CIRCULATION IN THE GINGIVA AS A PROGNOSTIC, DIAGNOSTIC AND FOLLOW-UP MARKER OF PERIODONTAL DISEASE AND AFTER PERIODONTAL SURGERY

FINAL REPORT 2019

Until we were able to obtain our planned instruments **we adapted the heat provocation test to the human gingiva to assess vascular reactivity in periodontal inflammation and smoking.** gingival blood flow (GBF) was recorded by Laser Doppler Flowmetry before and after heat provocation in healthy volunteers (n = 50). Heat was generated either by warm saline or a halogen lamp. The latter method was also utilized for a heat test in non-smoking and smoking patients with periodontal inflammation. The circulatory parameters were correlated to the inflammatory marker, i.e. gingival crevicular fluid (GCF) production measured by Periotron. Local application of heat caused a rapid, significant and transient increase in GBF regardless of the method used. The increase in the speed and not in the concentration of moving blood cells was responsible for increased GBF. Higher GCF values were correlated with increased peak flow, flux pulse amplitude and faster restoration of GBF after the test in non-smokers, but not in smokers. The heat test could be a valuable tool to check the vascular reactivity of gingival vessels. Moderate periodontal inflammation may facilitate gingival vascular responsiveness which can be suppressed by smoking.

Despite significant administrative obstacles among the first in the world we set up the new, versatile **laser speckle contrast imaging method** (LSCI, Perimed AB) with OTKA support for **human GBF measurements** to study basic science and relevant clinical questions under clinical conditions in dentistry.

For the **assessment of the test-retest reliability of this method** our first aim was to investigate the effect of factors inherent in oral mucosa measurement on intra-day and inter-day reliability. GBF was measured in seventy healthy subjects. First, measurements were obtained by varying the incidence angle of imaging, using a lip retractor. Second, 3 snapshots were taken with closure of the mouth in-between, and lips were retracted by a dental mirror. These were repeated 1 week later. Third, snapshots were taken either by direct view or using a mirror. Reliability was assessed based on coefficient of variation. Unlike retraction of the lips and the mirror, the incidence angle had an effect on mean GBF. The coefficient of variation within a subject was 6.4% with the mouth constantly open. With retraction, the intra-session, and the inter-day coefficient of variation were 8.3% and 10.5%, respectively. The coefficient of variation was 11.9% by alternating direct and indirect imaging. LSCI has good short- and long-term reliability regardless of lip retraction or an indirect view. **This technique seems to be appropriate for the long-term clinical non-invasive follow-up of gingival microcirculation.**

The **rate of GBF between the various areas of the gingiva in resting position and under challenge** is unknown. In this study, the LSCI method was used **to map spatial and temporal changes in GBF after transient compression.** Horizontal, vertical, and papilla base compressions were applied on the attached gingiva in 21 healthy patients. LSCI was used to determine dynamic changes in regional GBF during a five-second occlusion interval and subsequent reperfusion for twenty minutes. Resting GBF in the attached gingiva apical to the papillae was higher as compared to that in the midbuccal area of the teeth. During short-term

horizontal compression, ischemia was greater coronal than apical to the occlusion line. Postocclusive hyperemia was observed not only in the regions affected by ischemia but encompassed a wider area. Hyperemic response was more pronounced and prolonged in male than in female patients. GBF in the attached gingiva shows spatial differences. Our findings corroborate the apicocoronal orientation of blood circulation. Periodontal and papillary collaterals may have little role in the blood supply of the adjacent attached gingiva under physiological conditions.

