IN VITRO STUDY OF *STAPHYLOCOCCUS EPIDERMIDIS* AND *PSEUDOMONAS AERUGINOSA* ADHESION AND COLONISATION INTENSITY ON ORIGINALLY SYNTHESISED BIOMATERIALS WITH VARIOUS CRYSTALLISATION DEGREES AND MODIFIED SURFACES

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Objectives: In the last few years the use of originally synthesised biomaterials in practical medicine has been extensively utilised in Latvia, therefore it is important to carry out studies in order to determine one of the most relevant biomaterial characteristics – their microbial contamination risk. The objective of this study is to explore the adhesion and colonisation degree of two bacteria species on eight originally synthesised biomaterial surfaces.

Materials and methods: Eight sorts of glass-ceramic biomaterials were tested in the study. Glass-ceramics was obtained from glass powder by repeated heating it. Starting materials was three analogous composition batches which were melted in different obstacles. That gave glass powder various tend to crystallize. The crystalline phases and crystallinity rate of glass-ceramics was determined by XRD.

Sample A is sintered from previously crystallized powder, crystalline phases are $Na_4(Nb_8P_4O_{32})$, α-$Ca_2P_2O_7$ and $NaNbO_3$. Samples B and C are from glass powder with great tendency to crystallize and form $NaAlNb(PO_4)_3$ un $Ca_3(PO_4)_2$. And samples D and E are from glass powder with relatively much smaller tendency to crystallize, obtained crystalline phase is not identifiable by XRD. B+, D+ and E+ are the same as described before, just to modify the surface, samples were corroded 30s by H_2O_2, HF and HNO_3 mixture. E and D surfaces before corroding are very flat, while all other are rough.

Bacterial species used in the study are *Ps.aeruginosa* ATCC 27853 and *S.epidermidis* ATCC 12228.

Biomaterial discs were contaminated with 10^3 CFU/ml (colony forming units) of respective bacterial suspensions and after two hours of cultivation the adhesion intensity was determined. Colonisation intensity was determined after 48 and 72 hours. Adhesion and colonisation degree was evaluated with a scanning electron microscope (SEM), as well as a plate count method. CFU quantity was calculated as 1 mm^2 of biomaterial disc surface. Adhesion intensity on both biomaterial surfaces was compared by using the Fisher's chi- square test ($p<0.05$).

Results:

Table No 1 Bacterial Adhesion Intensity after 2 hours (CFU/mm^2):

	A	B	B+	C	D	D+	E	E+
S.epidermidis	0.050	0.005	0.005	0.026	0.010	0.048	0.005	0.010
Ps.aeruginosa	0.048	0.005	0.016	0.050	0.026	0.050	0.010	0.032

Table No 2 Bacterial Colonisation Intensity after 48 hours (CFU/mm^2):

	A	B	B+	C	D	D+	E	E+
S.epidermidis	186	132	106	196	79	96	53	117
Ps.aeruginosa	394	239	186	399	106	122	80	186

Table No 3. Bacterial Colonisation Intensity after 72 hours (CFU/mm^2):

	A	B	B+	C	D	D+	E	E+
S.epidermidis	1255	835	1042	1245	835	607	420	1265
Ps.aeruginosa	2926	2505	2090	3340	1670	1255	835	2505

Conclusions:

1. Using SEM it was determined that *Ps.aeruginosa* forms a denser layer with polypoid outgrowths and channels between them, the biofilm layer covers the entire biomaterial. *S.epidermidis* forms less frequently scattered, more compact (denser) colonies.
2. Using the plate count method it was determined that *Ps.aeruginosa* has a more distinct adhesion and colonisation degree on the entire biomaterial surface. After 72 h colonisation degree of *Ps.aeruginosa* is at least twice as much as of *S.epidermidis.*
   Colonisation intensity within a 48 and 72 hour period on different biomaterial surfaces varies.Colonisation intensity does not correlate with adhesion intensity.