Opposite effect of supernatants from selected periopathogens and oral lactobacilli cultures on ATP levels in human gingival fibroblasts

Anna K. Szkaradkiewicz[1], Tomasz M. Karpiński[2], Agnieszka Zeidler[2], Andrzej Szkaradkiewicz[2]

[1]Department of Conservative Dentistry and Periodontology, University of Medical Sciences in Poznań, Poland;
[2]Department of Medical Microbiology, University of Medical Sciences in Poznań, Poland

SUMMARY

Studies were performed on the effects of supernatants obtained from bacterial cultures, including cultures of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Lactobacillus acidophilus* strains on ATP levels in human gingival fibroblasts (HGF-1) and on their viability. ATP levels were evaluated using luminescence test and cell viability was estimated using a fluorescence test. In control cultures mean levels of ATP in HGF-1 amounted to 4.90±0.32 mln RLU. Supernatants of *P. gingivalis* and *A. actinomycetemcomitans* cultures were found to significantly reduce ATP production in HGF-1 (mean levels of ATP amounted to 3.41±0.33 and 3.55±0.3 mln RLU respectively), which was not accompanied by an increased proportion of dead fibroblasts. Supernatants of *P. intermedia* induced no significant alterations in ATP level in HGF-1. In turn, supernatants of *L. acidophilus* H_2O_2 (+) and H_2O_2 (-) cultures significantly increased ATP levels in HGF-1 (the mean levels amounted to 5.94±0.31 mln RLU and 5.88±0.28 mln RLU respectively). The results indicate that extracellular products of *P. gingivalis* and *A. actinomycetemcomitans* most probably represent mitochondria-targeted peptides, which reduce synthesis of ATP in HGF-1. In turn, extracellular products of *L. acidophilus* seem to represent exopolysaccharides (EPS) with pro-oxidant activity, which stimulate synthesis of ATP in HGF-1.

KEY WORDS: Periopathogens, *Lactobacillus acidophilus,* Microbial products, ATP, Human gingival fibroblasts.

Received December 19, 2013 *Accepted* September 1, 2014

INTRODUCTION

Periodontitis represents a chronic periodontal disease leading to the destruction of periodontal tissues.

Most frequently periodontitis is manifested in adults with a moderate or severe clinical course (Pihlstrom *et al.,* 2005). The frequency of manifestation and advancement of chronic periodontitis increases with patient age (Albandar and Rams 2002). Epidemiological studies have confirmed that chronic periodontitis belongs to the most frequent chronic diseases in humans. In West Europe around 36% individuals aging 35-44 years manifest moderate and around 10% severe forms of chronic periodontitis.

The proportions are higher in countries of East Europe, amounting to 45% and 30-40% respectively (Sheiham and Netuveli 2002).

Microbiological studies in humans and experimental investigations on animal models indicate that the aetiopathogenesis of periodontitis involves the principal role of a mixed microbial infection with specific species of pathogenic bacteria, termed periopathogens or periodontopathogens (Darveau 2010; Kebschull and Papapanou 2011).

The clinically important periopathogens include, i.a., *Porphyromonas gingivalis* and *Prevotella intermedia*, representing obligatory anaerobes and *Aggregatibacter actinomycetem-*

Corresponding author
Prof. Andrzej Szkaradkiewicz
Department of Medical Microbiology
University of Medical Sciences in Poznań
Wieniawskiego 3, str., 61-712, Poznań, Poland
E-mail: szkaradkiewicza@poczta.onet.pl

comitans, the species of facultative anaerobes (Cutler *et al.,* 1995; Socransky *et al.,* 1998; Henderson *et al.,* 2002; Henderson *et al.,* 2010). Results obtained to date indicate that colonizing periodontal tissue, periopathogens and their products may induce a pro-inflammatory response and may exert cytotoxic effects toward host cells (Kebschull and Papapanou 2011; Preshaw and Taylor 2011; Irshad *et al.,* 2012). In parallel, oral lactobacilli were documented to be able to inhibit the growth of periopathogens and may prevent the progression of periodontitis (Teanpaisan *et al.,* 2011; Szkaradkiewicz *et al.,* 2011). Nevertheless, no data have yet been obtained on an antagonistic interaction of periopathogens and oral lactobacilli on metabolic activity in gingival fibroblasts. The cells prevail in connective tissue of the periodontium and they assure the integrity of its structure (Koka and Reinhardt 1997).