Spreading vasodilation is an important means of increasing local GBF effectively during increased metabolic demands or in case of vascular injury. Our aim was to develop a technique **proving the presence of spreading vasodilation in the human keratinized** gingiva. Local vasodilation was evoked by the application of **nitric oxide** (NO) donor nitroglycerin into a well, fixed 2 mm above the marginal gingiva, in 20 subjects with healthy periodontal tissue. Either 1 or 8 mg/mL nitroglycerin solutions were dropped into the test well at the upper right second incisor, and saline was applied into the control well at the upper left first incisor. The GBF was recorded for 15 minutes by a laser speckle contrast imager below the well and in the surrounding area in the mesial, distal, apical and coronal directions. Gingival thickness was measured by an ultrasonic biometer. Peak GBF increase was similar after 1 mg/mL and after 8 mg/mL nitroglycerin application in the well (51% ± 12% vs 42% ± 8%) and in the apical region (33 ± 9% vs 55% ± 13%). While the lower dose of nitroglycerin increased GBF only in the apical region around the well, the higher dose induced significant elevations in all surrounding regions, with apical prominence. Hyperaemia lasted 10-14 minutes in the low-dose group whereas it extended beyond the observation period in the high-dose group. Neither the baseline nor the NO-induced peak GBF were correlated with gingival thickness. The role of the direct effect of NO in the regulation of perfusion was demonstrated in the human gingiva as well as the propagation of local vasodilation to distant, especially apical areas, probably by the mechanism of flow-mediated dilation. This mechanism may have a clinical importance for flap survival or wound healing.

Spreading vasoconstriction is a known phenomenon which is described in various tissues as a locally evoked vasoconstriction which could elicit remote vasoconstriction at distant areas. Epinephrine is widely used vasoconstrictor in dentistry, but its spreading vasoconstriction has not been demonstrated yet in gingiva. The aim of this study was to **investigate the local and remote effect of epinephrine in the attached gingiva**. GBF was measured by LSCI in 15 healthy volunteers. In group A two wells were fabricated from orthodontic elastic ligature and placed 2 mm apically from the free gingival margin at the midbuccal line of 12 and 21 teeth. GBF was measured in the wells and tightly apical, coronal, distal, mesial directions to the wells. In group B the wells were made on the surface of the corresponding teeth including the gingival sulcus and four measurement regions were from the gingival margin reaching the mucogingival line close to each other. After the baseline recording either 1 mg/ml epinephrine solution (test site) and physiological saline (control site) were applied into the wells and further 15 minutes recording was done. In group A the GBF did not changed in any regions. In group B the GBF decreased at the test site immediately after application of epinephrine and remained low for 15 minutes in all regions. The decrease was the largest close to sulcus which degree became lower to the apical direction. The attached gingiva seems to be impermeable for epinephrine thus epinephrine cannot evoke any vasoconstriction on the keratinized gingiva. Whereas application at the gingival sulcus could evoke strong, long-lasting vasoconstriction up to the mucogingival junction suggesting the permeability of sulcus and presence of spreading vasoconstriction in the human gingiva.

To study the **effect of systemic vasoactive reflex on GBF** we elicited a 35 mmHg increase in the mean systemic arterial blood pressure by a handshake test (maintaining 30% of maximum hand grip force for 3 minutes), however this did not influence the GBF, whereas it did the perfusion in the moving mucosa.

When the **gingiva was stimulated mechanically with a wire or a toothbrush**, GBF increased significantly. In smokers the effect of gum brushing on GBF was greater and more prolonged ($p < 0.001$).

LSCI is proved to be a reliable tool in flap monitoring in general surgery; however, it has not been evaluated **in oral surgery** yet. We applied the LSCI to **compare the effect of a xenogeneic collagen matrix** (Geistlich Mucograft®) **to connective tissue grafts (CTG) on the microcirculation of the modified coronally advanced tunnel technique (MCAT) for gingival recession coverage.** Gingival microcirculation and wound fluid were measured before and after surgery for six months at twenty-seven treated teeth. In males, the flap microcirculation was restored within 3 days for both grafts followed by a hyperemic response. During the first 8 days the GBF was higher at xenogeneic graft comparing to the CTG. In females, the ischemic period lasted for 7–12 days depending on the graft and no hyperemic response was observed. Females had more intense and prolonged wound fluid production. The LSCI method is suitable to capture the microcirculatory effect of the surgical intervention in human oral mucosa. The application of xenogeneic collagen matrices as a CTG substitute does not seem to restrain the recovery of graft bed circulation. Gender may have an effect on postoperative circulation and inflammation.

