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THE INFLUENCE OF LASER RADIATION IN DECONTAMINATION OF BACTERIAL PLAQUE IN PATIENTS WITH ORTHODONTIC TREATMENT. A MICROBIOLOGICAL „IN VITRO" INVESTIGATION

D. Mocuța[1], Carmen Todea[1], A. Lazea[1], E. Hogea[2], C. Muntean[3]

[1]*Faculty of Dentistry, University of Medicine and Pharmacy „Victor Babeș" Timișoara, Romania*
[2]*Faculty of Medicine, University of Medicine and Pharmacy „Victor Babeș" Timișoara, Romania*
[3]*Politehnica University Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, Timișoara, Romania*

Summary

Background: The aim of this study was to evaluate the antimicrobial effect of laser radiation and chlorhexidine 0,2% treatment for reducing the number of pathogen oral microorganisms, especially Streptococcus mutans, adjacent to orthodontic brackets.
Materials and methods: The suspension of microorganisms was obtained from those patients who wear orthodontic brackets. The inoculated probes with this suspension were exposed to decontamination methods, represented by the Erbium laser (2760nm, 80mj, 8 Hz), 980nm GaAlAs diode laser and 0,2% clorhexidine. After the treatment, the probes were collected from around the brackets and then were incubated aerobically at 37°C. To confirm the bactericid effect of treatment, it was adopted the colony forming units method. The Microsoft Excel program, one-way ANOVA and Tukey-Kramer test were used for the statistical process.
Results: According to colony forming unit method the best bactericide effect was observed in the Erbium laser group, followed by theGaAlAs diode group comparatively with negative control group. Following one-way ANOVA analysis it can be remarked a significant difference between groups,regarding the values, resulted from the application of different experimental treatments ($p < 0,05$).
Conclusions: The present study proves the highest efficiency of Er:YAG laser radiation on newly colony forming around orthodontic brackets decontamination.
Key words: Er:YAG laser, 980nm diode laser,0,2% Chlorhexidine, plaque, orthodontic brackets

Résumé

L'influence du rayonnement laser sur la décontamination de la plaque bactérienne chez les patients avec un traitement orthodontique. Investigation microbiologique "in vitro"

Introduction: L'objectif de l'étude a été d'évaluer l'effet antimicrobien du traitment par rayonnement laser et l'effet de la chlorhexidine 0,2% sur la réduction du nombre de microorganismes pathogènes oraux brackets orthodontiques adjacentes.
Materiels et methodes: Les suspensions de micro-organismes ont été obtenus chez des patients avec des brackets orthodontiques. Les échantillons inoculés avec ces suspensions ont été soumisé à des procédés de décontamination représentés par l'application du faisceau laser en utilisant le laser Er:YAG (2760nm, 80mj, 8 Hz), 980 nm GaAlAs diode laser et 0,2% de chlorhexidine. Après l'application du traitment, des échantillons ont été prélevés dans les crochets et ont été incubés dans un thermostate à 37°C. La méthode d'évaluation des résultats de l'analyse bactériologique a été le comptage des colonies nouvellement formées. Le traitement statistique a été réalisé avec Microsoft Excel, ANOVA et le test de Tukey-Kramer.
Résultats: Après le comptage des colonies nouvellement formées, on a trouvé que le meilleur effet bactéricide a été observé dans le groupe d'Er: YAG, suivi de l'efficacité du groupe diode GaAlAs par rapport au groupe de contrôle. Après l'analyse one-way ANOVA on peut voir une diffèrence significative entre les groupes en ce qui concerne les valeurs résultantes de l'application de différents traitments expérimentaux ($p < 0,05$).
Conclusions: La présente étude démontre l'efficacité supérieure de l'Er:YAG rayonnement laser pour décontaminer les colonies nouvellement formées autour des brackets orthodontiques.
Mots clés: Er:YAG laser, 980nm diode laser, 0,2% de chlorhexidine, plaque, brackets orthodontiques

Correspondence address: *Prof. univ. Dr. Carmen Todea*
Faculty of Dentistry, Department of Oral Rehabilitation and Dental Emergencies,
University of Medicine and Pharmacy"Victor Babeș", Timișoara, Romania
Bulevardul Revoluției din 1989 Street, No. 9, Timișoara, Romania
e-mail: carmentodea@gmail.com