The cellular adenosine triphosphate (ATP) level represents a significant exponent of cell metabolic activity and viability. It was already well documented that ATP is required for most cellular functions (Dietrich and Horvath 2010) and that it is produced mainly in mitochondria in all eukaryotic cells.

A decrease in cellular ATP production provides metabolic conditions leading to cell death by different mechanisms, including apoptosis, autophagy or necrosis (Lemasters *et al.,* 2002; Skulachev 2006).

Several techniques are used to estimate ATP concentration in cells (De Korte *et al.,* 1985; Lundin *et al.,* 1986). However, the bioluminescence method provides the most sensitive and specific measurement (Higashi *et al.,* 1985; Weyermann *et al.,* 2005). It has already been well documented that the emitted light intensity is linearly related to the ATP concentration (Crouch 2000).

Taking the above into account, this study aimed to evaluate the effect exerted by supernatants isolated from cultures of clinical strains, including selected periopathogens and oral lactobacilli, on levels of ATP in human gingival fibroblasts. In parallel, in the context of our aim it was important to analyze whether the action of the studied supernatants was followed by alterations in percentage of viable and dead fibroblasts.

MATERIALS AND METHODS

Patients

The studies were performed on bacterial strains selected from 34 patients (30-52 years of age, mean 39.5±5.3 years) with chronic periodontitis among whom 16 patients suffered from moderate and 18 patients from severe chronic periodontitis.

Bacterial cultures

Bacteria of *Porphyromonas* and *Prevotella* genera were cultured in Columbia Agar enriched with 7% ovine blood, in anaerobic conditions, using GENbag anaer (bioMerieux), at the temperature of 37°C for 48-72 hours. Rapid ID 32 A test strips (bioMerieux) were used to identify the bacteria. Bacteria of *Aggregatibacter* genus were cultured in a chocolate agar, based on Columbia Agar with 7% ovine blood, in the atmosphere of 5% CO_2, using GENbag CO_2 (bioMerieux), at a temperature of 37°C for 48-72 hours. Identification involved API NH test (bioMerieux), supplemented by examination of the growth ability around factors X and V and examination of the ability to produce catalase.

Lactobacillus spp. was cultured on Rogosa agar and the cultured isolates obtained in anaerobic conditions were identified using API 50 CHL (bioMerieux). The capacity of hydrogen peroxide production among *Lactobacillus* strains was defined in culture of the obtained isolates in the presence of 5% CO_2 at a temperature of 37°C for 48 hours in a differentiating medium, TMB-Plus agar, prepared according to Rabe and Hillier (2003). Development of an altered colour of the growing colonies (appearance of a blue colour) indicated production of hydrogen peroxide.

Production of supernatants

Cultures of individual bacteria provided material to obtain 0.5 McF bacterial suspension in 2 ml physiological saline. Two ml of RPMI-1640 (Sigma) were supplemented with 0.1 ml bacterial suspension and cultured for 24 hours in anaerobic conditions. Subsequently the cultures were passed through sieves to obtain supernatants. The supernatants were also subjected to inactivation at temperature of 56ºC for one hour and at 100ºC for 30 minutes.

Cell cultures
Gingival fibroblasts, HGF-1 (CRL-2014, ATCC) were cultured in T-25 and T-75 culture vessels (Nunc) in an incubator at a temperature of 37°C, in an atmosphere of 5% CO_2. The culture medium involved DMEM (ATCC) solution enriched with 10% foetal bovine serum (FBS, Sigma), supplemented with penicillin 100 U/ml and streptomycin 20 mg/ml (Sigma). The culture medium was replaced three times a week.

