Study of *Candida albicans* Colonies on a New Polymer used to Create Complete Dentures. I

DAN-NICOLAE BOSINCEANU[1], IOAN GABRIEL SANDU[2,3], DANA GABRIELA BOSINCEANU[1], NORINA CONSUELA FORNA[1]*

[1] University of Medicine and Pharmacy Gr. T. POPA , Faculty of Dental Medicine, 16 Independence Blv., 700101, Iasi, Romania

[2] Gheorghe Asachi Technical University of Iasi, Faculty of Material Science and Engineering, 61A D-trie Mangeron Blv., 700050, Iasi, Romania

[3]Romanian Inventors Forum, 3 Sf. Petru Movila, Str. Bl. L11, Sc. III, Et. 3, Ap.3, 700089, Iasi, Romania

This study aims to assess the quality of materials used in removable dentures sphere namely classic heat-curing acrylate and the same type of acrylate improved by crosslinked polymerization using as template vitamin B12 (cyanocobalamin). A major path followed is the biofilm formation of Candida albicans and its adherence to the surface of these materials.

Keywords: Candida albicans, complete dentures, polymer, dentures fabrication, acrylate

Biofilms are the immobilized cells in an organic polymer matrix of microbial origin. The ability to form biofilms, considered recently the attribute only for a few species is now seen as an attribute for almost all microorganisms. It also became known that the ways in which bacteria build their biofilms extremely vary from one species to another under the influence of different environmental conditions [1 - 5]. At the same time, infections caused by *Candida albicans* remains the most common infections [6 - 8]. One main reason is the very wide range of manifestations of candidia which grows only in the host tissue but formed biofilms in the medical devices, like implants, biofilm cells showing cell characteristics completely different from their natural counterparts [9 - 15].

In this paper are presented some aspects regarding the achievement dentures, acrylate polymerization method based on the vitamin B12 addiction, so that microbial biofilm formation to be as limited as possible, to improve the oral health of the tissues of the mouth.

Experimental part

Materials and methods

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 McFarland standard tubes (Bio Meriuex France) (figs. 1 and 2).

The growth environment is originally designed for the cultivation of dermatophytes. It is currently used for the isolation and cultivation of all fungus. Peptones are a source of growth factors supporting nitrogen. The glucose provides the energy source for the growth of microorganisms and higher glucose concentration provides an advantage for the fungus growth (osmotically stable), while most of the bacteria do not tolerate the high concentration of sugar. In addition, the low *p*H is optimal for the fungus, but not for the bacteria.

Fig.1. Agar Sabouraud growth

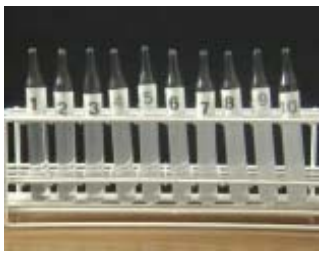

Fig.2. Standard tubes McFarland

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

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 clinical use due to its availability and stability. Specimen dimensions were $1cm^2/1cm^2$(fig. 3).

Fig.3. Acrylates amples classic and with vitamin B12

The numbering from 1 to 15 has been obtained by conventional polymerization method, with the vitamin B12 and the one from 1B-15B was obtained by template polymerization using as a template B12, polymerized by mixing it with the monomer. Ratio polymer: monomer was kept constant, following the classic recipe polymerization syndicated by the manufacturer.

The samples were processed and then polish with paste gloss Bimsstein Abraso Starglanz from Bredent Company. Polishing brushes were used with 2x2 inserts, special fabric Abraso Scwabbel Acrylic with 8mm diameter from the same company and the gloss was obtained with Acrylic Brush diameter 10 to 35mm.