Background

The oral cavity is heavily colonized by a complex, relatively specific, represented by a sessile microbial community, with a strong interdependent connection of microorganisms adhered to each other and/or on a dental surfaces, being organized in so-called oral biofilm, respectivelyplaque (1,2). This biofilm was described as a three dimensional complex with microorganisms embedded in a extracellular polymeric matrix of substances. The dental plaque contains a significant variety of bacterial species, many of these being responsable for the oral cavity infections. Among them, one of the most common problems is represented by the enamel demineralization, which represents a direct consequence of the balance disruption between demineralization and remineralization process (3,4). Decades of epidemiological, biochemical and animal studies reported the streprococcus group as a predominance in oral biofilm, the majority and also incriminated in caries initiation being the Streptococcus mutans (S.m.). As such, a great clinical importance consequence due to the biofilm structure organization and expression pattern of microorganisms genes alteration is the reducing of pathogens susceptibility to antimicrobial substances. The biofilm age and structure can restrict the penetration way of the antimicrobial agents to biofilm, leaving unaffected cells in the plaque depth (5). Other specialized studies attest that the species of streptococcus group are the first bacteria which colonize the oral surfaces, their share is 70% in plaque and as a result, S.m. is the first odonthopatogen agent present in supragingival dental plaque. Having the caries and periodontal diseases as a starting point due to the accumulation of plaque on hard and soft oral tissues, conventional mechanical debridement and a good oral hygiene can lead to a temporary microorganisms reduction from dental plaque (6).

Current trends of dental therapy and prevention are pursuing the adoption of minimally invasive therapeutic strategies. As such, numerous studies have reported the laser bactericidal effect on pathogenic microorganisms from oral cavity (7). Using laser radiation in dentistry includes numerous applications in various specialities thanks to minimally invasive action manner. As such, hard and soft tissue removal, bacterial decontamination, edema reducing, improvement to stop the painful symptoms, healing stimulation, tissue regeneration are just some of the benefits of using laser equipment.

From another point of view, orthodontic treatments have become a necessity not only to children and teenagers, but also to adults. In the last two categories, minimally invasive treatments involve the use of fixed orthodontic appliances. In the case of retention biofilm around orthodontic brackets, additional oral hygiene methods are needed to obtain a significant reduction of pathogenic oral microorganisms which colonize in greater numbers on the strength of predisposing factors existence, represented by brackets design. As such, those patients who are under an orthodontic treatment require a proper education in achieving aright oral hygiene and also regular presentations to periodic inspections. The use of 0,2% CHX as an antiseptic subtstance, commonly used in dentistry and studied for its effects is chlorhexidine gluconate. Widely used chlorhexidine mouth wash in various concentration specific clinical indications or dental gel composition recorded two important properties namely, reducing the Streptococcus mutans number in plaque and saliva levels and the longer use of chlorhexidine may lead to fewer carious lesions in experimental studies (8,9). However the long-lasting 0,2% CHX use may lead to adverse effects related to microbial resistance, dental chromatic alteration or altered taste.

In the absence of plaque control, an early enamel demineralization appearance, as white spots lesions, is something usual.

The aim of this study, given these considerations, is to analyze the reducing bacterial colonies around orthodontic brackets by using laser radiation compared with conventional treatment based on 0,2% Chlorhexidine (CHX).

Materials and Methods

This study was performed at the Department of Oral Rehabilitation and Dental Emergencies,Faculty of Dentistry, in collaboration with Pediatric Dentistry and Microbiology Departments from University of Medicine and Pharmacy „Victor Babes", Timisoara. The experimental research included a clinical stage and a laboratory stage on human extracted teeth. The study was performed with 18 human third molars freshly extracted in which the ceramic orthodontic brackets were stitched and after this part all of them were placed on bacterial culture medium. For obtaining bacterial culture it was used unstimulated saliva, from patients who wear orthodontic braces, which was mixed with synthetic culture medium liquid (broth) in order to develop microbial colonies brackets adjacent.

The microorganisms samples were obtained respecting the same protocol of May-Lei Mei (10). Three 22-25 years old patients were selected for collecting the saliva samples.The participation of patients to study was conditioned by oral and written information for their role in this situation, also, the informed consent it was obtained after full disclosure and understanding all information and way of progress by each patient. It was obtained Institutional Ethical Committee of Medicine and Pharmacy „Victor Babeș" Universit Timișoara approvals.

