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Evaluation of the microbial, cytotoxic and physico-chemical properties of the stainless steel crowns used in pediatric dentistry

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Preformed stainless steel crowns are used in pediatric dentistry to obtain full crown restoration of primary molar teeth. They are consider the best restoration in terms of durability and effectiveness. The purpose of this study is to evaluate microbial, cytological and physio-chemical properties to determine whereas stainless steel crown are biocompatible, safe for surrounding tissue and helpful in avoiding micro-organisms influence on the tooth tissue. Based on the results, it was determined that stainless steel crowns used in pediatric dentistry represent no cytotoxic risk to the surrounding tissues, have a low probability of developing hypersensitivity to the coronal material and also that their biological properties make them suitable to use in pediatric dentistry for the reconstruction of damaged primary molar tissue.

Key words: *pediatric crowns, primary teeth, teeth restoration, microbial biofilm, physico-chemical properties, cytotoxicity, material composition*

1. Introduction

Pediatric dentistry encounters many challenges in daily practice. First and foremost among them is the difficult cooperation associated with the age of the patient. The treatment needs of young patients are high, based on the U.S. Department of Health and Human Services, 23% of children aged 2–5 years have caries in deciduous teeth, while for children between 6–8 years this number rises to 52% [9]. The method of treatment and subsequent restoration should, therefore, be as minimally traumatic as possible and, at the same time, should be associated with the best possible prognosis to avoid the need for retreatment or complications such as pain, endodontic treatment, tooth extraction, or systemic complications such as fever, weakness, weight loss, periapical abscesses, metastatic abscesses and odontic sepsis [1]. The restoration of the tooth is therefore a very important element, determining the success of the treatment. Various materials are used for restoration in deciduous teeth [37]. Many studies suggest that the restoration for deciduous molars that determines the best prognosis are prefabricated stain-

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less steel crowns (SSC) [10], [16], [27], [31], [35]. They are ready-made full-crown restorations, available in kits. Each kit has several crown sizes dedicated to a particular deciduous molar. The crown is then placed according to the manufacturer's instructions by cementing it on the tooth with luting cement [17]. The advantages of such a restoration are the achievement of a full-crown restoration with a long term survival rate, the possibility of restoring teeth with advanced destruction, teeth after endodontic treatment or teeth with developmental disorders of the hard tissues of the tooth [34]. However, many authors point out the possible risk aspects of using a permanent restoration such as prefabricated pediatric crowns in the oral environment. The first aspect is the physico-chemical composition of the crowns and their effect both on the surrounding oral soft tissues and entire body. The second aspect of this composition is connected directly to microbiological aspect and the potential associated risk of inflammatory reaction. Last but not least aspect is the cytotoxic aspect of the biomaterial from which the crown is made. In this work, the results of a study of stainless steel crowns used in pediatric dentistry, evaluating their properties in a broad aspect, will be presented. Physio-chemical assay were performed by using FTIR spectroscopy, XRD patterns to identify the composition of SSC and optical profilometer for measuring the surface roughness of the crown. Then the microbiological aspect of SSC was evaluated. The susceptibility of the stainless steel crown biomaterial to microbial adhesion and biofilm formation was appraised. For this purpose, quantitative and qualitative assessment of adhesion and biofilm formation ability was performed using *Streptococcus mutans* (ATCC 25175), *Lactobacillus rhamnosus* (ATCC 9595), *Candida albicans* (ATCC 90028) and *Candida albicans* (ATCC 10231) strains. The biofilm-forming capacity was then evaluated using Leica SP8 confocal microscopy with an HC Fluotar L 25× water objective. Next, the histological properties of the crowns were evaluated using the Balb/3T3 normal mouse fibroblast line (clone A31, American Type Culture Collection ATCC® CCL-163™, Manassas, USA) which is one of the models used to evaluate in vitro cytotoxicity of biomaterials.

2. Materials and methods

The subject of the study is 3M ESPE Stainless Steel Crowns used in pediatric dentistry manufactured for the European market in 3M Germany GmbH. The crowns are used for the restoration of first and second deciduous molar, each comes with 6 available crown sizes numbered from size 2 to 7. The crowns are distributed in kits containing 48 or 96 crowns, it is also possible to purchase a package containing 2 crowns of a certain size. The tests were performed on crown catalog number E-LL-4 (lower left second molar size 4). Size 3 and 4 are commonly used sizes in clinical practice. Tests with the use of computer microtomography 1172 SkyScan, Bruker showed that the wall thickness of these crowns is in the range (0.11–0.21) mm (Fig. 1B). According to safety data sheet the composition of the crown contains 100% stainless steel CAS Nbr 12597-68-1. These crowns were chosen for the study because of their widespread use in pediatric dentistry for restoring deciduous molars. These crowns are pre-trimmed, belled and crimped for fast and easy placement and the manufacturer assures their superior longevity.

