The development of resins represented a great step forward in dental technique, the first thermopuremiserable acrylic resins being developed in 1936 [1].

According to EN ISO 1567 there are four types of resins: thermo polymerized resins (upper 65°C) (either bi-component, or monocomponent), self polymerized (lower 65°C), thermoplastic resins in powder form, photo-polymerized and microwave polymerized resins [2].

Different acrylic resins are widely used today both in pediatric dentistry and orthodontics. They are used for the manufacturing of space maintainers, interceptive devices or removable orthodontic appliances (R.O.A.).

Uncontrolled biofilm formation is a major concern in individuals receiving medical devices such as implants, removable appliances, intubation tubes and catheters. Microorganisms embedded in a hydrated polymeric matrix of biofilms are much more tolerant to antimicrobial agents than are planktonic microorganisms [3].

Although seemingly that only the surface of the R.O.A. is contaminated, the microorganisms that form biofilms also penetrate in the pores and cracks of the acrylic resin generating a real reservoir of bacteria.

Acrylic baseplates of removable orthodontic appliances worn by children were contaminated by mutans streptococci (MS) colonies/biofilms in all cases after 1 week [4].

Biofilms on removable orthodontic appliances act as reservoir of microorganisms, capable of modifying the environmental condition of oral cavity and are difficult to be removed with routine hygiene measures [5].

A contaminated acrylic space maintainer can induce local infections (oral mucosa stomatitis) as well as systematically ones (of upper airways, lungs, kidneys). Dentists, orthodontists, dental-lab technicians dealing with infected R.O.A. have also a high risk through possible cross-contamination.

All R.O.A. are composed of porous materials in which microorganisms (fungi, bacteria and viruses) infiltrate, attach and form biofilms, a 3-dimensional protective matrix in which they build-up. Especially Candida albicans penetrates deeply the pores and cracks of the polymer [6].

Acrylic resin with persistent antimicrobial activities represents a promising method for preventing bacteria and fungus-induced stomatitis, an infectious disease commonly associated with the wearing of removable orthodontic appliances [7].

Oral biofilms are functionally and structurally organized polymicrobial communities embedded in a self produced extracellular matrix of exopolymers on mucosal, dental and/or oral device surfaces [8]. The oral cavity is a unique environment in the human body, characterized by near-constant presence of liquid water (in saliva), short term extreme temperature fluctuation, externally exposed hard surfaces and by wide variation in carbon and nitrogen input, including a basal component (saliva) that is complex but contains only limited bacterial energy source [9]. The oral
The oral environment is colonized by a complex microbiota that grows and lives as diverse biofilms on all mucosal and dental surfaces. This microbiota includes protozoa, yeasts, mycoplasmas, Archeae, and bacteria. Bacteria are the most numerous and diverse group from which only about half of are culturable [8]. There are over 700 different bacterial species in the oral microflora which colonize the teeth, tongue, oral mucosa, hard palate, carious lesions, periodontal pocket and other surfaces or synthetic materials [10].

The properties of the oral environment determine which organisms colonize, grow and predominate, and result in biofilms with distinct species composition in various habitats of the oral cavity. As a result of the dynamic balance imposed by numerous microbial interactions, component species at a colonization site can remain relatively stable over time but environmental changes may lead to rearrangement in community structure and composition, which can predispose the host site to disease [11].

Candida species are commensal fungi that are found in 30-50% of human oral cavities. Under certain conditions from commensal fungi can transform into opportunistic pathogens that can lead to superficial mucosal and systemic infections. Moreover, Candida species are considered the primary causative agents of denture stomatitis, an oral pathology in denture-bearing patients, particularly under the maxillary prosthesis [12].

**Experimental part**

For this study, two types of materials were chosen in order to test their influence on biofilm formation. For biofilm quantification a strain of Candida spp. was selected among 12 strains isolated from 23 healthy pediatric patients, 7 to 12 years of age, wearing space maintainers or removable orthodontic appliances for at least 4 months at the time of 12 years of age, wearing space maintainers or removable orthodontic appliances. For biofilm formation the strains isolated from 23 healthy pediatric patients, 7 to 12 years of age, wearing space maintainers or removable orthodontic appliances were used to test their influence on biofilm formation. For this study, two types of materials were chosen in order to test their influence on biofilm formation. For biofilm quantification a strain of Candida spp. was selected among 12 strains isolated from 23 healthy pediatric patients, 7 to 12 years of age, wearing space maintainers or removable orthodontic appliances.

**Results and discussions**

Results of the microtiter plate assay revealed the strain which had the best capacity to develop a denser biofilm (170 µL) to each well for distaining, for 30 min, and then the OD was measured at 540 nm using an ELISA reader.

The materials used in this experiment were as follows: the cold-cure acrylic Palapress®vario (Heraeus-Kulzer GmbH, Hanau, Germany) and the visible light acrylic polymerizable system Eclipse (Dentsply DeTrey, Konstanz, Germany).

Palapress®vario is a pourable, cold-curing powder and liquid denture base material. The mixing ratio that we used was: 10 g of methylmethacrylate copolymer powder to 7 mL acrylic liquid.

Eclipse is a visible light polymerizable system with a substantially reduced manufacture time for removable orthodontic appliances or space maintainers. Eclipse is composed of urethane oligomers, a class of materials which has found wide acceptance in various dental applications and is free of methyl, ethyl, propyl or butyl monomers. From the producer technical data (www.dentsplymea.com) we noticed an extended hydrolytic stability and the fact that plaque growth on devices appears to be equal to or better than that of a typical acrylic appliance. The material was light polymerized in a special developed processing unit from the Department of Dental Technology, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy.