The patients inclusion criteria: patients undergoing fixed orthodontic treatment for at least 6 months, without clinically detectable caries, periodontal disease absence and the respective pockets with a depth of 4 mm or more, the absence of systemic diseases, and in general without any medication. These patients discontinued all means of oral hygiene for 24 hours before the collection of saliva samples.

Exclusion criteria of patients: recent established orthodontic treatment (≤5 months), active caries, periodontal disease, systemic diseases and consumption of drugs with antibacterial effect. Five ml of saliva were collected from each patient in sterile containers, and after completing the collection of the three patients, all samples were submitted in one sterile container.

The study included 18 human third molars extracted for orthodontic reasons, infectious complications like pericoronitis, which were kept 72 hours in a 0,9% sodium chloride solution until to begin the experiment.

The teeth selection criteria were: intact vestibular surface without developped defects, no cracks, no caries or white spots lesions, without previous exposure to chemical treatments such as hydrogen peroxide and without changes caused during the extraction by elevator or pliers (11).

After samples selection, it was performed an ultrasonic scaler cleaning and brushing with fluoride-free paste. The buccal surface of each tooth was demineralized for 30 seconds with 37% orthophosphoric acid (Ormco Etching Solution, Ormco Corporation, USA), followed by washing for 20 seconds and drying, becoming a white opaque enamel demineralized specific process. The enamel surface was treated with primer (3M, Unitek, CA, USA), following that ceramic brackets (Ceramic .022 Roth, Ortho Classic, USA) to be stitched with Transbond Plus Color Change(3M, Unitek, CA, USA), following the manufacturer's instructions, and for curing was used LED light (420-480 nm, 1500 mV/cm^2, Rainbow Curing light, China) for 30 seconds (10 seconds to the mesial, distal and occlusal surface) (12).

After applyingthe brackets, each sample was stored in a sterile container. Each container was getting 20 ml synthetic culture medium liquid (broth) and from saliva mixture was taken 280μl salivawith a microbiological pipette and then were mixed with the broth,where samples were stored. At the end of process, all containers were aerobically incubated (Jouan IG150 Infrared Controlled CO2 Incubator, Germany) for 24 hours at 37°C in order to obtain microbial colonies brackets adjacent.

The next day, from each bracket were collected biofilm samples for seeding on agar-blood culture medium (COLUMBIA AGAR + 5% blood ram, Mediclim, Romania) following to be aerobically incubated for 24 hours at 37°C in order to develop microbial colonies.

The samples were randomized into 4 groups (n=3/group)(table 1).

All the chosen conventional treatments(0,2% CHX) were performed on the same day by one person (M.D.) and the remaining treatments were carried out by a person skilled in this field (T.C.), taking place in the same working conditions, to exclude a possible subjective error, thus following the manufacturer's instructions.

For group 2, decontamination was accomplished with GaAlAs Diode (λ 980nm Biolitec Laser Systems, USA) 1W, 3J, 4 seconds/application, pulsed working mode (18-20 beats/application), 3 applications around brackets enamel area. Decontamination for group 3 was carried out with Er:YAG laser (Fotona, FIDELIS plus II, Slovenia) 8Hz, 80mj, 5 applications, 5 seconds/application, pulsed mode (VSP). In group 4 was applied 0,2% CHX gluconate gel (Elugel 0,2%, Oral Care, France) on the buccal surface bracket adjacent, leaving for acts 40 seconds and then removed by flushing with water.

Microbiological sampling around the brackets was performed using sterile bacteriological loops which were deposited in broth solution for aerobically incubation at 37°C for 24 hours. On the third day it was counting the microbial colonies from the prior culture medium to its treatment, by one qualified person in the microbiology field. After obtaining the microbial colonies on the culture medium, were performed, in a randomized mode, smears for optical microscope observing, especially Streptococcus mutans (OPTIKA, B-600Tiph, Italy, 100x/1,25 oil PH, PLAN). The smears were stained with hematoxylin-eosin followed by the standard preparation protocol (13).

Statistical analysis was performed by using Microsoft Excel program (2012 Windows version), one-way analysis of variance (ANOVA) and Tukey-Kramer test.

