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Research Article

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Regeneration Plate 3.0 – Improvement and Maintenance of Intestinal Health by Reduction of Oxidative Stress and Inflammation

Peter C. Dartsch*

Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Auf der Vosshardt 25, D-49419 Wagenfeld, Germany

***Corresponding author:** Peter C. Dartsch, Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Auf der Vosshardt 25, D-49419 Wagenfeld, Germany

ABSTRACT

Background: Sleep is an integral biological necessity and is understood to possess recuperative and regenerative properties. Sleep deprivation has been associated with diseases and an increase in morbidity and mortality. Several recent studies have suggested a strong relation between insufficient sleep and gastrointestinal diseases, especially when triggered by inflammatory processes. Accordingly to these findings it has been reported that sleep deprivation in both humans and experimental animals causes a progressive increase in circulating white blood cells, mainly neutrophils as well as an increase in various circulating proinflammatory molecules.

Experimental: Against this background we used cultured intestinal epithelial cells to investigate the positive impact of a specially designed device, Regeneration Plate 3.0, which is positioned under the bed during sleep and is stated to improve systemic health on the cellular level by reducing oxidative stress which acts on the body. The plate produces a vital field with a frequency pool containing all important regeneration frequencies within a radius of 90 cm. The body's own energy field only resonates with those frequencies that are required for an optimal supply of energy to the cells. The field strength of the vital field is adjusted in such a way that the energy system cannot be over-energized. In addition, we also used an in vitro model with inflammation-mediating cells (= functional neutrophils) to examine whether the Regeneration Plate 3.0 might be able to reduce the generation of reactive oxygen radicals during an inflammatory process.

Results: The results demonstrate that the Regeneration Plate 3.0 was able to reduce oxidative stress acting on intestinal epithelial cells. After 24 hours, the percentage of surviving cells after exposure to 2 mM hydrogen peroxide and the Regeneration Plate 3.0 for 8 hours was 27.7 ± 5.7%, while the viability of the untreated control cells was 14.7 ± 2.9% (mean values ± standard deviations). The difference between both experimental groups was statistically highly significant at the $p \leq 0.01$ level. Moreover, the generation of superoxide anion radicals by functional neutrophils was reduced by nearly 50% in comparison to untreated control cells. Again, the difference between both groups was statistically highly significant at the $p \leq 0.01$ level.

Conclusions: Both beneficial effects of the Regeneration Plate 3.0 shown in the present in vitro study can act on the body during sleep and might enhance not only intestinal health, but also systemic health and well-being.

Keywords

Vital field
Regeneration Plate
Sleep
Intestinal health
Oxygen radicals
Oxidative stress
Intestinal cells
IPEC-J2
Functional neutrophils
HL-60
Oxidative burst
Inflammation
Cell culture

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INTRODUCTION

Sleep is an integral biological necessity and insufficient sleep or sleep deprivation are disadvantageous factors not only for individual health and well-being, but also for public safety [1-6]. However, the ability to relax the body and mind during sleep may be affected by the surrounding environment [7].

Sleep is understood to possess recuperative and regenerative properties and sleep deprivation has been associated with diseases and an increase in morbidity and mortality. In this context, the term "regeneration" means the recovery phase in which the body can recharge its energy and reserve stores after a high level of physical or mental stress.

Several recent studies have suggested a strong relation between insufficient sleep and gastrointestinal diseases, especially when triggered by inflammatory processes [8-11]. Accordingly to these findings it has been demonstrated that sleep deprivation in both humans and experimental animals causes a progressive increase in circulating white blood cells, mainly neutrophils [12] as well as an increase in various circulating proinflammatory molecules [13,14]. Sleep deprivation has been directly related to oxidative stress, inflammation and multi-organ injury of liver, lung, heart and intestine [15-19].

Taken together, an undisturbed sleep which allows a regeneration of the body is essential for systemic health. Against this background we used cultured intestinal epithelial cells to investigate the impact of a specially designed device, Regeneration Plate 3.0, which is positioned under the bed during sleep and is stated to improve systemic health on the cellular level.