Our next aim was to **characterize the time course of graft vascularization and incorporation following vestibuloplasty procedures** by measuring GBF. Our secondary aim here was to determine the relative contribution of the recipient wound bed's environment to the graft's neovascularization with corroboration by simultaneous quantitative determination of Vascular Endothelial Growth Factor (VEGF) expression. Five patients with inadequate width of keratinized gingiva in the anterior mandible were treated by vestibuloplasty with the use of apically repositioned split thickness flap combined with a collagen membrane. Intraoral photographs and LSCI GBF measurements were taken at baseline and at 17 follow-up visits for one year. Thirty regions of interest of the augmented mucosa and the surgically unaffected surrounding mucosa were evaluated, GBF values were analyzed in relation to intraoral photographs. VEGF was determined from the wound fluid. At 6 months, the mean width of keratinized gingiva increased ($p<0.01$), but the thickness was unchanged. Scar formation was observed within all augmented cases, and was higher centrally (81%) and apically (69%) than at the coronal side of the graft (18%). Perfusion of the graft edges coronally and laterally was higher than at the apical side. The rate of increase in graft's perfusion at the peripheral zones varied at the coronal and lateral side, but was higher than at the central zone until day 11 (coronally) and until day 14 (laterally). At the apical side perfusion of all zones in the graft increased similarly. The VEGF expression was more abundant at the apical side than either at the coronal or at the lateral sides. Revascularization mainly occur from the lateral and coronal sides rather than from the alveolar mucosa. Prolonged ischemia and increased VEGF expression might relate to scar tissue formation.

Our aim was also to **measure the pre-, intra- and post-operatively the circulation of the transplanted free forearm fasciocutan flaps during oral reconstruction surgary and to prospectively monitor their revascularizations and healings** using LSCI. Four female patients (55-72 years old) were included in this study who got fasciocutaneous forearm flaps to

cover lesions after removal oral removal tumors. Circulation of the free flaps and recipient regions were recorded by LSCI before, during, twice weekly after surgery, weekly thereafter and every 4 weeks for six months. Intraoral photographic documentation and blood pressure measurements were performed. All patients recovered without complications. As the two-month perfusion values remained unchanged at the end of follow-up in the surrounding mucosa (301±32) we considered it as reference. Postoperative ischemia was observed in the first 4 days after surgery (101±10 on day 2). From day 5, the flap blood flow started to increase, reaching the hyperemic peak (169%±25) on day 13 [5.-22.] and then gradually decreased to the reference value. In the second postoperative month the circulation of the flap implanted in the oral cavity was more pronounced (127±17) than before surgery on the forearm (47±2.6), but it was lower than that of the surrounding mucosa (301±32).

Our data show that based on the changes in flap perfusion monitored by LSCI **the optimal timing of early implant placements may be assessed individually.**

Postoperative complications may occur during the healing of palatal donor sites due to disturbed blood circulation of palatal tissues. Therefore **for assessment of palatal wound healing** blood flow was measured by LSCI in seven patients after connective-tissue harvesting. The slope in blood-flow elevation within the first 3 days as well as time needed for maximum reperfusion were calculated. Each surgical site was assessed by clinical examination on day 3. In donor sites with secondary-intention wound healing, postoperative blood flow was elevated with significant delay compared to the surrounding tissues and to the primarily healed wound. Reperfusion time and healing score were strongly correlated ($r = 0.87$, $P < .001$), as were the slope and clinical rank ($r = -0.85$, $P < .001$). LSCI proved to be an objective method to assess individual wound-healing time and to predict the quality of wound healing.