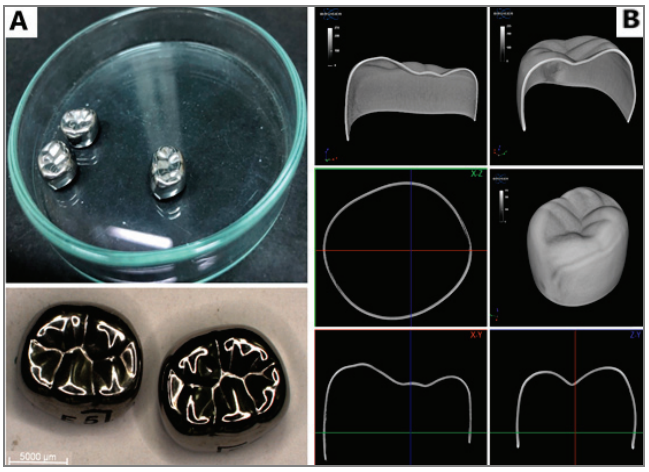

Fig. 1. Pediatric stainless steel crowns used in the procedure. A) Images obtained in an optical microscope; B) restoration and cross-sections of one of the crowns obtained using a 1172 SkyScan computer microtomograph, Bruker

The purpose of the study was to analyze the composition, properties of the stainless steel crown biomaterial, its potential cytotoxic properties and its ability to form a bacterial biofilm, which is crucial in terms of maintaining oral health. One stainless steel crown was used for physico-chemical and histological examinations each. Microbiological examination required 8 crowns as samples for quantitative method, 4 crowns as samples for microscopic examination and 1 crown as a negative control. The study samples were sterilized in a Vacuklav 24B+ autoclave (Germany, Melag), using the steam method in compliance with the European standard EN 13060. Sterilization was carried out at 121 °C, 1.1 bar, 20:30 minutes.

2.1. Physico-chemical assay

X-Ray Diffraction (XRD) measurements were made on the X'Pert PRO X-ray diffractometer (Cu Kα1, 1.54060 Å) (PANalytical, Malvern Panalytical Ltd., Malvern, UK); FT-IR spectra (Fourier Transform Infrared) measurements were performed on a Thermo Scientific Nicolet iS50 FT-IR spectrometer equipped with an ATR module (iS50 ATR) (Waltham, MA, USA). The source of infrared radiation was a HeNe laser. Scanning electron micro-scope (SEM) micrographs were made on an FEI Nova NanoSEM 230 microscope (Hills-boro, OR, USA). The energy dispersive spectra were measured on FEI Nova NanoSEM 230 scanning electron microscope (Hillsboro, OR, USA) equipped with energy dispersive spectrometer (EDS; EDAX Genesis XM4; Hillsboro, OR, USA). Roughness measurement was carried out using a Leica DCM8 optical profilometer at 20× magnification. The reconstruction of the sample crown was obtained using the computer microtomograph 1172 SkyScan, Bruker (Kontich, Belgium), with the parameters of the X-ray source: 100 kV/100 µA, with a resolution of 8 µm, a unit rotation step of 0.49 degrees and a exposure time of 1165 ms [21].

2.2. Microbiological assay

To evaluate the susceptibility of the stainless steel crown biomaterial to microbial adhesion and biofilm formation, reference strains from the American Collection of Type Cultures were selected: *Streptococcus mutans* (ATCC 25175), *Lactobacillus rhamnosus* (ATCC 9595), *Candida albicans* (ATCC 90028) and *Candida albicans* (ATCC 10231) [14]. The dental material consisted of prefabricated pediatric steel crowns from 3M (Fig. 1). The crowns were steam sterilized at 121 °C.

In the quantitative method, a suspension of microorganisms corresponding to a McFarland density of 1.0 (3×10^8 CFU/ml) for bacteria and 0.5 (1.5×10^6 CFU/ml) for fungi was prepared from fresh cultures of the strains analysed. To obtain suspension of strains, liquid Brain Heart Infusion Broth – BHI (Biomaxima, Lublin, Poland) with 5% sucrose, liquid Man-Rogosa-Sharpe Broth – MRS (Biomaxima, Lublin, Poland) with 5% sucrose and liquid Sabouraud Dextrose Broth (Biomaxima, Lublin, Poland) with 5% sucrose were used, correspondingly, for *S. mutans* and *L. rhamnosus* and *C. albicans*. Sterile stainless steel crowns were inserted into the suspension of microorganisms prepared in this way [38]. After the incubation period (*S. mutans* – 37 °C, 48 h under aerobic conditions with elevated CO_2, *L. rhamnosus* – 37 °C, 48 h under anaerobic conditions; *C. albicans* – 37 °C, 48 h under aerobic conditions), the materials were rinsed 3 times in NaCl and shaken in 1 ml of 0.5% saponin solution (Sigma-Aldrich, Poznań, Poland) for 1 minute. The obtained suspension of microorganisms, desorbed from the surface of the material, was quantitatively inoculated onto solid media suitable for the microorganism (BHI AGAR, MRS AGAR, SABOURAUD AGAR – Biomaxima, Lublin, Poland). After the incubation period, the grown colonies were counted and the number of colony-forming units per millilitre of suspension (CFU/ml) was evaluated [30], [33], [36]. The CFU/ml value was calculated according to the formula:

$$\text{CFU/ml} = \text{average number of colonies} \times \text{inverse of dilution} \times 10$$

The test was conducted in 2 repetitions.