* *email: dbossu1@gmail.com; Tel.: +40741049893*

Fig.4. Specimens test tubes

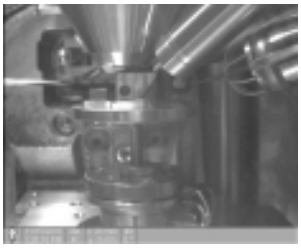

Fig.5. Electronic microscope Quanta 200 3D Dual Beam

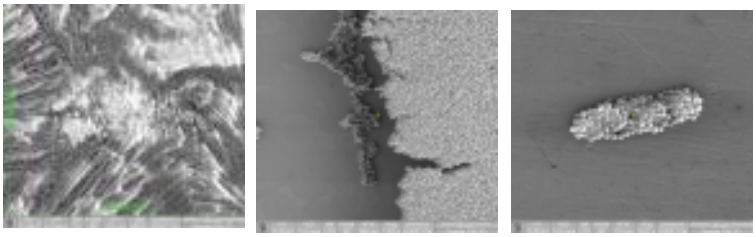

Fig.6. Sample acrylate colonized with Candida albicans after 24 h, colony appearance: a - island, b - compact, c - rare cell clusters

From a stock culture preserved at -80°C was inoculated plate with agar Sabourand which was then incubated 24 hours at T = 36 ± 1°C of the culture obtained was prepared, a slurry with a density of 5 McFarland (~107CFU/mL) in distilled water (fig. 6). The suspension was distributed in sterile tubes each containing a test specimen (1.0mL suspension/ tube). The tubes were then maintained for 90 min at 36 ± 1°C, necessary for access into the surface of yeast cells tested materials (fig. 4).

Then the samples have been placed in tubes containing 3mL broth-Sabourand 8% glucose and incubated under the same conditions of T°C, time (24h and 48h, respectively, during the occurrence and extension of the biofilm). At the expiration of two incubation periods the medium was removed, samples were washed in distilled water, allowed to stand10 min on filter paper for air drying then were then examined under an electron microscope Quanta 200 3D Dual Beam, in which are embedded two systems, namely, SEM electron microscope which provides a magnification of 100X, and a high-resolution digital format which is a FIB ion beam system capable of fast and precise grinding of different geometries (of μm) of the sample material, revealing sub-surface structure, obtaining sections deposition layers etc. Ionic system also provides a high resolution image (fig.5).

Under the microscope, the following parameters were watched at 24h and 48h, respectively, 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 "-");

- phe extension of the biofilm: 0 - rare cell clusters, 1 - biofilm island, 2 - compact biofilm;

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

In the part one of this study, we shall analyze only the first parameters after 24h. The rest of them will be analyzed in the next parts of this paper which will be published later.

Results and discussions

The images captured by scanning electron microscope revealed the samples obtained by polymerization of acrylate classic without template molecule B12 after 24 h (figs.6a, b and c).

Of the 15 samples acrylate classic analyzed after 24 h, all had their biofilm formed on the surface, the degree of extension of the biofilm from 0, absence of two of the samples, the value of 1 for six of the samples and the amount of two to seven samples. The thickness has also different values between 0-5μm for two of the samples, between 5-10μm for three samples, between 10-15μm for six samples, between 15-20μm for three samples and one sample with width between 20-25μm. Therefore the final score of the samples had value 0 for two of the samples, the value 2 for two samples, a value of three for five samples, the two samples 4, the three samples 5 and 6 for a sample value, as also have been the data summarized in the table below (table 1).

Of the15 samples enriched with vitamins B12 acrylate, cross linked by the polymerization recipe, biofilm formation was observed on all specimens, with its extension values ranging from 0 to 4 of the test, one of the new samples, two values for the two samples, and biofilm thickness of 0 for three samples, one to eight samples, two to three samples and 3 for a single sample. So finally score value was 0 for three samples, one for one sample, 2 for the six

Samples	Presence of Biofilm	Biofilm Extension	Width	Final Score
1	+	0	0	0
2	+	1	2	3
3	+	1	1	2
4	+	1	1	2
5	+	2	3	5
6	+	2	2	4
7	+	0	0	0
8	+	1	2	3
9	+	2	4	6
10	+	2	3	5
11	+	2	2	4
12	+	2	3	5
13	+	1	2	3
14	+	2	1	3
15	+	1	2	3