Gingival thickness (GT) has a great importance in periodontal flap design, gingival recession and soft tissue aesthetic of implant-borne prosthetic restorations. We also **set the new PIROP ultrasonic biometer for GT measurements and determined its reproducibility** by comparison with the invasive transgingival probing technique. Materials and Methods: GT was measured in 25 periodontally healthy volunteers both by PIROP and an endodontic spreader on the attached gingiva. Reproducibility was assessed by calculating standard deviation (SD) in five repeated measurements and Pearson correlation coefficient (r). Agreement between the two methods was evaluated based on Bland–Altman limits of agreement (LoA). Results: No systemic bias in GT was observed between the two methods (1.13±0.07 mm in PIROP vs 1.04±0.03 mm in spreader, p=0.218). The repeatability of the PIROP was better than the spreader method (SD=0.14 [0.13-0.17] mm versus 0.20 [0.17-0.23] mm, p<0.001). With five repetitions the measurement error of the PIROP decreased from ±0.14 mm to ±0.06 mm. The correlation among the repeated observations were strong (r=0.86) for the ultrasonic, weak (r=0.34) for the invasive method. The LoA between the two methods was +0.75 to -0.58 mm. Conclusion: PIROP is a highly reliable device for routine gingival thickness measurements. As the repeated measurement is easy and quick it is recommended to repeat the measurement a few times to improve the precision in individual case.

Our proposed animal experiments were significantly hampered by several years lasting animal cage permit issues. To investigate **the effects of endothelin-1 (ET-1) and to clarify the role of its receptors (ETA and ETB) were investigated on the gingival vasculature in rats.** GBF was monitored by laser-Doppler flowmeter at the upper incisal papilla in 5 groups of animals. In the 1st group iv. vehicle and cumulative doses of ET-1 was administered, 2nd and 3rd group

was pretreated with selective ETA antagonist BQ-123, or selective ETB antagonist BQ-788 before ET-1, 4th group with cyclooxigenase inhibitor indomethacin and nitric oxide (NO) synthase blocker L-NAME and then with ET-1, 5th group same as the 4th, but BQ- 123 was also applied as pretreatment. Mean arterial blood pressure (MABP) was continously recorded in femoral artery. From the 6th experimental animal group papilla was excised for immunohistochemistry. ET-1 caused after an abrupt, transient decrease, a dose dependent sustained increase in both MABP and gingival vascular resistance (GVR) in group 1. In 2nd group GVR decreased compared to the 1st group, in 3rd and 4th increased and no change was found in 5th. ETB receptor immunostaining was detected in both endothelium and smooth muscle cells of arteries and veins, whereas ETA receptor expression appeared only in vascular muscular tissues. Our data suggest that ET-1 is a potent vasoconstrictor agent in gingiva. By binding to ETA and ETB receptors located on vascular smooth muscle cells ET-1 induces vasoconstriction, on the other hand through the stimulation of ETB receptors located on endothelium evokes vasodilation as well. Furthermore, the selective ETB receptor stimulation results in more powerful vasodilation through prostaglandin, NO and a recently unknown molecule release than vasoconstriction. It is interesting to note that ET-1 is also produced endogenously under normal condition in gingiva and may suppress the resting blood flow through ETA receptors what might balance the baseline release of vasodilators (eg. NO). Disruption of the fine equilibrium between gingival vasoconstrictors and vasodilators can cause alterations in functional adaptation.