For qualitative evaluation, a suspension corresponding to a density of 1.0 on the McFarland scale (3×10^8 CFU/ml) for bacteria and 0.5 (1.5×10^6 CFU/ml) for fungi was prepared from an 18-hour culture of the analyzed reference strains. The liquid medium Brain Heart Infusion Broth – BHI (Biomaxima, Lublin, Poland) with 5% sucrose, liquid medium Man-Rogosa-Sharpe Broth – MRS (Biomaxima, Lublin, Poland) with 5% sucrose and liquid medium Sabouraud Dextrose Broth (Biomaxima, Lublin, Poland) with 5% sucrose were used to obtain suspension of strains, correspondingly for *S. mutans* and *L. rhamnosus* and *C. albicans*. Dental material was introduced into the suspension prepared in this way and the whole thing was incubated (48 h, 37 °C, aerobic conditions for *C. albicans*, anaerobic for *L. rhamnosus* and at elevated CO_2, microaerophilic for *S. mutans*). After the incubation period, the biomaterial was rinsed 3 times in NaCl and transferred to 2 ml liquid TSB medium with 100 µl of 1% (10 mg/ml) 2,3,5-phenyltetrazolium chloride (TTC) for bacteria and TSB with 0.1% (1 mg/ml) 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) with 10 µl Menadione in acetone for fungi. The incubation period and detection criteria depended on the tetrazolium compound used. For *S. mutans* and *L. rhamnosus*, a TTC assay was performed. After the incubation period (24 h, 37 °C), the samples were washed and the presence of red formazan formed as a result of TTC reduction by live microorganisms was evaluated. The degree of TTC reduction was determined according to a 5-point scale: 0 – no reduction of TTC, 1(+) – spot reddening of the surface

of the biomaterial, 2(++) – reddening of the surface of the biomaterial, 3(+++) – reddening of the entire surface of the material and pinking of the substrate, 4(++++) – strongly reddened entire surface of the material and reddening of the substrate. For *C. albicans*, an MTT assay was performed. After a 3 h incubation period (37 °C), the samples were rinsed and the presence of purple formazan formed as a result of MTT reduction by live yeast cells was evaluated. The degree of MTT reduction was determined according to a 5-point scale: 0 – no reduction of MTT, 1(+) – spotty appearance of purple colour on the surface of the biomaterial, 2(++) – purple part of the surface of the material, 3(+++) – purple colour of the entire surface of the biomaterial and substrate stained in this colour, 4(++++) – strongly purple entire surface of the material and substrate. Then, for confocal microscopy, a bacterial suspension was prepared from fresh cultures of the strains analysed, with a McFarland density of 1.0 for *S. mutans* and *L. rhamnosus* and a density of 0.5 for *C. albicans*. 1 ml of the suspension thus prepared was incubated with the biomaterial (48 h, 37 °C; aerobic with elevated CO_2, anaerobic or aerobic). After the incubation period, the material was rinsed in NaCl and introduced into the LIVE/DEAD dye mixture. After a 30-minute incubation period, the material was washed and evaluated using a Zeiss Cell Observer SD confocal microscope. Samples were covered with a No. 1.5 coverslip before imaging and imaged on a Leica SP8 confocal microscope using an HC Fluotar L 25× water objective (numerical aperture of 0.95) [7], [11], [29]. The confocal aperture size was 1 AU. The LIVE dye (Syto9) was excited with a 488 nm laser (recorded emission was in the 493–539 nm range), the DEAD dye (propidium iodide) was excited with a 561 nm laser (recorded emission was in the 592–676 nm range). In addition to the fluorescence of the dyes, the reflected light of the 561 nm laser was collected to visualize the coronal surface. For each sample, several representative fields of view were imaged and scanned in the *Z*-axis (confocal stack thickness 20–90 μm, optical section spacing 0.566–1 μm). Background noise was reduced using a median filter (3 × 3 pixels). Visualizations were made in Imaris software – LIVE dye in the images in green, DEAD dye in red, coronal surface in blue.

2.3. Cytotoxicity assay

Cytotoxicity assays were performed on the Balb/3T3 mouse fibroblast line (clone A31, American Type Culture Collection ATCC® CCL-163™, Manassas, U.S.A), which is one of the models used to evaluate *in vitro* cytotoxicity of biomaterials (PN-EN ISO 10993-5:2009 Biological evalution of medical devices – Part 5: Tests for *in vitro* cytotoxicity) [35]. Balb/3T3 cells were cultured in DMEM medium with 4.5 g/L glucose, 25 mM HEPES (Lonza), supplemented with 1% *L*-glutamine with streptomycin and penicillin (Sigma-Aldrich®, St. Louis, MO) and 10% fetal calf serum (CS, Sigma-Aldrich®, St. Louis, MO, USA), under standard conditions: 37 °C, 5% CO_2, at constant humidity, HERA cell CO_2 150i incubator, (Thermo Scientific, Waltham, MA, USA). Balb/3T3 cells were trypsinized (0.25% Trypsin – EDTA, Sigma-Aldrich®), resuspended in complete culture medium and seeded in a 6-well plate (TPP, Trasadingen, Switzerland) at 1.5×10^5 cells/well. After 24 h, test material was placed in the wells with fibroblasts. After 24 h of incubation of the fibroblasts with the material, the morphology of the cells was evaluated under an inverted CKX53 phase-contrast microscope (Olympus, Tokyo, Japan). Under the disc, near the disc, and in the rest of the well. The control in the study was a culture conducted in full medium and under standard conditions, which was not in contact with the test material. The degree of cytotoxicity of the test describing in PN-EN ISO 10993-5:2009 Biological evalution of medical devices – Part 5: Tests for *in vitro* cytotoxicity), where changes in cell morphology are described as none (where no changes are observed), low (some cells degenerated under the sample), moderate (degenerated cells only under the sample and up to 1 cm around the material) and high (degenerated cells in the whole cell culture). The above grades are numbered from 0 to 4. Changes above grade 2 are considered to be cytotoxic effects of the tested material [35].