Evaluation of ATP levels
ATP levels in cultures of gingival fibroblasts (HGF-1) were evaluated using a luminescence test (CellTiter-Glo Luminescent Cell Viability Assay, Promega). The tests were performed in the presence of a buffered physiological saline, PBS (15 µl /10^5 HGF-1 cells/135 µl medium) and 10% supernatants obtained from cultures of individual bacteria (15 µl /10^5 HGF-1 cells/135 µl medium). In preliminary experiments we analyzed the effects of culture medium in the presence of 5%, 10%, 15% and 20% of the supernatants originating from the above-mentioned bacterial cultures. The most pronounced alterations in RLU levels were found to take place in 10%-20% supernatants. However, effects of 5% supernatant concentrations proved to be insignificantly different from the control. Therefore, in all our experiments we applied a 10% concentration of supernatants from bacterial cultures.
ATP levels in HGF-1 gingival fibroblasts were estimated in the presence of a supernatant isolated from every isolated bacterial strain, in three repetitions on three consecutive days. Estimations of supernatant effects were conducted before their heat treatment and after exposing them to a temperature of 56ºC for 30 minutes and 100ºC for 30 minutes. The medium involved DMEM (ATCC) solution enriched with 10% foetal bovine serum (FBS, Sigma). The prepared cells were incubated for 24 hours in an incubator at 37ºC in the presence of 5% CO_2. Then, the cells were rinsed with the culture medium and tested for ATP. Each of the samples was supplemented with 150 µl of the prepared reagent (substrate plus buffer), mixed for 2 minutes and incubated for 10 minutes at room temperature. The results were read using a luminometer (GloMax, Promega). The light emitted in the presence of ATP was quantitated in relative light units (RLU). The intensity of emitted light quants was directly related to ATP content in the tested sample.

Testing of viability in gingival fibroblasts
The viability testing in HGF-1 gingival fibroblasts took advantage of the Live/Dead Viability/Cytotoxicity Kit (Invitrogen, USA) fluorescence test. This test quickly discriminates live from dead cells by simultaneously staining with green-fluorescent calcein-AM to indicate intracellular esterase activity and red-fluorescent ethidium homodimer-1 to indicate loss of plasma membrane integrity. The studies were conducted in Lab-Tek Chamber Slide (Nunc) culture chambers in a buffered physiological saline (PBS, 50 µl/0.5x10^6 HGF-1 cells/450 µl medium) or in the presence of 10% supernatant obtained from cultures of individual bacterial strains (50 µl/0.5x 10^6 HGF-1 cells/450 µl medium). Viability of HGF-1 gingival fibroblasts was tested in the presence of every supernatant, isolated from individual bacterial strains, in three repetitions on three consecutive days. The effect of the supernatants was tested before and following their heat treatment at a temperature of 56ºC for 30 minutes and at 100ºC for 30 minutes. The medium involved DMEM (ATCC) solution, enriched with 10% foetal bovine serum (FBS, Sigma). The prepared cells were incubated for 24 hours in an incubator at the temperature of 37ºC in 5% CO_2. Subsequently, the cells were washed in the culture medium and tested for their viability. The readouts were made at zero time and following 6, 12, 18 and 24 hours, using a Nikon Eclipse E200 fluorescence microscope (magnification of 1000x).

Statistical analysis
Results obtained in the studies were analysed using the computer software STATISTICA 8 for Windows. The statistical analysis included non-parametric tests, permitting calculation of a broad range of various position measurements (mean) and dispersion (standard deviation). In the comparative analysis related to ATP level in studied groups the one-way ANOVA with Tukey-Kramer test was applied. In the analysis of gingival fibroblast viability the non-parametric

Kruskal-Wallis test was applied. In every test, hypotheses were verified at the significance level of p=0.05.

RESULTS

In the presented experiments 36 bacterial strains were isolated from the patients, including 15 strains of *Lactobacillus acidophilus* (with 6 strains producing H_2O_2 and 9 strains unable to produce H_2O_2), 11 strains of *Porphyromonas gingivalis*, 6 strains of *Prevotella intermedia* and 4 strains of *Aggregatibacter actinomycetemcomitans*.

The results obtained illustrating changes in ATP levels in HGF-1 gingival fibroblasts, under effect of supernatants obtained from cultures of individual strains of bacteria in specific species were analysed together providing ranges and means of the obtained values ± SD in Tables 2 and 3.

