
14B	+	2	4	6
15B	+	2	4	6

normality of both distributions with Shapiro-Wilk and Kolmogorov-Smirnov tests.

The frequency tables 4, 5 show scores of 7 and 8 (46.7%, 20%) in classic acrylate samples, frequencies no longer meet in acrylate B12 group .We can check normality tests using normal distributions.

Table 6 includes the results of normality tests. In essence, they tested the degree of overlap between the variable analyzed the cumulative distribution and the cumulative distribution of a variable whose distribution follows Gauss curve. We are interested in Shapiro-Wilk test, given that the number of values is considered small. If *p* is less than or equal to 0.05, then we reject the hypothesis of normality of distribution (distribution deviates from normality). In both groups we find deviations from normal distribution. We can use the tests in this case nonparametric statistical tests: Wilcoxon test nonparametric equivalent for paired samples *t* test (table 7).

Tabel 3
ELEMENTS OF DESCRIPTIVE STATISTICS FINAL SCORES AFTER 48h

Statistics

		acrilat_clasic	acrilat_B12
N	Valid	15	15
	Missing	0	0
Mean		6.73	4.80
Std. Error of Mean		.248	.405
Std. Deviation		.961	1.568
Skewness		-.498	-.775
Std. Error of Skewness		.580	.580
Kurtosis		-.334	-.534
Std. Error of Kurtosis		1.121	1.121
Minimum		5	2
Maximum		8	7

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	5	2	13.3	13.3	13.3
	6	3	20.0	20.0	33.3
	7	7	46.7	46.7	80.0
	8	3	20.0	20.0	100.0
	Total	15	100.0	100.0	

Table 4
FREQUENCY OF CANDIDA FOR CLASSIC ACRYLATE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	2	13.3	13.3	13.3
	3	2	13.3	13.3	26.7
	5	5	33.3	33.3	60.0
	6	5	33.3	33.3	93.3
	7	1	6.7	6.7	100.0
	Total	15	100.0	100.0	

Tabel 5
FREQUENCY OF CANDIDA FOR B 12 ACRYLATE

Tabel 6
TABLE NORMALITY

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
acrylat_classic	.276	15	.003	.872	15	**.037**
acrylat_B12	.284	15	.002	.858	15	**.023**

a. Lilliefors Significance Correction

Starting from the null hypothesis which argues that the two groups did not differ statistically significant we try to show that the working assumption that there are significant differences between the two groups is valid.

According to the Wilcoxon test there is a significant difference statistically between final scores of the two groups at 48 h. (Wilcoxon: N = 15, z = 3.034, two-tailed = 0.002).

In a future paper we are going to analyze the width and extension of the two groups and if there are statistically significant difference between final scores of the two groups at 48 h.

Ranks

		N	Mean Rank	Sum of Ranks
acrylate_B12 - acrylate_classic	Negative Ranks	12[a]	7.38	88.50
	Positive Ranks	1[b]	2.50	2.50
	Ties	2[c]		
	Total	15		

a. acrilat_B12 < acrilat_clasic

b. acrilat_B12 > acrilat_clasic

c. acrilat_B12 = acrilat_clasic

Test Statistics

	acrylate_B12 - acrylate_clasic
Z	-3.034[a]
Asymp. Sig. (2-tailed)	**.002**

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

Tabel 7
WILCOXON TEST TABEL

Conclusions

The data obtained lead to the following conclusions:

To characterize the properties of Candida biofilms are used different experimental models in our case fragments of acrylate for dentures for obtaining biofilms in vitro .In addition in this study was obtained by microscopic image and statistical tests compare the amount of biofilm formed in the two classical types of materials acrylate acrylate polymer in the presence of vitamin B12 after 48h.

Using experimental method of achieving biofilm technique and classic acrylic fragments B12, directly and by using statistical tests of Kolmogorov-Smirnov normality and Saphiro-Wilk, proved that Candida biofilm formed in greater amount to 48 h.

According to the Wilcoxon test there is a significant difference statistically between final scores of the two groups at 48 h. (Wilcoxon: N = 15, z = 3.034, two-tailed = 0.002).

The the frequency tables aware of cell clusters in group B12 acrylate rates (13.3%), while none exist at group acrylate and 2 scores appearance indicates lower biofilm thickness in group B12 acrylate (26.7%) acrylate group compared to classic where none exist.

References

1. LEE, S.Y., LAI, Y.L., HSU, T.S**.,** Journal of Oral Rehab., **110**, no. 2, 2002, p. 179.
2. LEGGAT, P.A., KEDJARUNE, U., Int. Dent. J., **53,** no**.** 3, 2003, p. 126.
3. BAYRAKTAR, G., GUVENER, B., BURAL, C., URESIN, Y, Influence of polymerization method, curing process, and length of time of storage in water on the residual methyl methacrylate content in dental acrilic resins, Journal of Biomedical Materials Research, **76B**, no. 2, 2006, p. 340.
4. LEFEBRE, C.A., SCHUSTER, G.S., MARR, J.C., KNOERSCHILD, K.L., The effect of the cytotoxicity of eluates from denture base resins, Int.J.Prosthodont., **8**, 1995, p.122.
5. COLOSI, I., COSTACHE, C., JUNIE, M., Rezistenþa biofilmelor de Candida la antifungice, Fungi &Mycotoxins, **3,** no. 1, 2009, p. 242.
6. RAMAGE, G., LOPEZ RIBOT, J.L.,Techniques for antifungal susceptibility testing of Candida albicans biofilms, Methods Mol. Med., **118**, 2005, p. 71.
7. DORKO, E., JENCA, A., PILIPCINEC, E., DANKO, J, SVICKÝ, E, TKACIKOVA, L. Candida-associated denture stomatitis, Folia Microbiol., **46**, no. 5, 2001, p. 443.
8. BOSINCEANU, D.N., SANDU, I.G., BOSINCEANU, D.G., FORNA, N.C., Rev. Chim. (Bucharest), **65**, no. 4, 2014, p. 466.
9. ANDRES, E., FOTHERGILL, H., MECILI, M., Efficacy of oral cobalamin (vitamin B12) therapy, Expert Opin Pharmacother, **11**, no. 2, 2010, p. 249.
10. AVON, S.L., GOULET, J.P., DESLAURIERS, N., Removable acrylic resin disk as a sampling system for the study of denture biofilms in vivo, Journal of Prosthetic Dentistry, **97**, no. 1, 2007, p. 32.
11. BAENA MONROY, T., MORENO MALDONADO, V., FRANCO MARTINEZ, F., Candida albicans, Staphylococcus aureus and Streptococcus mutans colonization in patients wearing dental prosthesis, Med. Oral Patol. Oral Cir. Bucal., **10**, no. 1, 2005, p. 27.
12. BUYUKERKMEN, B., OZTURK, A., Effect of polymerisation techniques on residual monomer amount released from different acrylic resins, Materials Researchs Innovations, **14**, no. 5, 2010, p. 381.
13. CHEN, T., KUSY, R.P., Effect of methacrylic acid: Methyl methacrylate monomer ratios on polymerization rates and properties of polymethyl methacrylates, Journal of Biomedicals Material Research, **36**, 1997, p. 190.
14. DHIR, G., BERZINS, D.W., DHURU, V.B., Physical properties of denture base resins potentially resistant to Candida adhesion, J. Mater. Sci. Mater. Med., **19**, no. 2, 2008, p. 959.
15. DOUGLAS, L.J., Candida biofilms and their role in infection, Trends in Microbiology, **11,** no. 1, 2003, p. 30.

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