RESULTS

The resulting colonies after treatment application were counting on the last day of experiment. Also, it has been chosen to achieve, at this stage, the corresponding smears to the previous, for optical microscope examination (fig. 1,2,3).

Following the inoculation of the samples with pathogenic oral microorganism resulted values almost identical after the newly formed colonies counting process. Colony count shows a value greater than 108 of oral microorganism number for 1ml saliva and S.m. presence is 70% of the total.

By applying different types of treatment in order to reduce the pathogenic oral microorganisms number, S.m. more specifically, resulted lower values, compared to the pre-treatment values. Values less than 10^5 were recorded for experimental groups,the negative control group being an exception. Also, there was a significant reduction in S.m. presence, percentage varies depending om efficiency of treatment applied. First of all, to calculate the normal distribution of values in vitro study results, was used Microsoft Excel program (2012 Windows version). Mean and standard deviation (SD) of microorganisms number developed after treatment application, were calculated. For this in vitro study, it was resorted to one-way analysis of variance (ANOVA) and according to the results it was applied the Tukey-Kramer test, for differences determination between types of experimental treatments. The significance level was set at 5%($p<0,05$) and 95% confidence level.

Further, analysis of the results was based on study and interpretation specific S.m. values. Within table 2 and

Table 1 - Working groups

Group number	1	2	3	4
Treatment type	No treatment	980 nm GaAlAs Diode Laser, 1W, 3J	2,760 nm Er:YAG laser, 8Hz, 80mj	0,2% CHX
Probe number	2,9,12	4,6,8	1,5,10	3,7,11

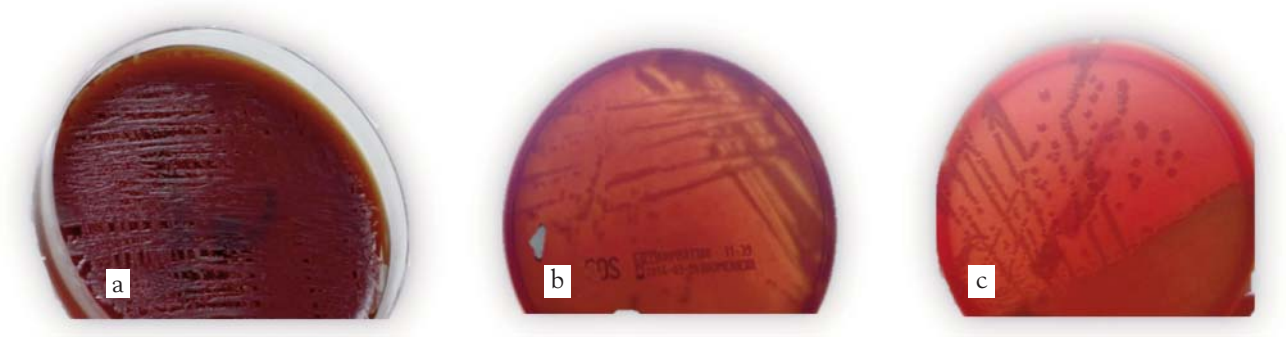


Figure 1 - Microbial colony counting prior to its experimental treatment (a) and after Er:YAG laser (b) and GaAlAs diode (c) application

Figure 2 - Smear performed pre-(a) and post Er:YAG laser treatment (b) on group 3