3. Results

3.1. Physico-chemical assay results

The tests of physico-chemical properties of pediatric stainless steel crowns were divided into several stages. The first of them was the analysis of the phase composition of the material from which the crowns were made (X-Ray Diffraction and FT-IR spectra). Elements contained in crowns, especially in the process of use,

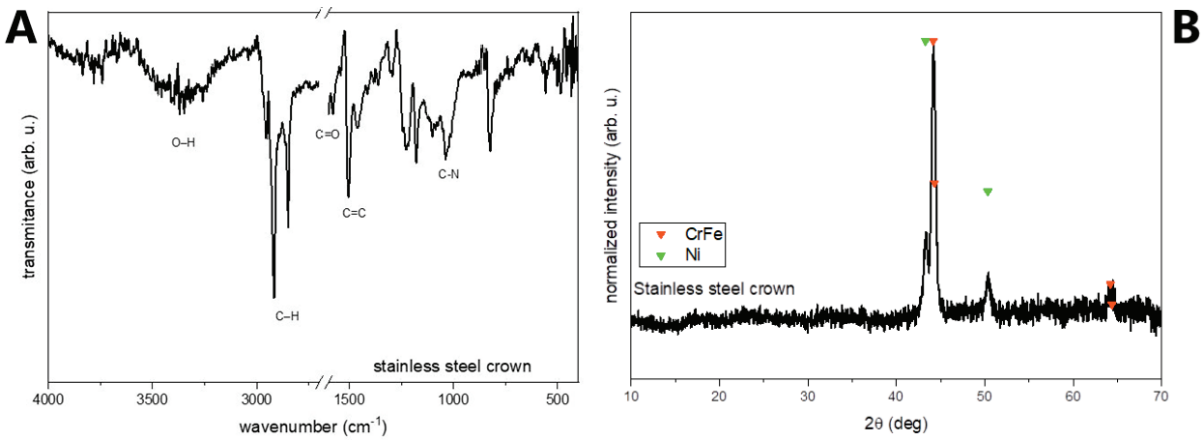

Fig. 2. A) FTIR spectrum of stainless steel crown. B) XRD pattern of stainless steel crown

may be released into the oral cavity and may be toxic to the tissues around the crown.

In Figure 2A, the FTIR spectrum with assigned characteristic bands for the stainless steel crowns is presented. The band at 1031 cm^{-1} originate from C-N stretching vibration, the bands at 1459 cm^{-1} and 1502 cm^{-1} are related to the C=C stretching vibrations, the band 1574 cm^{-1} comes from C=O stretching vibrations and intense bands at 2848 cm^{-1} and 2913 cm^{-1} originate from C-H asymmetrical stretching vibrations. There is also visible weak O-H stretching vibration with maximum at 3350 cm^{-1}. XRD patterns of stainless steel crowns are presented in Fig. 9.

In Figure 2B, the XRD pattern of stainless steel crown is shown. There are characteristic peaks visible for CrFe alloy and for Ni, which means that this crown is made from steel with nickel addition. To check the exact composition of stainless steel crown, EDS meas-