The dynamics of alterations in ATP levels in HGF-1 gingival fibroblasts subsequent to their exposure to supernatants of individual strains of analysed bacterial cultures are presented in Figure 1 and Table 1. Supernatants of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* cultures were found to induce a significant decrease in ATP level already after 6 h and the decrease persisted for a period of 24 h. On the other hand, supernatants from cultures of *Prevotella intermedia* exerted no effect on ATP levels in gingival fibroblasts. In turn,

TABLE 1 - *Mean levels of ATP (luminescence in millions of RLU) in cultures of gingival fibroblasts (HGF-1) at zero time and following 6, 12, 18 or 24 h incubation with supernatants obtained from cultures of studied bacteria.*

Studied cells	*Incubation times*				
	0 hour	*6 hours*	*12 hours*	*18 hours*	*24 hours*
Control (gingival fibroblasts HGF-1) with 10% PBS	4.73±0.34	4.77±0.27	4.82±0.38	4.80±0.33	4.84±0.35
HGF-1 with 10% supernatant of *L. acidophilus* H2O2 (+)	4.74±0.44	5.66*±0.53	5.73*±0.49	5.79*±0.56	5.89*±0.52
HGF-1 with 10% supernatant of *L. acidophilus* H2O2 (-)	4.81±0.39	5.75*±0.46	5.81*±0.45	5.85*±0.42	5.93*±0.47
HGF-1 with 10% supernatant of *P. intermedia*	4.71±0.36	4.52±0.41	4.55±0.38	4.52±0.46	4.61±0.57
HGF-1 with 10% supernatant of *A. actinomycetemcomitans*	4.76±0.47	3.70*±0.40	3.68*±0.39	3.60*±0.44	3.52*±0.46
HGF-1 with 10% supernatant of *P. gingivalis*	4.68±0.51	3.52*±0.42	3.47*±0.45	3.48*±0.47	3.47*±0.43

*Significant difference compared to zero time.

TABLE 2 - *Mean levels of ATP (luminescence in millions of RLU) in gingival fibroblasts (HGF-1) following 24 h incubation with supernatants of the examined periopathogens.*

Studied cells	*Unheated supernatants*	*Supernatants exposed to 56ºC for 30 minutes*	*Supernatants exposed to 100ºC for 30 minutes*
	Mean ± SD [range]		
Control (gingival fibroblasts HGF-1) with 10% PBS	4.84±0.35 [4.37-5.45]	4.78±0.51 [4.21-5.35]	4.81±0.48 [4.26-5.29]
HGF-1 with 10% supernatant of *P. intermedia*	4.61±0.57 [3.89-5.28]	4.72±0.69 [4.12-5.07]	4.77±0.56 [4.03-5.21]
HGF-1 with 10% supernatant of *A. actinomycetemcomitans*	3.52*±0.46 [2.83-4.28]	3.65*±0.45 [3.06-4.41]	4.68±0.52 [3.64-5.06]
HGF-1 with 10% supernatant of *P. gingivalis*	3.47*±0.43 [2.57-4.15]	3.53*±0.36 [2.69-4.37]	4.61±0.49 [3.48-5.15]

*Significant difference compared to the control group.

supernatants from *Lactobacillus acidophilus* cultures significantly augmented ATP levels in gingival fibroblasts already after 6 h and the effect persisted for a period of 24 h.

In parallel, the studies documented a significant decrease in ATP content taking place under effect of 56°C heat-treated or untreated supernatants obtained from cultures of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* strains. Supernatants of the bacterial cultures subjected to heat treatment at 100°C induced no decrease in ATP levels in gingival fibroblasts (Table 2).

In turn, the level of ATP in the fibroblasts did not differ from control values after exposure of the cells to supernatants of *Prevotella intermedia* strains (Table 2).

The unheated supernatants of *Lactobacillus ac-*

TABLE 3 - *Mean levels of ATP (luminescence in millions of RLU) in cultures of gingival fibroblasts (HGF-1) following 24 h incubation with supernatants of Lactobacillus acidophilus.*

Studied cells	*Unheated supernatants*	*Supernatants exposed to 56°C for 30 minutes*	*Supernatants exposed to 100°C for 30 minutes*
		Mean ± SD [range]	
Control (gingival fibroblasts HGF-1) with 10% PBS	4.84±0.35 [4.37-5.45]	4.78±0.51 [4.21-5.35]	4.81±0.48 [4.26-5.29]
HGF-1 with 10% supernatant of *L. acidophilus* H2O2 (+)	5.89*±0.52 [5.16-6.95]	5.82*±0.48 [5.22-6.78]	4.29±0.55 [3.43-5.13]
HGF-1 with 10% supernatant of *L. acidophilus* H2O2 (-)	5.93*±0.47 [5.42-7.24]	5.85*±0.57 [5.29-6.82]	4.37±0.41 [3.58-5.36]

*Significant difference compared to the control group.