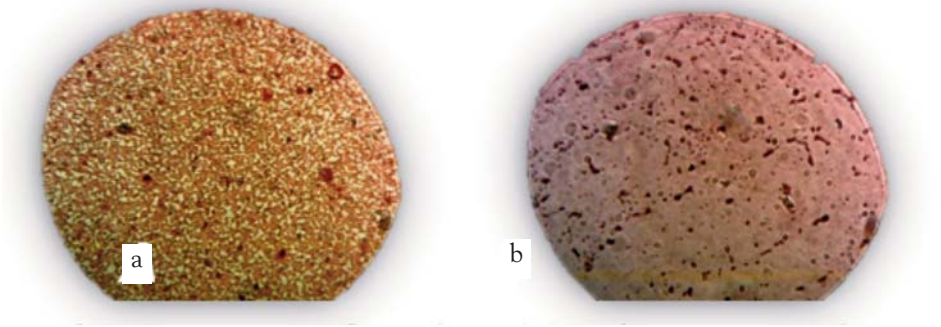
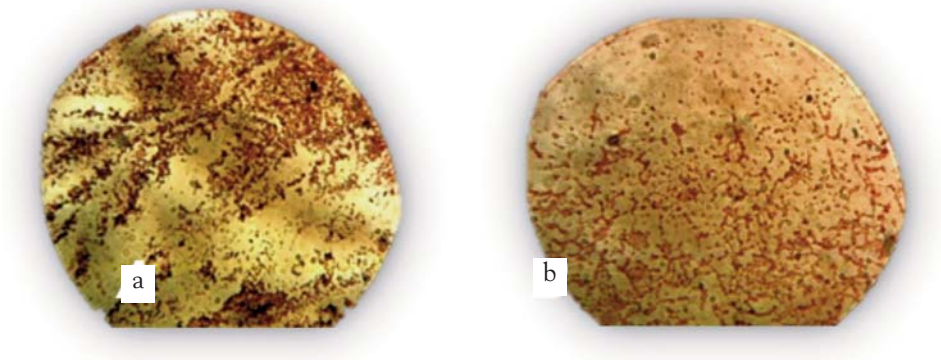


Figure 3 - Smear performed pre-(a) and post GaAlAs Diode treatment (b) on group 2



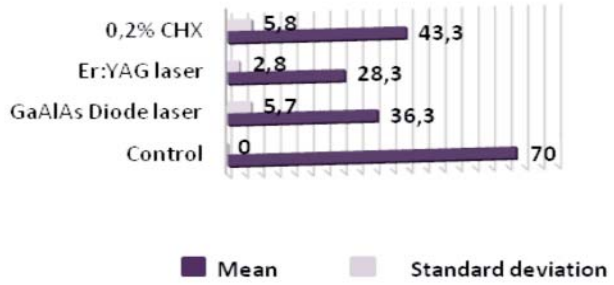
graphic 1 can be seen the average values for each group, but also, it was calculated the standard deviation for S.m. percent. As it is well noted for control group 1, values being identical, the deviation is null.

To analyze the average of results, was choosen the lowest S.m. percentage resulted in each experimental group. The highest average values is clear in group 1 where no treatment was applied, considered negative control, while the lowest average is observed in group 3 where Er:YAG laser was applied.

After one-way ANOVA analysis, it can be noted a significant difference between groups in terms of values

Table 2 - Mean and standard deviation calculation

Group number	1	2	3	4
Treatment type	No treatment	980 nm GaAlAs Diode laser	2760 nm Er:YAG laser	0,2% CHX
Probe 1 S.m.%	70 %	30 %	25 %	40 %
Probe 2 S.m.%	70 %	40 %	30 %	50 %
Probe 3 S.m.%	70 %	40 %	30 %	40 %
Mean	70	36,3	28,3	43,3
Standard deviation	0	5,7	2,8	5,7



Graphic 1 - Mean and standard deviation of S.m. percent

resulted from different experimental treatments application ($p < 0,05$).

After Tukey test made on S.m. values, it is showed a significant difference between groups results after treatment application, compared to samples control group ($p < 0,05$). From the statistic point of view, it can be noted, that is a significant difference between the Er:YAG group and 0,2% CHX group ($p < 0,05$).

Discussion

Despite efforts to bring an improvement for patients hygiene efficiency carrying fixed orthodontic appliance, prolonged retention of bacterial biofilm around retentive areas of these devices, unwanted effects make their presence at the end of treatment. The most commonly effects are represented by white spots lesions which continues to demonstrate that there is still a great combination between them and fixed orthodontic treatment (14). This in vitro research was focused on analyzing the effectiveness of several types of dental plaque reducing treatments, for the enamel areas orthodontic brackets adjacent, pathogenic oral microorganisms accumulation favourite areas. The results, after treatment application for reducing pathogenic oral microorganisms, reveal statistically significant differences compared with negative control group. Moreover, these results indicating the evidence on which were applied treatments show a significant difference, compared with control groups which shows that, indeed, it was recorded a reduction for pathogens oral bacteria number, expecially S.m.