Table 1
EVIDENCE OF ACRYLATE SCORE COLONIZED WITH CANDIDA ALBICANS ACRYLIC AFTER 24h

Samples	Presence of Biofilm	Biofilm Extension	Width	Final Score
1B	+	0	0	0
2B	+	1	1	2
3B	+	1	1	2
4B	+	0	1	1
5B	+	1	2	3
6B	+	1	2	3
7B	+	2	3	5
8B	+	1	2	3
9B	+	0	0	0
10B	+	1	1	2
11B	+	0	0	0
12B	+	1	1	2
13B	+	1	1	2
14B	+	1	1	2
15B	+	2	1	3

Table 2
EVIDENCE OF ACRYLIC SCORE VITAMINE B12 COLONIZED WITH CANDIDA ALBICANS AFTER 24h

Table 3
ELEMENTS OF DESCRIPTIVE STATISTICS FINAL SCORES

		Classic Acrylate	Acrylate_B12
Normal	Valid	15	15
	Missing	0.0	0.0
Mean		**3.20**	**1.80**
Stadium Error of Mean		0.449	0.296
Stadium Deviation		1.740	1.146
Skewness		-0.447	-0.538
Stadium Error of Skewness		0.580	0.580
Kurtosis		-0.083	-1.054
Stadium Error of Kurtosis		1.121	1.121
Minimum		0.0	0.0
Maximum		6	3

Table 4
FREQUENCY OF CANDIDA FOR CLASSIC ACRYLATE

Final scores		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	2	13.3	13.3	13.3
	2	2	13.3	13.3	26.7
	3	5	33.3	33.3	60.0
	4	2	13.3	13.3	73.3
	5	3	20.0	20.0	93.3
	6	1	6.7	6.7	100.0
	Total	15	100.0	100.0	

Final scores		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	3	20.0	20.0	20.0
	1	2	13.3	13.3	33.3
	2	5	33.3	33.3	66.7
	3	5	33.3	33.3	100.0
	Total	15	100.0	100.0	

Table 5
FREQUENCY OF CANDIDA FOR B 12 ACRYLATE

samples, 3 and 5 for four test samples, were also synthesized as the data in the table 2.We are interested in whether, after 24 h, between the two groups-group classic acrylate and acrylate group B12-differences statistically significant. For statistical analysis we used SPSS v.17 and MicrosoftExcel 2007.

We are interested if , after 24 h, between the two groups-group classic acrylate and acrylate group B12-differences statistically significant. Fot statistical analysis we used SPSS v. 17 and MicrosoftExcel 2007.

From the table 3 we find that the average scores descriptive statistics for the classic acrylate (value **3.20**) is higher than the average scores of the acrylate B12 (value **1.80**). To determine if this difference is statistically significant we have to use statistical tests, not before checking the normality of both distributions with Shapiro-Wilk and Kolmogorov-Smirnov tests.

From frequency tables, we find the presence of scores of 4,5 and 6.0 (13.3, 20, 6.7% of the total) in the acrylate frequencies that no longer are met in acrylate_B12 lot.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Signifiant	Statistic	df	Signifiant
Classic Acrylate	0.188	15	0.163	0.930	15	**0.270**
Acrylate_B12	0.236	15	0.024	0.840	15	**0.053**

a. Lilliefors Significance Correction

Table 6
TESTS OF NORMALITY

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Classic Acrylate	**3.20**	15	1.740	0.449
	Acrylate_B12	**1.80**	15	1.146	0.296

Tablel 7
STANDARD DEVIATIONS

Paired Samples Correlations

		N	Correlation	Signifiance
Pair 1	Classic Acrylate and acrylate_B12	15	-0.086	0.761

Tablel 8
CORRELATION TABLE SET VALUES

Paired Samples Test

		Paired Differences					t	df	Signifiance (2-tailed)
		Mean	Stadium Deviation	Stadium Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Classic Acrylate - acrylate_B12	1.400	2.165	0.559	0.201	2.599	2.505	14	**0.025**

Tablel 9
SETS VALUES DIFFERENCES

We find in both groups we do not deviate from the distribution normal. Based on the null hypothesis that claims that between the two groups did not differ statistically significant we demonstrate the working hypothesis that the average scores of the two groups of samples is significantly different.