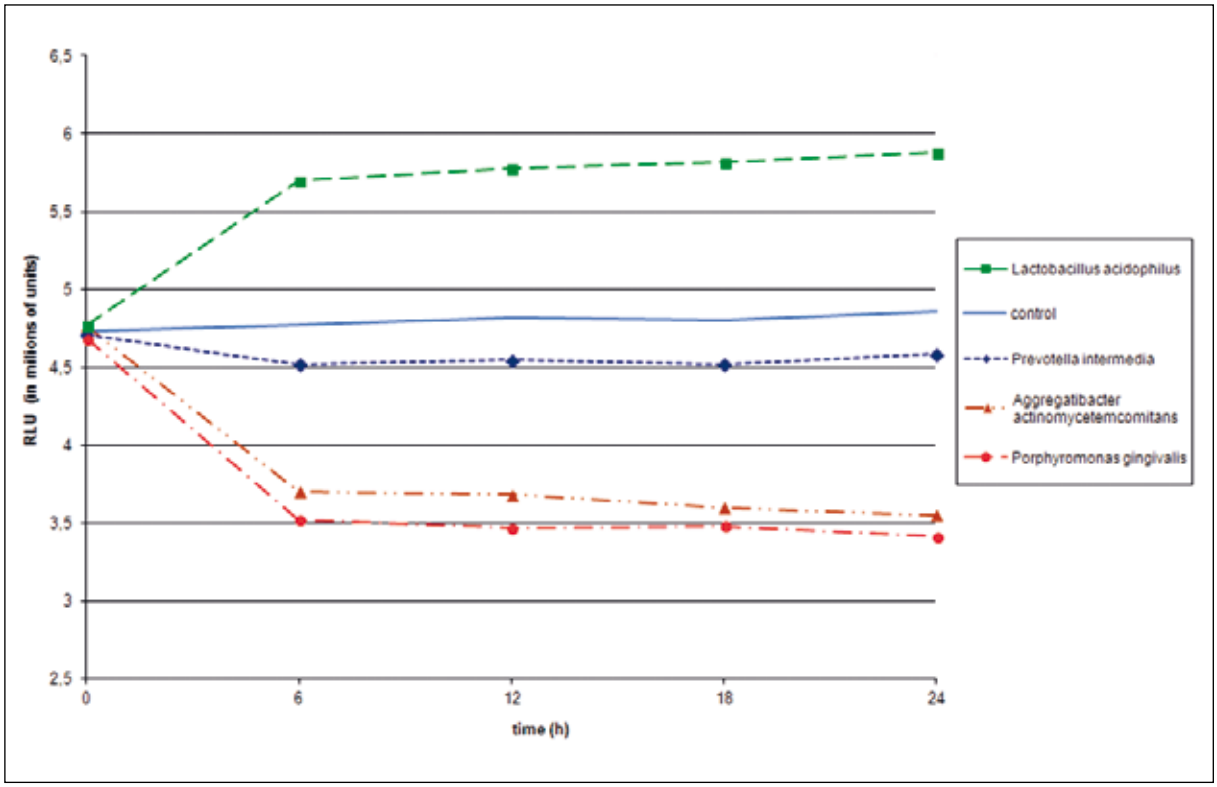

FIGURE 1 - *Graphic presentation of mean ATP levels (luminescence in millions of RLU) in cultures of gingival fibroblasts (HGF-1) at zero time and following 6, 12, 18 or 24 h incubation with supernatants obtained from cultures of studied bacteria.*

idophilus strains or the supernatants exposed to a temperature of 56°C induced a significant increase in ATP level in HGF-1 gingival fibroblasts while the supernatants exposed to the temperature of 100°C induced no increase in ATP level in the gingival fibroblasts (Table 3).
In studies on the viability of HGF-1 gingival fibroblasts following any duration of incubation, i.e. at zero time or following 6, 12, 18 or 24 hours viability of gingival fibroblasts ranged between 94% and 98%. No significant differences were disclosed between control samples and samples containing supernatants isolated from cultures of individual bacterial strains.