In the present study, the possible **localization and role of vascular endothelial growth factor receptor type 2 (VEGFR2) in the regulation of gingival venules** in a rat model of experimental diabetes are examined. Six weeks after streptozotocin premedication, Wistar male rats presenting blood sugar levels >20 mmol/L were selected for investigation. The VEGFR2 antagonist ZM323881 [5-((7-benzyloxyquinazolin-4-yl)amino)-4-fluoro-2-methylphenol-hydrochloride] (20 μg/mL) was dripped onto the gingiva between the mandibular incisors. Changes in diameter of the selected gingival venule were measured by vital microscopy combined with digital photography at specified times. Immunohistochemical staining was used to localize VEGFR2. For controls, the same protocol was used on animals with normal blood sugar levels and healthy gingiva. There was a significant difference between the baseline venule diameter of the diabetic and the control groups (47 ± 1 and 28 ± 2 μm, respectively). After 15, 30, and 60 minutes of local application of ZM323881, significant vasoconstriction was observed in the venules of diabetic rats compared with the baseline (81.4% ± 4.6%, 81.8% ± 4.4%, and 80.6% ± 5.1%, respectively). The control group showed no change in the venule diameter. The immunohistochemical analysis showed significantly increased VEGFR2 expression in the mast cells along the venules in the diabetic group, whereas mast cells were rarely found in the control group. The findings suggest that VEGF expression is increased in gingiva in experimentally induced diabetes. After VEGFR2 activation, the mast cell-derived vasodilatory and inflammatory mediators may contribute markedly to the concomitant changes in the microcirculation.

Due to a fire at the University of Physical Education in Nov. 2015 damaged our lab in an adjacent building, which was further exacerbated by the use of high pressure water during the fire fighting. Instruments/monitors/microscopes were heavily damaged what pulled back our real 3D subproject. Our methodical **transparent, true 3D qualitative and quantitative microscopic innovation** consists of 3 separate developments: 1) The triethanolamine based optical clearing solutions, which are able to homogenize the refractive indexes of tissue

components, thus increasing their transparency. 2) The assembling of a true 3D investigating system, including the so called „stereoconverter", an optical device, which is able to increase the magnification of the conventional light microscope by 5-8 fold without decreasing its depth of field (DOF). This results in a relative increase of DOF at appropriate magnification. 3) The development of a true 3D measuring and modeling computer software based on stereophotogrammetric methods, for the analysis of spatial structures and functions. Several orofacial structures (gingiva, masticatory muscles, lip, tongue, submandibular salivary gland, etc.) of rats were investigated both post mortem and in vivo using the system above. Beside soft tissues, we also developed hard tissue (tooth, bone) specific clearing solutions in vitro. With the aid of our clearing materials, we were able to make the structures of deeper tissue layers examinable, e.g. to study their microcirculatory system. Due to the increase of magnification and the relative enlargement of DOF, extremely thick tissue sections or blocks can be displayed. By application of our true 3D measuring and modeling computer system, the microscopic structures can be covered by a spatial point cloud and spline surfaces. Mathematical analysis of these surfaces can be performed and shaded photorealistic three-dimensional models and animations of them can also be achieved. The true 3D way of thinking instead of planar approach can result in paradigmatic change also in the field of microscopy and may lead to the development of a new scientific branch i.e. the "transparent, true three-dimensional qualitative and quantitative microscopy". The identification and modeling of the delicate spatial structures of soft and hard tissues might not only provide substantial, new, qualitative and quantitative knowledge, but also might influence their therapies.

Due to the above issues informed you in our yearly reports we have done the followings as well for OTKA. We investigated **the role of biofilm lysine decarboxylase (LDC) in the pathomechanism of periodontitis.** Lysine, a nutritionally essential amino acid, enters the oral cavity in gingival crevicular fluid (GCF). During oral hygiene restriction (OHR), LDC in dento-gingival biofilms converts lysine to cadaverine. Lysine depletion impairs the dental epithelial barrier to bacterial proinflammatory products. Antibodies to LDC from Eikenella corrodens (Ecor-LDC) inhibit LDC activity and retard gingival inflammation in beagle dogs. Whether E. corrodens is the major source of LDC in dental biofilms and whether the lysine analog tranexamic acid (TA) inhibits LDC activity, biofilm accumulation, and GCF exudation in a human gingivitis model were examined. Antibodies raised in goats to LDC-rich extracts from E. corrodens cell surfaces were used to inhibit Ecor-LDC and detect it in biofilm extracts using Western blots. Ecor-LDC activity was measured at pH 4.0 to 11.0 and its TA dissociation constant (Ki) at pH 7.0. Young adults used a 5% or 10% TA mouthwash three times daily during OHR for 1 week. Ecor-LDC antibodies and TA inhibited biofilm LDC. Ki of TA for Ecor-LDC was 940 μM. TA reduced plaque index (PI) by downshifting the PI correlation with biofilm lysine content after OHR without TA. GCF was correspondingly suppressed. However, greater TA retention in saliva partially relieved GCF suppression but not biofilm lysine depletion. TA slightly inhibits LDC but strongly reduces biofilm by inhibiting bacterial lysine uptake. Unfortunately, TA may impair dental epithelial attachments by also inhibiting lysine transporter uptake. Ecor-LDC inhibitors other than lysine analogs may maintain sufficient lysine levels and attachment integrity to prevent periodontal inflammation.