MATERIAL AND METHODS

Regeneration Plate 3.0

According to the manufacturer, the Regeneration Plate 3.0 should be positioned under the bed. The plate produces a vital field with a frequency pool containing all important regeneration frequencies within a radius of 90 cm. The body's own energy field only resonates with those frequencies that are required for an optimal supply of energy to the cells. The field strength of the vital field is adjusted in such a way that the energy system cannot be over-energized. The effect of the regeneration plate on the user can only be seen energetically and includes all known recovery support of energetics such as deep restful sleep, harmonization for body, mind and soul, removal of energetic blockages, opening of the energy flow, and, finally, protection against unwanted environmental pollution and influences such as geopathogenic interference zones, electromagnetic fields and others. Thus, the energy potential of the cells is gently built up again during sleep.

For the experiments described here, the Regeneration Plate 3.0 powered by Qi-Quant technology, was kindly provided by Qi Life Energy GmbH, A-8775 Kalwang, Austria.

Cell culture

Intestinal epithelial cells (IPEC-J2; ACC-701; Leibniz Institut, DSMZ, Braunschweig, Germany) were routinely grown in a mixture of Dulbecco's Modification of Eagle's Medium and Ham's F12 (1:1) supplemented with 10% growth mixture and standard amounts of penicillin/streptomycin and cultivated in an incubator at 37°C in an atmosphere of 5% CO_2 and 95% air at nearly 100% humidity. Cells were routinely cultivated as mass cultures and were regularly subcultured twice a week. For the experiments cells were taken from 80-90% confluent mass cultures.

Human promyelocytes (cell line HL-60; ACC-3; ECACC 98070106; Leibniz Institute DSMZ, Braunschweig, Germany) were routinely cultivated in RPMI 1640 culture medium with 10% growth mixture and standard amounts of penicillin/streptomycin and incubated in an incubator at 37°C and an atmosphere of 5% CO_2 and 95% air and almost 100% humidity. The non-adherent cells were routinely cultivated as suspension mass cultures and were subcultured twice a week. By addition of 1.5% dimethylsulfoxide to the culture medium, cells were differentiated over a period of 6 days into functional neutrophils, which are capable to generate superoxide anion radicals after stimulation by a phorbol ester [20-22].

Examination of intestinal epithelial cell viability after exposure to oxidative stress

In order to investigate the ability of intestinal epithelial cells to survive exogenous oxidative stress with and without the positive impact of the Regeneration Plate 3.0, the cells were seeded at a density of 50,000 cells/ml into 96-well plates. After complete attachment and spreading of the cells within 48 hours, cells were exposed to 2 mM hydrogen peroxide with and without the regeneration plate 3.0 by using two separate mini-incubators (Figure 1A). The exposure period of the cells with the regeneration plate 3.0 was 8 hours. Both mini- incubators were about 20 meters distant with several house wall between them. This guaranteed that there was no interference between the two cell samples. After 24 hours the cells were washed with phosphate-buffered saline and fresh culture medium containing 10% of the water-soluble tetrazolium dye XTT (sodium 3′-[1-[(phenylamino)-carbony]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-