DISCUSSION

This study demonstrated that supernatants originating from 24 hour cultures of *P. gingivalis* or those of *A. actinomycetemcomitans* interact with human gingival fibroblasts, significantly reducing their production of ATP. The effects of reduced cellular ATP levels have not been detected following exposure to supernatants of *P. intermedia*. In parallel, it was found that the activity of *P. gingivalis* supernatants or *A. actinomycetemcomitans* supernatants was not reduced by their exposure to a temperature of 56°C for 30 min. On the other hand, following exposure to 100°C for 30 min, the supernatants lost their activity. In contrast to polysaccharides and thermostable peptides, large proteins usually cannot withstand heat treatment at 56°C for 30 min. It has been well-documented that strains of *P. gingivalis* and *A. actinomycetemcomitans* produce toxins which belong to large proteins.
They include the secreted by *P. gingivalis* cysteine proteases (gingipains: HRgpA-95kDa, RgpB-50kDa and Kgp-110kDa), which play a significant role in tissue destruction, and the *A. actinomycetemcomitans*-produced cytolethal distending toxin composed of 3 subunits with molecular weight ranging from 24 to 35 kDa, which inhibits human gingival fibroblast divisions, particularly the progression from G_2 to mitosis (Imamura 2003; Shenker *et al*. 2004; Belibasakis *et al*. 2004). In turn, polysaccharides are not secreted by the tested periopathogens, representing a component of lipopolysaccharide they form a component of cell wall structure in the bacteria. Thus, the obtained data allow us to conclude that the substances released by *P. gingivalis* and by *A. actinomycetemcomitans* which reduce ATP levels in gingival fibroblasts may represent thermostable peptides. Their cytotoxic activity might result in a decreased production of ATP.
However, we failed to detect an increased proportion of dead cells following exposure to the supernatants. Therefore, the data indicate rather that the substances produced by *P. gingivalis* and *A. actinomycetemcomitans*, most probably involving peptides, do not exert lytic effects toward the plasma membrane, and their toxicity is directly linked to mitochondrial damage.
Such a potential for the unique activity of peptides seems probable in view of the data documenting cell-permeable peptides which efficiently enter humans cells and can target the mitochondria with a high specificity (Fisher *et al*. 2005; Horton *et al*. 2008). The decreased energy potential of a cell may, in a secondary manner, be followed by an inhibited proliferation of fibroblasts and induction of cell death by apoptosis.
In turn, the levels of ATP in gingival fibroblasts have not been altered by supernatants of *P. intermedia* and, therefore the periopathogen does not produce substances inducing intracellular disturbances. The conclusion seems to be supported by the earlier data indicating that sonicated bacterial extracts from *P. intermedia* do not inhibit growth of fibroblasts (Yamasaki *et al*. 1998). On the other hand, in our studies on supernatants of 24 hour cultures of *L. acidophilus* strains their use was followed by a significant increase in ATP levels in gingival fibroblasts. In addition, the increase in ATP levels could not be related to H_2O_2 produced by the *L. acidophilus* strains. Results obtained in the range are consistent with our earlier observations (Szkaradkiewicz and Stopa 2013). At present, the identity of the human gingival fibroblast ATP-production stimulating factor remains unknown. However, its (their) thermal stability demonstrated in our experiments indicates that it represents a polysaccharide. This conclusion is supported by studies proving that lactobacilli, including *L. acidophilus*, may release high amounts of exopolysaccharides (EPS) (Cern-

ing 1995; Laws *et al.,* 2008). To date, variable EPS functions have been described, acting, i.a., as colonization promoters, agents protective against the environment, structure stabilizers in a biofilm and as signalling molecules (Badel *et al.,* 2011). This study pointed out for the first time that the probable EPS released by *L. acidophilus* strains may exhibit pro-oxidant activity, stimulating ATP synthesis in human gingival fibroblasts.