Chronic periodontitis is controlled without antibiotics by scaling and root planing (SRP) to remove dental biofilm. We previously reported that the epithelial barrier to bacterial proinflammatory products is impaired when biofilm lysine falls below the minimal content of normal blood plasma. We therefore **examined whether low biofilm lysine contents were**

associated with being refractory and requiring antibiotics to supplement SRP therapy. Sixteen periodontitis patients and 6 periodontally healthy volunteers (HV), respective mean ages 57±6 and 36±8 years, were examined. Periodontitis patients received SRP and surgery, and HV received prophylaxis. At quarterly maintenance/prophylaxis visits during the subsequent year, therapeutic response was good (GR, n=9) or poor (PR, n=7; 5 cigarette smokers). Biofilm cadaverine, lysine and other amino acid (AA) contents were determined by liquid chromatography. Cadaverine mole fraction of lysine plus cadaverine (CF) indicated biofilm lysine decarboxylase activity. Biofilm lysine was 0.19±0.10 and 0.20±0.09 μmol/mg in GR and HV, but 0.07±0.03 μmol/mg in PR (Kruskal-Wallis $p<0.01$). All AAs were depleted in biofilm from smokers, but only lysine in biofilm from non-smokers. CF was inversely associated with clinical attachment level (CAL) at baseline before therapy in all patients ($R2=0.28$, $p<0.01$) and with CAL change after therapy in GR ($R2=0.49$, $p<0.05$). Lysine and cadaverine contents discriminated PR from GR and HV (Wilks' Lambda=0.499, $p <0.012$). Refractory responses requiring antibiotic therapy result from smoking and/or microbial infections that starve the biofilm and epithelial attachment of lysine. Biofilm CF associates with periodontitis severity pre-therapy and extent of therapeutic response post-therapy.

The initial response to experimental gingivitis (EG) is the exudation of gingival crevicular fluid (GCF). Individual variation in GCF is reproducible and parallels its reduction after therapy (Trombelli et al., J Clin Periodontol 2008). High and low GCF exudation rates also associate with different groups of biofilm bacteria after EG (Huang et al. ISME.J 2014). We have already reported (Lohinai et al. J Periodontol 2012) that biofilm accumulation (plaque index, PI) and GCF exudation after a week of EG are a consequence of how much lysine remains not converted to cadaverine by lysine decarboxylase (LDC) in the biofilm. Lysine in that study was positively and linearly related to PI, but by a second degree polynomial curve to GCF (top of the GCF curve is at minimal plasma lysine). All individual points in the study were either above the regression line (high) or below it (low). The present study's aim was therefore to examine whether these relationships were statistically distinct GCF responses to EC. A quadratic polynomial regression model was fitted using GCF exudation as the outcome. Significance of GCF responder status (main effect), and its interactions with lysine content and lysine content-squared were determined using an F-ratio test with 3 degrees of freedom to compare the sum of squares explained by the independent terms relative to the sum of squares error. A stronger fit of the high and low responder curves ($R^2 \geq 0.90$) compared to the combined data ($R^2=0.37$) was found and the difference between these curves was highly significant (F Ratio 41.77, $p<0.0001$). These **GCF responses were also independent of biofilm lysine content, suggesting the presence of two independent responder groups** in the data. **The rate of GCF exudation appears high or low after a week of EG. The response is intrinsic to each individual and likely influences their biofilm development and disease.**