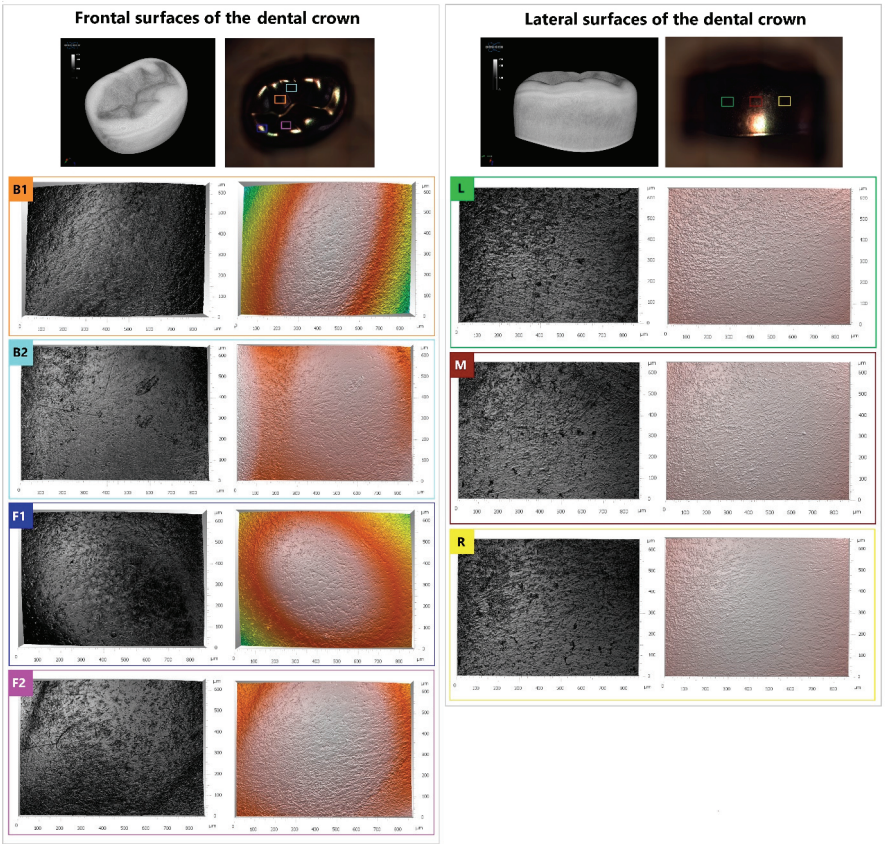

Fig. 3. Profilograms obtained with the Leica DC8 optical profolometer, for different places of the lateral (L-M-R) and frontal (B1-B2-F1-F2) surfaces of the crown. L – left lateral region, M – middle, R – right lateral crown region; B1 and B2 – the front surface of the crown, F1 and F2 – the top surfaces of the crown

urements were performed at three different areas of the sample. The averaged composition of crown is presented in Table 1.

Table 1. Average wt. % of elements in stainless steel crown

Element	wt. %
Fe	66.45
Cr	17.84
Ni	8.06
C	4.81
Al	2.05

Pediatric crown roughness tests were carried out in 7 places of the crown, dividing it into the lateral surface of the crown, and then the roughness was determined for the L-M-R areas, and the frontal surface of the crown, differentiating the areas of the masticatory surface (B1, B2) and crown sutures (F1, F2). The tests were carried out using the Leica DCM8 optical profilometer using 20× magnification, the analysis area of each area was 600 × 800 [um].

Based on the obtained profilograms (Fig. 3), 2 roughness profiles were analyzed (in the longitudinal and transverse directions), on the basis of which the following roughness parameters were determined: *Ra*, *Rq*, *Rz* (Table 2).

The mean values of roughness parameters are summarized in Table 2. The roughness of the material is related to the shape, but – above all – to the finishing treatment of the implant, especially to polishing. Therefore, the lowest roughness values are found in areas with large flat, easily polished surfaces (M), which are located on the lateral surface of the implants, and the largest are in the areas of crown rubble (B1).

3.2. Microbial adhesion and biofilm formation results

In Figure 4, a macroscopic image of the quantitative culture of the suspension desorbed from the surface of the biomaterial is shown. The obtained values of colony-forming units for individual microorganisms are summarized in Fig. 5 and Table 3.

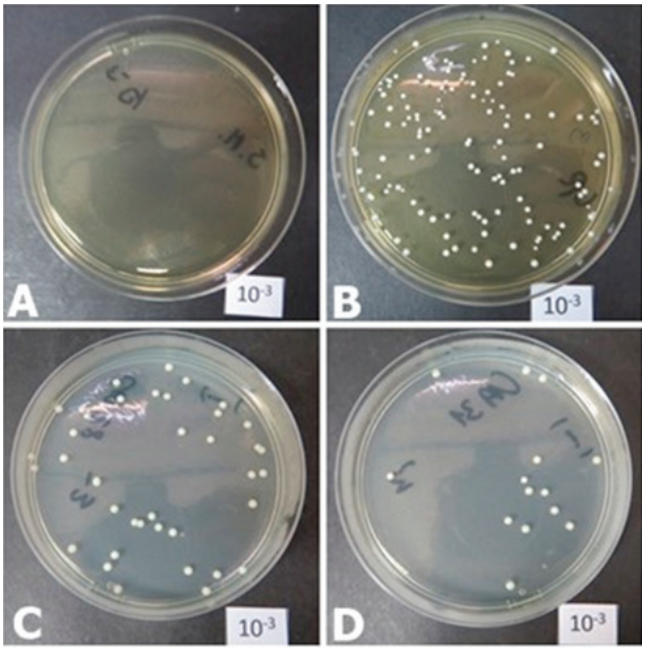

Fig. 4. Quantitative culture of the suspension of analyzed strains (A – *S. mutans* 25175; B – *L. rhamnosus* 9595C; C – *C. albicans* 90028; D – *C. albicans* 10231)

In Figure 5, a graph containing the level of adhesion of individual microorganisms to the coronal surface is presented.