The results correspond to earlier observations documenting *in vivo* a stimulatory effect of *L. acidophilus* supernatants on proliferation of fibroblasts (Halper *et al.,* 2003) and on the growth and development of embryonic cells *in vitro* (Li *et al.,* 2005). Recently it was also noted that EPS may exert a protective effect on human endothelium cells, manifesting a significant capacity in scavenging of free radicals (Lin *et al.,* 2012). Therefore, it seems probable that EPS of *L. acidophilus* exerts both pro-oxidant and antioxidant activity.

Our results demonstrate that extracellular products of the periopathogens *P. gingivalis* and *A. actinomycetemcomitans* most probably representing mitochondria-targeted peptides, reduce synthesis of ATP in human gingival fibroblasts, which may inhibit their proliferation and may induce cell death by apoptosis. In turn, extracellular products of *L. acidophilus*, most probably representing exopolysaccharides with pro-oxidant activity, promote synthesis of ATP in human gingival fibroblasts.

Competing interests

The authors declare no competing interests.

ACKNOWLEDGEMENTS

This study was supported by the University of Medical Sciences in Poznań, Poland (Grant no. 502-14-02209324-08456).

REFERENCES

Albandar J.M., Rams T.E. (2002). Global epidemiology of periodontal diseases: an overview. *Periodontol. 2000*. **29**, 7-10.

Badel S., Bernardi T., Michaud P. (2011). New perspectives for Lactobacilli exopolysaccharides. *Biotech. Adv.* **29**, 54-66.

Belibasakis G.N., Mattsson A., Wang Y., Chen C., Johansson A. (2004). Cell cycle arrest of human gingival fibroblasts and periodontal ligament cells by *Actinobacillus actinomycetemcomitans*: involvement of the cytolethal distending toxin. *APMIS.* **112**, 675-685.

Cerning J. (1995). Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. *Lait.* **75**, 463-472.

Crouch S. (2000). Biocompatibility testing ATP bioluminescence. *Med. Device Technol.* **11**, 12-15.

Cutler C.W., Kalmar J.R., Genco C.A. (1995). Pathogenic strategies of the oral anaerobe, Porphyromonas gingivalis. *Trends Microbiol.* **3**, 45-51.

Darveau R.P. (2010) Periodontitis: a polymicrobial disruption of host homeostasis. *Nature Rev. Microbiol.* **8**, 481-490.

De Korte D., Haverkort W.A., van Gennip A.H., Roos D. (1985). Nucleotide profiles of normal human blood cells determined by high-performance liquid chromatography. *Anal. Biochem.* **15**, 197-209.

Dietrich M.O., Horvath T.L. (2010). The role of mitochondrial uncoupling proteins in lifespan. *Pflugers Arch.* **459**, 269-275.

Fischer R., Fotin-Mleczek M., Hufnagel H., Brock R. (2005) Break on through to the other side-biophysics and cell biology shed light on cell-penetrating peptides. *ChemBioChem.* **6**, 2126-2142.

Halper J., Leshin L.S., Lewis S.J., Li W.I. (2003) Wound healing and angiogenic properties of supernatants from *Lactobacillus* cultures. *Exp. Biol. Med.* **228**, 1329-1337.

Henderson B., Ward J.M., Ready D. (2010) *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*: a triple A periodontopathogen? *Periodontology 2000.* **54**, 78-105.

Higashi T., Isomoto A., Tyuma E., Kakishita E., Uomoto M., Nagai K. (1985) Quantitative and continuous analysis of ATP release from blood platelets with firefly luciferase luminescence. *Thromb. Haemost.* **53**, 65-70.

Horton K.L., Stewar, K.M., Fonseca SB., Guo Q., Kelly S.O. (2008). Mitochondria-penetrating peptides. *Chem. Biol.* **15**, 375-382.

Imamura T. (2003). The role of gingipains in the pathogenesis of periodontal disease. *J. Periodontol.* **74**, 111-118.

Irshad M., van der Reijden W.A., Crielaard W., Laine M.L. (2012) In vitro invasion and survival of *Porphyromonas gingivalis* in gingival fibroblasts; role of the capsule. *Arch. Immunol. Ther. Exp.* **60**, 469-476.