From each type of material, square coupons were made (1.5x1.5 cm). A 200 µm of Candida spp. inoculum that matches 0.5 McFarland standards in saline solution (0.9%), diluted 1:30 in SDB growth medium was placed on each coupon. The coupons containing the culture medium and Candida spp. were incubated at 37°C for 48h in 100% UR chamber.

After the incubation period the coupons were rinsed with distilled water, stained with acridine orange (AO) in acetate buffer solution (Sigma) for 2 min at room temperature, rinsed in pure water, air dried at room temperature and examined using the Leica DM 2500 microscope’s 63x oil immersion objective with 1.3 numerical aperture. Fifteen randomly selected fields (176x132 µm) on the surface of each coupon were analyzed by microscopy and image processing. The fields were scanned at 400 Hz using a 488 nm argon laser at 25% intensity, also using the Leica DM 2500 microscope’s 63x oil immersion objective with 1.3 numerical apertures. The confocal images obtained were analyzed using COMSATAT 2, special computer software designed for biofilm quantification [14, 15]. The following parameters were measured and compared: biomass, average thickness and maximum thickness, surface to volume ration, coverage percentage, diffusion distance and average colony size.

**Fig.1. Removable orthodontic appliances with poor hygiene and biofilms under the secondary orthodontic springs**
The CLSM images showed that the biofilms formed were predominantly composed of blastospore cells on both types of surfaces tested (fig. 3). These findings can be related to the pH value of the culture media or to other components in the culture media (glucose or sucrose), as it was described in other studies [16].

The analysis of biofilms using Comstat 2 software showed that in both materials used, the average maximum thickness of the biofilm was above 10 μm (table 1). Differences were observed between the average thickness of the biofilm. The thickest Candida spp. no. 28 biofilm was formed on Palapress acrylic material with an average thickness of 10.84 μm. Mean biofilm thickness provides a measure of the spatial size of the biofilm and is the most common variable used in biofilm literature. Santana et al. (2013) obtained similar results regarding average thickness, using a Candida albicans strain (ATCC 90028) on poly(-methylmethacrylate) (PMMA) acrylic resin coated with saliva and YNB (yeast nitrogen base) culture media supplemented with glucose (1%) or sucrose (1%) [16].

No significant differences were observed in biomass development of the biofilm between the two types of materials tested (table 1). Lower values of biomass were obtained in a study on PMMA resin after 48 h in YNB supplemented with 100 mM glucose [17]. Faot et al. (2014) in a study on efficacy of citric acid denture cleanser and the effects of residual biofilm and recolonization, obtained higher biovolume for the biofilm formed by Candida albicans (ATCC 90028) on PMMA in YNB supplemented with glucose 100 mM for 72 h (the medium was changed every 24 h) [18].

Average and maximum diffusion distance indicate the distance over which nutrients and other substrate components have to diffuse to the voids to the microorganism within the biofilm colony [15]. Insignificant differences were observed regarding the average and maximum diffusion distance in the Candida spp. no. 28 biofilm formed on tested materials.

Differences were observed in average colony size. The area sizes of microcolonies at the substratum provide valuable information about the organization of the biofilm community. The differences recorded could be due to material characteristics. This is consistent with recent studies showing that a significantly higher proliferation of Candida albicans was observed on the surface of denture base materials with the highest polar contribution to surface free energy (i.e. Eclipse, Mucopren), and this result supports the assumption that there might be a relationship between the polar contribution to surface free energy of the substratum material and Candida albicans proliferation [19]. The biofilm formed on Palapress material surfaces is characterized by tall (10 μm average thickness, 16 μm maximum thickness) and large microcolonies (43 μm²) compared to the biofilms formed on Eclipse material surfaces which are characterized by low (8 μm average thickness and 13 μm maximum thickness) and small microcolonies (29.9 μm²).

In regards to the coverage percentage, the biofilm formed on Eclipse material surfaces covers 66% of the total area, while on Palapress material the biofilm covers only 40% of the total area. These results explain why there are no differences in biomass between the biofilms formed on both materials despite the thickness and average colony size. While the biofilm formed on Palapress material has microcolonies that are taller and larger, it present less microcolonies on a given surface, compared to the number of microcolonies formed by Candida spp. no. 28 on Eclipse material. In a study on biofilm-forming ability and pathogenicity, Candida isolates were cultured on two different materials (silicon and acrylic resin). The biofilm formed on silicone surfaces had a significant higher biomass (2.17-6.61 mg) compared to the biofilm formed on acrylic resin surfaces (0.25-1.50 mg) [20]. Estivill et al. (2011) in a study on biofilm formation of 84 strains of Candida, three clinical materials (Teflon™, PVC, polyurethane) were tested and concluded that C. albicans, C. parapsilosis and C. tropicalis produced more biofilm on
Teflon™ compared to PVC and polyurethane, whereas *C. glabrata* and *C. krusei* biofilms showed no differences among the three materials [21].

The *Candida albicans* colonies on a new polymer used to create complete dentures were studied in [22].

**Conclusions**

*Candida* spp. was able to form biofilm on both plastic materials tested. Differences were observed in average thickness and maximum thickness of the biofilm. The thickest biofilm was formed on Palapress®vario material. The microcolonies of the biofilm formed on Palapress®vario were larger and they occupied a greater area compared to those formed on Eclipse material. The coverage percentage was higher for the biofilm formed on Eclipse material which explains the insignificant differences in the biomass values between the biofilms formed on the tested materials.

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**References**

1. ARDELEAN L., BORTUN C., MOTOC M., RUSU L., Mat. Plast., 47, no.4, 2010, p.433
6.*** www.msi-lab.com