Table 2. Comparison mean and SD value of *Ra*, *Rq* and *Rz* roughness parameters for different regions of the pediatric crown

	top-to-base crown direction			transverse direction		
	Lateral surface of the dental crown					
µm	*Ra*	*Rq*	*Rz*	*Ra*	*Rq*	*Rz*
L	0.880 ± 0.16	1.216 ± 0.26	6.514 ± 1.58	0.740 ± 0.03	0.990 ± 0.05	5.084 ± 0.25
M	0.412 ± 0.01	0.513 ± 0.02	2.487 ± 0.36	0.565 ± 0.03	0.799 ± 0.01	4.508 ± 0.35
R	0.427 ± 0.02	0.522 ± 0.06	2.590 ± 0.81	1.561 ± 0.29	1.876 ± 0.32	5.230 ± 0.59
	Lateral surface of the dental crown					
µm	*Ra*	*Rq*	*Rz*	*Ra*	*Rq*	*Rz*
B1	1.156 ± 0.36	2.053 ± 0.73	7.396 ± 2.29	4.870 ± 0.16	5.387 ± 0.75	9.180 ± 2.77
B2	0.642 ± 0.04	1.020 ± 0.13	4.156 ± 0.17	2.181 ± 0.21	2.780 ± 0.36	7.408 ± 2.11
F1	0.881 ± 0.33	1.162 ± 0.44	4.486 ± 1.14	1.768 ± 0.07	2.851 ± 0.26	10.543 ± 1.95
F2	0.771 ± 0.09	0.985 ± 0.1	5.102 ± 0.65	6.674 ± 1.37	7.725 ± 1.31	15.389 ± 4.93

Table 3. Number of colony-forming units per milliliter of suspension (CFU/ml)

Culture strains	*S. mutans*		*L. rhamnosus*		*C. albicans 28*		*C. albicans 31*	
	I	II	I	II	I	II	I	II
CFU/ ml	0	0	4.3×10^6	5.4×10^6	3.8×10^5	1.2×10^5	1.9×10^5	1.4×10^5
Mean	0		$4.85 \times 10^6 \pm 7.78 \times 10^5$		$2.5 \times 10^5 \pm 1.84 \times 10^5$		$1.65 \times 10^5 \pm 3.54 \times 10^4$	

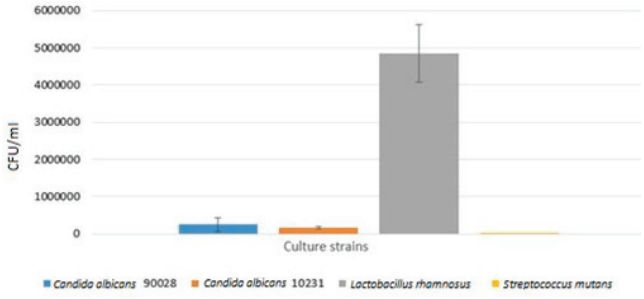

Fig. 5. Level of adhesion of microorganisms to the surface of dental crowns

The qualitative method of biofilm formation by the degree of reduction of TTC and MMT by the analyzed reference strains is shown in Figs. 6 and 7 and in Table 4.

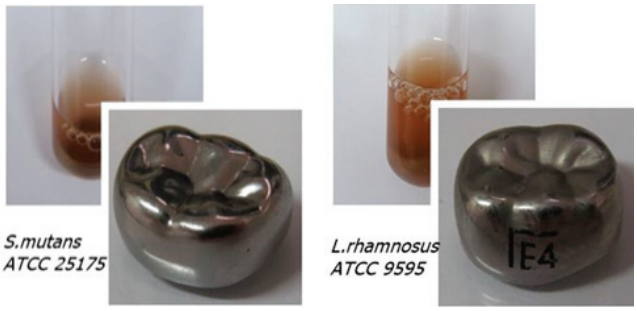

Fig. 6. The degree of TTC reduction by strains of *S. mutans* and *L. rhamnosus*

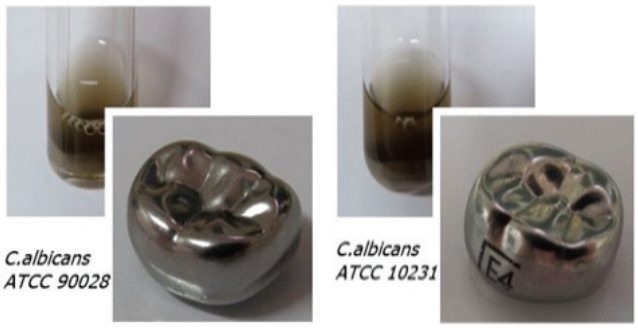

Fig. 7. The degree of MTT reduction by model strains of *C. albicans*

The overall results shows no adhesion properties of the *S. mutans* strain to the surface of the pediatric stainless steel crowns. The highest adhesion properties to the surface of the dental material were noted for *L. rhamnosus* strain. Both *C. albicans* 90028 and *C. albicans* 10231 presented similar low adhesion to the surface of the dental material.

3.3. Confocal microscopy results

The results of confocal microscopy visualisation are shown below. Selected cross-sections of the tested biomaterial and result of the adhesion of individual microorganisms are shown in Fig. 8.

The results shows low adhesion for both *C. albicans* strains whereas *L. rhamnosus* and *S. mutans* show greater adhesion to the stainless steel surface of the crown. Both *L. rhamnosus* and *S. mutans* were presented mostly by alive microbial colonies.

3.4. Histology results

The evaluation of the morphological changes of the fibroblasts after temporary contact with the dental crown and their degree of cytotoxicity are given in Fig. 9. The cells, fibroblasts showed normal morphology, no differences were observed at the edge of the sample, 1 cm from the sample and further away from the sample in relation to the control culture. The tests carried out showed a lack of cytotoxicity of the dental crowns, the degree of toxicity was found to be 0 on a four-step scale for assessing toxicity.