The control of biofilm growth is essential for the maintenance of good dental health. The aim of this research was to examine **the antibiofilm properties of non-toxic substances as sodium bicarbonate ($NaHCO_3$) and newly developed, Hungarian patented, highly pure chlorine dioxide (ClO_2)** on *Streptococcus mutans*. *S. mutans* biofilm formation and preformed biofilm destruction were examined after exposure of different concentrations of $NaHCO_3$ and highly pure ClO_2 (Solumium) with crystal violet staining (total biomass analysis), MTT assays (metabolic activity) and confocal laser scanning microscopy (ratio of dead/live bacteria in different biofilm depth) and analysed by ANOVA. Our results revealed significant inhibition of *S. mutans* biofilm formation with $NaHCO_3$ or more powerful with ClO_2 in comparison with

biofilm formation alone. $NaHCO_3$ displayed much less demolition on preformed biofilm than ClO_2. These observations mirrored the differences found in investigation of their minimal inhibitory concentration. Our data demonstrate that both $NaHCO_3$ and highly pure ClO_2 are powerful inhibitors of biofilm development, but show less effects on the biofilm destruction. Clinical application of these agents is suggested as effective non-toxic biocides for the prevention of dental biofilm formation and therefore the consequent tooth decay.

Success of endodontic treatment depends on the eradication of microbes from the root canal system. The complexity of the infected root canal system is the main obstacle in achieving a complete cleaning. During the root canal treatment the presence of remaining mechanically untouched areas increases the importance of chemical disinfection. The irrigants should be able to penetrate into the side-canals. The aim was to study **the antibacterial effectivity of root canal irrigants' gas phase and their ability to redissolve while preserving efficacy.** We used inoculation loops to place 1 uL of Enterococcus feacalis suspension (containing $9x10^7$ CFU/ml bacteria) above 37C° 10 mL of 0.12% hyperpure chlorine-dioxide (ClO2), 2% chlorhexidine-digluconate (CHX), 2.5% sodium-hypochlorite (NaOCl), 10% potassium-iodide (KI) or distilled water in airtight bottles. One and 10 minutes later the surviving bacteria were plated and two days later the growing colonies were counted. In a further experiment, a Durham tube was filled up with bacterial suspension. Durham was put in a closed Eppendorf in which the level of irrigants' was lower than the orifice of Durham. After 10 minutes in 37C° thermostat, the surviving bacteria were measured by the mentioned method. Wilcoxon/Mann-Whitney test was used for statistical analysis. Gas phase of ClO2 killed all the bacteria already in one minute. After ten minutes the potassium-iodide reduced the amount of bacteria from 9x107CFU/ml to 3x101CFU/ml, sodium-hypochlorite decreased initial bacterial count only with one order of magnitude, while chlorhexidine-digluconate and distilled water did not alter the bacteria amount. In the study of redissolving ClO2 eradicated totally the bacteria, potassium-iodide showed a slight reduction, while chlorhexidine-digluconate and sodium-hypochlorite were inefficient. The ClO2 is volatile and have powerful antibacterial effects in both gas and redissolved phases.

Our students won the following prizes by OTKA support: 1st (2016, 2017), 2nd (2015, 2019) and 3rd (2018, 2019) prizes in Student Conference of Medical, Dental and Pharmaceutical Sciences of Semmelweis University, National Excellence Scholarship (2016), 2nd prize in XXXIII. National Scientific Students' Conference, Medical and Health Section, Special Jubilee Award of the University of Pécs (2017), 1st prize in Rector Competition of Semmelweis University (2017), 3rd prize in VIII. International Scientific Conference, Science4health2017, Peoples' Friendship University of Russia (RUDN University), Moscow (2017).

Thank you for your support!