Kebschull M., Papapanou P.N. (2011). Periodontal microbial complexes associated with specific cell and tissue responses. *J. Clin. Periodontol.* **38** (Suppl. 11), 17-27.

Koka S., Reinhardt R.A. (1997). Periodontal pathogen-related stimulation indicates unique phenotype of primary cultured human fibroblasts from

gingiva and periodontal ligament: implications for oral health disease. *J. Prosthet. Dent.* **77**, 191-196.

Laws A.P., Chadha M.J., Chacon-Romero M., Marshall V.M., Maqsood M. (2008). Determination of the structure and molecular weights of the exopolysaccharide produced by *Lactobacillus acidophilus* 5e2 when grown on different carbon feeds. *Carbohydrate Res.* **343**, 301-307.

Lemasters J.J., Qian T., He L., Kim J.S., Elmore S.P., Cascio W.E., Brenner D.A. (2002) Role of mitochondrial inner membrane permeabilsation in necrotic cell death, apoptosis, and autophagy. *Antioxid. Redox. Signal.* **4**, 769-781.

Li W.I., Brackett B.G., Halper J. (2005). Culture supernatant of *Lactobacillus acidophilus* stimulates proliferation of embryonic cells. *Exp. Biol. Med.* **230**, 494-501.

Lin Y., Liu J., Hu Y., Song X., Zhao Y. (2012). An antioxidant exopolysaccharide devoid of pro-oxidant activity produced by the soil bacterium Bordetella sp. B4. *Biores Technol.* **124**, 245-251.

Lundin A., Hasenson M., Persson J., Pousette A. (1986). Estimation of biomass in growing cell lines by ATP assay. *Methods Enzymol.* **133**, 27-42.

Pihlstrom B.L., Michalowicz B.S., Johnson N.W. (2005) Periodontal diseases. *Lancet.* **366**, 1809-1820.

Preshaw P.M., Taylor J.J. (2011). How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *J. Clin. Periodontol.* **38** (Suppl. 11), 60-84.

Rabe L.K., Hillier S.L. (2003). Optimization of media for detection of hydrogen peroxide production by *Lactobacillus* species. *J. Clin. Microbiol.* **41**, 3260-3264.

Sheiham A., Netuveli G.S. (2002) Periodontal diseases in Europe. *Periodontol. 2000* **29**, 104-121.

Shenker B.J., Besack D., McKay T., Pankoski L., Zekarat A., Demuth D.R. (2004) *Actinobacillus actinomycetemcomitans* cytolethal distending toxin (Cdt): evidence that the holotoxin is composed of three subunits: CdtA, CdtB, and CdtC. *J. Imunol.* **172**, 410-417.

Skulachev V.P. (2006). Bioenergetic aspects of apoptosis, necrosis and mitoptosis. *Apoptosis.* **11**, 473-485.

Socransky S.S., Haffajee A.D., Cugini M.A., Smith C., Kent R.L. Jr. (1998) Microbial complexes in subgingiwal plaque. *J. Clin. Peridontol.* **25**, 134-144.

Szkaradkiewicz A.K., Karpiński T.M., Zeidler A., Wyganowska-Świątkowska M., Szkaradkiewicz A. (2011). Protective effect of oral lactobacilli in pathogenesis of chronic periodontitis. *J. Physiol. Pharmacol.* **62**, 685-689.

Szkaradkiewicz A.K., Stopa J. (2013). Effect of supernatants from *Lactobacillus acidophilus* culture on ATP levels in human gingival fibroblasts. *Curr. Issues Pharm. Med. Sci.* **26**, 140-143.

Teanpaisan R., Piwat S., Dahlen G. (2011). Inhibitory effect of oral *Lactobacillus* against oral pathogens. *Lett. Appl. Microbiol.* **53**, 452-459.

Weyermann J., Lochmann D., Zimmer A. (2005). A practical note on the use of cytotoxicity assays. *Int. J. Pharmac.* **288**, 369-376.

Yamasaki M., Nakata K., Imaizumi I., Iwama A., Nakane A., Nakamura H. (1998). Cytotoxic effect of endodontic bacteria on periapical fibroblasts. *J. Endod.* **24**, 534-539.