Dental stainless steel crowns did not show cytotoxic effects using the method of direct contact method. Dental crowns did not alter the morphology of fibroblast cultures of lineage Balb/3T3. The degree of cytotoxicity of the dental crowns, was determined to be 0 degree. This indicates the absence of toxicity of the dental crowns evaluated.

Table 4. The degree of TTC and MTT reduction for the analyzed reference strains

	Control group [–]	*S. mutans*	*L. rhamnosus*	*C. albicans 28*	*C. albicans 31*
TTC reduction	–	–	+++	×	×
MTT reduction	–	×	×	+	++

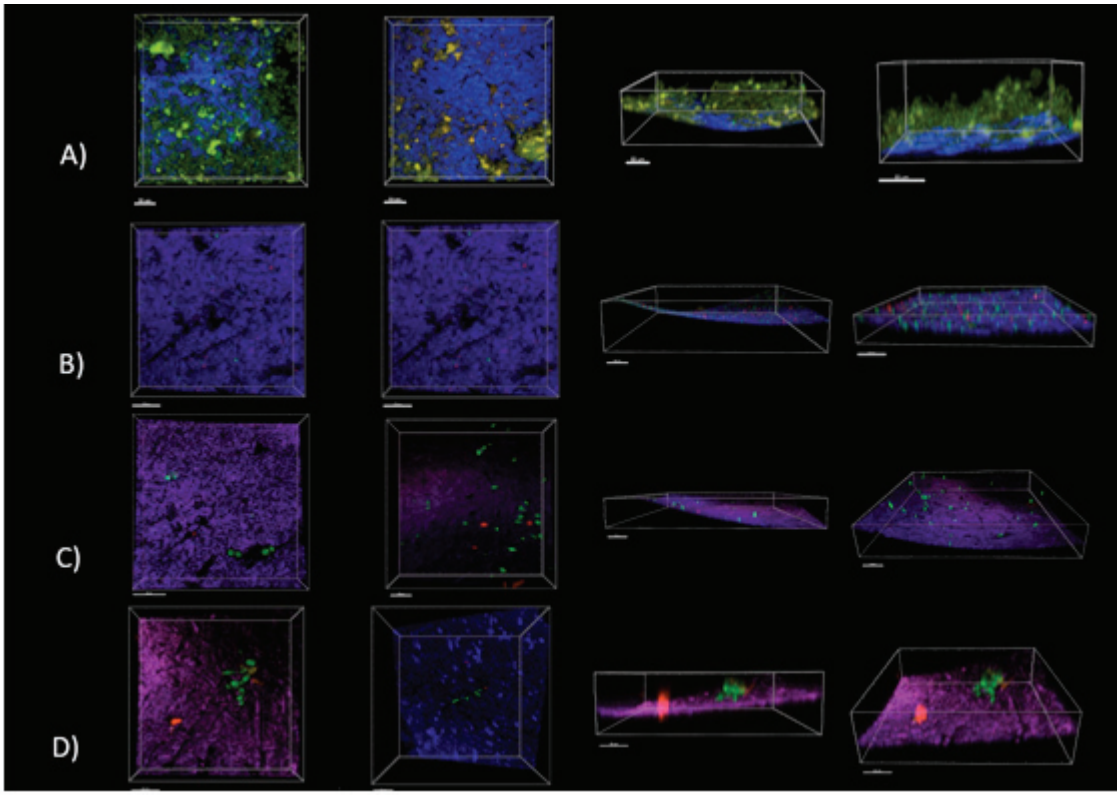

Fig. 8. Adhesion to the coronal biomaterial surface of selected microorganisms which are part of natural oral microbial flora, including: A) *S. mutans*, B) *L. rhamnosus*, C) *C. albicans* 28, D) *C. albicans 31*