Of the three tables we can conclude that the value for t (value 2.505), degrees of freedom (df = 14) and the significance level p = 0.025 < 0.05, so the test result is statistically significant. We note that the average difference between the two groups is 1.4, so the trend scores in the lot acrylate is to be greater than existence in the lot acrylate B12.

Conclusions

Scanning electron microscopy is currently used for the detection of biofilms on medical tools and implantable devices. Due to the excellent properties of illumination and detection, this type of microscopy is the most used tool for identifying biofilm.

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 test for paired samples, Wilcoxon test samples pairs proved that *Candida biofilm* formed in greater amount at 24h. The average difference between the 2 groups is 0.533, so the trend scores in the batch acrylate extension is to be greater than the existing one in the lot acrylate B12 24 h.

Biologically speaking, this new polymer obtained by cross linked polymerization , with vitamin B12 is better than the classic acrylate, reducing the colonization with *Candida albicans*, such a frequent phenomena among denture wearers.

References

1. ARENDORF, T.M., WALKER, D.M., Arch. Oral. Biol., 25, 1980, p. 1.
2. COLOSI, I., COSTACHE, C., JUNIE M., Fungi & Mycotoxins, 3, no. 1, 2009, p. 242.
3. DORKO, E., JENCA, A., PILIPCINEC, E., DANKO, J., SVICKY, E. , TKACIKOVA, L., Folia Microbiologica , 46, no. 5, 2001, p. 443.
4. LEFEBRE, C.A., SCHUSTER, G.S., MARR, J.C., KNOERSCHILD, K.L., Int. J. Prosthont., 8, 1995, p.122.
5. LEGGAT, P.A., KEDJARUNE, U., Int. Dent. J., 53, no. 3, 2003, p. 126.
6. VASILAS, A., MOLINA, L., HOFFMAN, M., HAIDARIS, C.G., Archives of Oral Biology, 37, 1992, p. 613.
7. PODARIU, A.C., ARDELEAN, L., JUMANCA, D., GALUSCAN, A., RUSU, L.C., Rev. Chim. (Bucharest), **63**, no. 7, 2012, p. 720.
8. GRIGORIU, A.-M., LUCA, C., HOROBA, E., DUNCA, S., Rev. Chim. (Bucharest), **64**, no. 6, 2013, p. 606.
9. LOVELL, L.G., BERCHTOLD, K.A., ELLIOT, J.E., LU, H., BOWMAN, C.N., Polymers for Advanced Technologies, 12, 2001, p. 335.
10. MCCABE, J.F., WALLS, W.G., Applied Dental Materials, 8th Edition, Blackwell Science, London, UK, 1998.
11. RAMAGE, G., LÓPEZ-RIBOT, J.L., Methods Mol. Med., 118, 2005, p.71.
12. GAMAN, S., BORTUN, C.M., MERCUT, V., ARDELEAN, L., RUSU, L.C., Rev. Chim. (Bucharest), **63**, no. 8, 2012, p. 797.
13. BOLOS, O.C., BORTUN, C.M., CERNESCU, A., ARDELEAN, L., BOLOS, A., RUSU, L.C., Mat.Plat., **50**, no. 1, 2013, p. 28.
14. BORTUN, C.M., CERNESCU, A., ARDELEAN, L., Mat. Plast., **49**, no. 1, 2012, p. 5.
15. IOSIF, L., AMZA, O.E., PREOTEASA, E., AMZA G., PREOTEASA, C.T., DUMITRASCU, C., Mat. Plast., **48**, no. 1, 2011, p. 104

Manuscript reiceved: 19.12.2013