As it is known, potentially cariogenic bacteria can be identified on dental plaque, normally, but they are less competitive in the presence of neutral pH and in a smaller proportion of the total bacterial community entering the constitution of dental plaque. In this case, by means of balanced diet, the level of potentially cariogenic bacteria is clinically insignificant, while, in the remineralization and demineralization process is in an equilibrum state (15).

If the frequency of carbohydrate consumption increases, the dental plaque will be exposed for a long time to a critical pH, resulting the hard dental structure demineralization (pH about 5.5) (16). To prevent these problems, especially those caused by S.m., it is important to know and realize a proper decontamination of the oral cavity using well proven antiseptic substances. As antiseptic substance, commonly used in dentistry and studied for its effects, chlorhexidine gluconate is effective in reducing S.m. number from dental plaque and saliva. The disadvantage of its using, is because the orthodontic treatment establishes for a long-term and well-known adverse effects of CHX will be installed shortly, which will raise concerns to patient on staining and taste alteration over time. From another point of view, the laser radiation was found to be superior to antiseptic substances, as a result of the experimental tests results, obtained and reduced newly microbial colonies formed number after treatment application (17-19).

The antibacterial effect of different types of laser radiation was and is extensively studied. Studies show that Er:YAG and diode lasers are of particulary significant efficiency on Echerichia coli and Enterococcus faecalis, which is considered as a viable tool in the root canals decontamination. In other in vitro conducted studies, for antibacterial effect of the Er:YAG laser radiation, was clearly demonstrated the bactericidal effect on Porfiromonas gingivalis and Actinobacillus actinomycetemcomitans (20-22). It was expected that these studies should extend to prevention field. Demineralization is an initial stage of caries process installation. Establishing the effective treatments for demineralization prevent or arresting it, represents the red thread leader of modern prevention. As such, this study, raises a key issue opposite demineralization around brackets prevention.

In what concerns the incidence of bacteria reduced by laser radiation,it must be considered several factors which play a decisive role on this line: energy laser radiation, the water content of tissue acted upon, volume, thickness and strength of the cell wall, absorption property and bacteria migration to tissues,as well as, the penetration degree in the enamel prisms, respectively dentinal tubules (23).

In this research, evidence inoculation dates of stay was of 24 hours. On the clinical situation, this period is certainly one longer, the degree of dental hard surface impairment is probably much increased (significantlly) than for this samples study. Observing all these situations, it can be said that, to hold/gain efficiency relatively good, is important that energy laser equipments can have effects both in surface and deepness, depending on the degree to which enamel structure is affected and respectively of the dentine. The results of this study provide a strong evidence that by reducing the oral microorganisms number, especially S.m., are record after using the Er:YAG laser and it can be noted the difference between Er:YAG laser equipment and the ones who use 0,2% CHX.

By increasing bacterial resistance to antibiotics, conventional mechanical and chemical decontamination, has been widely questioned in terms of effectiveness of these therapies. To reduce these shortcoming problems, laser therapy can be a possible alternative to antibacterial therapy for preventing the undesirable effects of prolonged plaque retention from enamel surface (24).

Traversing the advantages of using laser equipment to the detriment of conventional antimicrobial agents: non-

invasively mode, repeatability, increased selectivity, absence of drug resistance installation, effective destruction of the target microorganisms within a few minutes, all depending upon the emitted laser energy density(on the contrary, the conventional antimicrobial agent, a period of few hours or days which are a necessity to achieve the desired effect), but ultimately, antimicrobial effects may be limited depending on the acted place and the restriction given by different forms of fiber (25,26).

It was insisted on analyzing results, especially S.m. number, since they are the most predominant in dental caries initiation. Even if there have been recorded reductions of S.mnumber, after the diode laser application with low level energy, it is necessary in the future to be able to achieve further research regarding its application for generally reducing pathogenic oral microorganisms, because of the different susceptibilities existence to different laser energy of specific cariogenic bacteria and other bacteria present in the microbial biofilm.

Conclusions

Orthodontic treatments establish for a long term what it will cause a sharp use of CHX, by setting up such microbial resistance, chromatic dental alteration and taste alterations.

The present study demonstrates the superior efficiency of Er: YAG laser radiation decontamination newly formed colonies around orthodontic brackets. The advantages of this method are the nonivasively mode, repeatability, high selectivity, without the presence of resistance to drugs, effective reduction of target microorganisms in a few minutes.

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