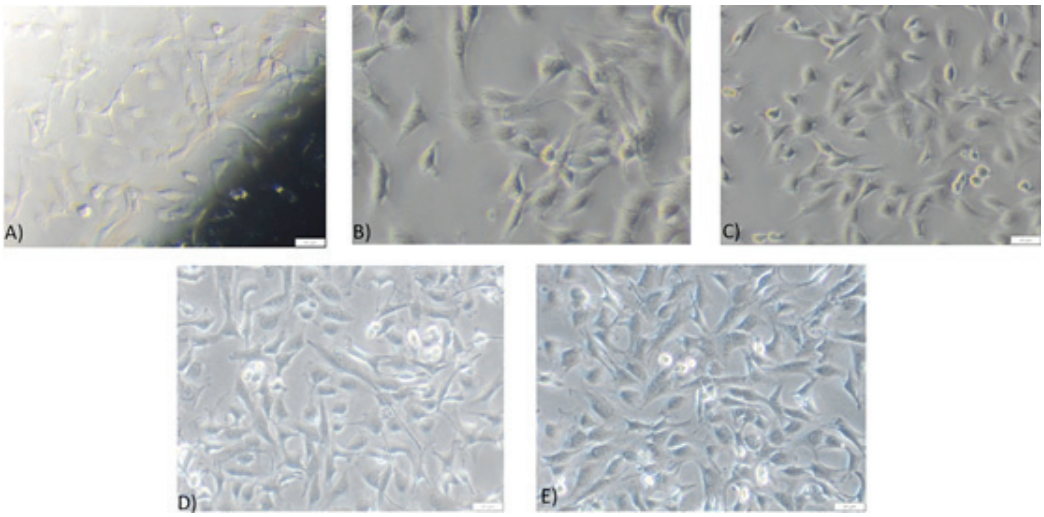

Fig. 9. Morphological image of Balb/3T3 fibroblasts after 24 h incubation with dental crown, direct contact: A) at the edge of the sample, B) 1 cm from the edge of the specimen, C) further away from the specimen, D) under the specimen, E) control culture without contact with the test material. 100× magnification

4. Discussion

Every material that is introduced longitudinally into the oral cavity becomes a location where a great quantity of biological processes take place. This is the result of electrochemical corrosion, enzymatic, microbiological, thermal and occlusal stresses of SSC material [7], [29], [39]. These aspects are undoubtedly influenced by the surface of the biomaterial, including also its roughness. So far, due to the new solution in pediatrics, which are crowns, there are very few studies in the field of physicochemical research that would comprehensively examine the chemical composition, roughness of

materials and the biological response of this material [4], [6]. Roughness tests conducted on an optical profilometer allowed for a non-invasive determination of the roughness value (Table 2), which in the tested values of the parameters *Ra*, *Rq* and *Rz* is lower than that which characterizes natural enamel [13]. Nevertheless, it is noteworthy that the roughness is different for different regions of the crown, and the highest values were observed on the frontal surfaces in direct contact with the oral environment. Such a small roughness is the effect of polishing the surface, which can be seen especially in the lateral regions of the crown. The biomaterial, especially the surface becomes a niche for bacteria formation. Streptococcus mutans colonization is the most relevant in term of caries outbreaks. It is a caries pioneering bacteria as a result of its ability to synthesize extracellular glucans and fructose homopolymers. This allows for the creation of an environment favorable for the colonization of subsequent microorganisms and the formation of a caries-forming biofilm [11], [20], [25], [26]. They possess high surface free energy and are, therefore, able to adhere to the steel crown surface by van der Waal's attractive forces [40]. *L. rhamnosus* is a probiotic bacteria and it interferes with caries biofilm formation by various processes, such as the formation of biosurfactants and the reduction of biofilm acidity [23], [28]. Arezoo Tahmnourespour et al. noted the anti-biofouling effect of *L. Rhamnosus* biosurfactant in their study. As illustrated in Figs. 2 and 4. *L. rhamnosus* has the ability to adhere to the surface of the dental crown. The quantitative and qualitative assessment of adhesion showed a low adhesion of *S. mutans* to the dental crown surface, but confocal studies showed the presence of *S. mutans* in the Imaris software visualisation. *S. mutans* on the coronal surface displayed in Fig. 5 in green colour indicating viable colonies. *L. rhamnosus* colonies are also present. It is, therefore, important that the crown surfaces, like other restorations, are kept in good condition through proper hygiene. The reason for re-treatment and failure especially in Class II cavities (involving the proximal surfaces of the teeth) is often marginal failure or secondary caries, which is practically absent in SSC due to full crown coverage whereas in comparison, in the case of resin-modified glass ionomer cement, this is a common reason for having to re-treat the tooth [12]. This suggests that, especially when dealing with a large cavity, restoration with SSC should be first choice [32]. In the case of *C. albicans* 28 and *C. albicans* 31 adhesion, both strains showed a low presence on SSC surface. According to Fig. 6, the placement of SSC in the close contact to the fibroblast cell shows no cytological stress to the fibroblasts colonies, which can imply lack of cytotoxicity of the SSC material and be considered as safe longevity restoration with no potential cytotoxicity risk [14]. The SSC biomaterial can be a source of metal ions, and from the viewpoint of hypersensitivity and allergy, Ni ions in particular are the most important one. Several studies have shown that the Ni ion content in saliva remains at low non-toxic levels and is highest in the first few days after application of the crown [2], [3], [18], [22], [24]. The amount of ions increases when the gingival margin of the crown is cut and bent for better adaptation [5], [8], [15]. Older generations of crowns showed a higher nickel content whereas current crowns are made of low-nickel steel. This is done in order to reduce the disruptive effects of nickel ions on the organism. Based on the physico-chemical properties, studies were performed to check and confirm purity and composition of SSC. It can be concluded that studied SSC have pure phase of CrFe steel with Ni addition. The FTIR measurements results also did not show any additional vibrations beside the typical for stainless steel materials. The EDS analysis confirmed pure character of SSC, showing also that nickel content is about 8%, which confirms manufacturer declaration.

5. Conclusions

The biological response to SSC material shows no stress to the surrounding tissue. Microbial properties of biomaterial displays low adhesive properties, combined with full-crown tissue covering the risk of failure and need of re-treating seems to be low. Material contains low level of nickel (about 8%), which can cause hypersensitivity or allergy, however clinical data shows that this is rarely the case. Overall SSC used in pediatric dentistry are considered to be a suitable method of primary molar restoration.

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