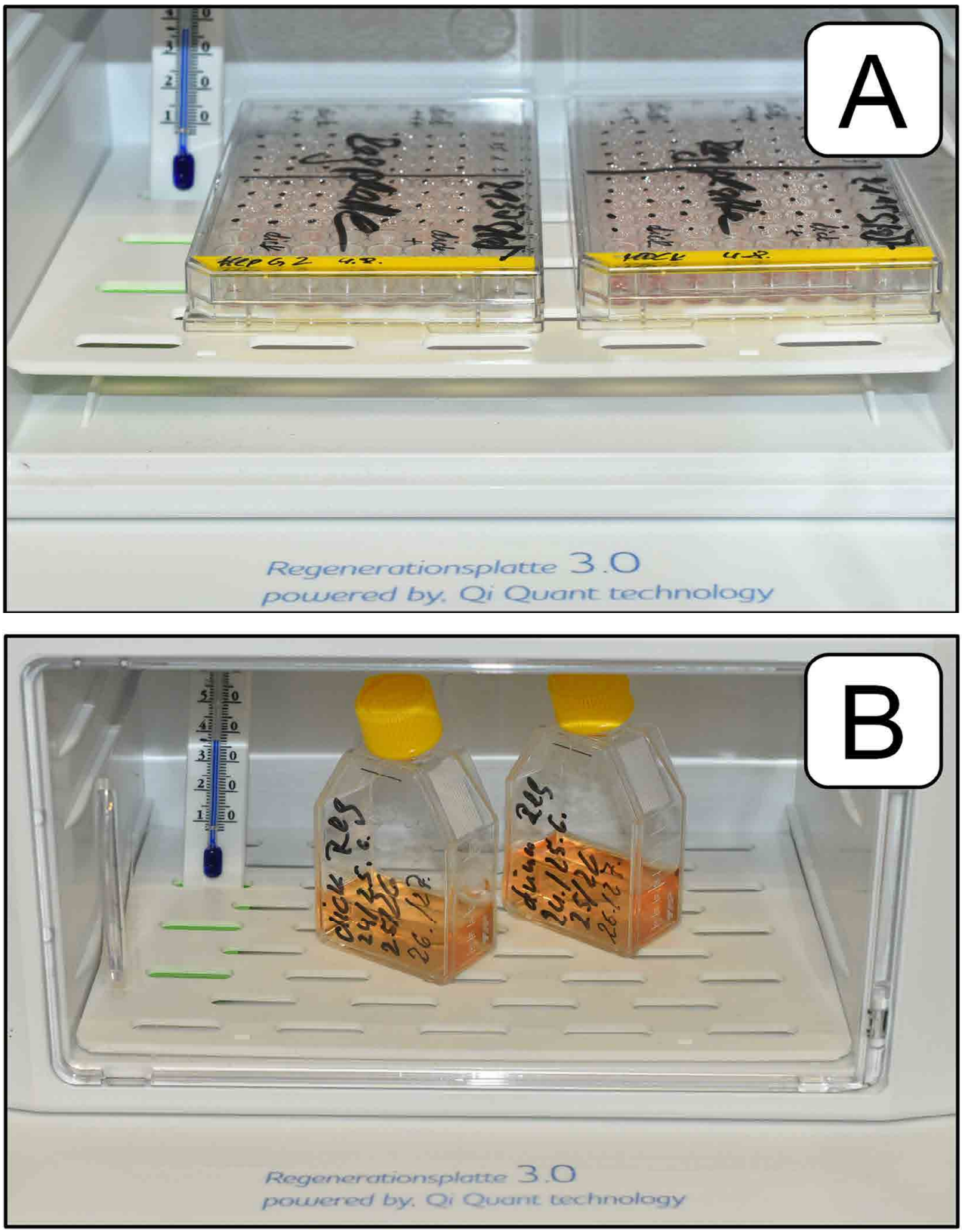

Figure 1: Experimental setup for the exposure of adherent cells during oxidative stress by addition of 2 mM hydrogen peroxide to the culture medium (A) and of human promyelocytes during their daily 8-hour exposure period in the course of their differentiation into functional neutrophils (B).

Research Article

sulfonic acid hydrate; Xenometrix, Allschwil, Switzerland) was added. Due to the activity of the mitochondrial enzymes in metabolically active cells, the initially slightly yellowish dye was cleaved and an orange color developed. The extent of the color change was proportional to cell vitality. This dye is widely used in a colorimetric assay for examination of cell viability and proliferation [23-25]. The optical density (= color change of the dye) was recorded at t = 0 and definite time points at ΔOD = 450 minus 690 nm with the Elisa reader (BioTek ELx808 with software Gen 5 version 3.00) and finally calculated with Microsoft Excel. A total of three experimental series with duplicate parallel wells was conducted.

Examination of superoxide anion radical generation by functional neutrophils

A second in vitro model was used to investigate whether the Regeneration Plate 3.0 can influence the generation of endogenous superoxide anion radicals by functional neutrophils. Throughout the 6-day differentiation period, the cells were exposed to the Regeneration Plate 3.0 for 8 hours per day in the mini-incubator (Figure 1B). Cells that were kept in the second mini-incubator without the Regeneration Plate 3.0 served as corresponding controls. As already described, two separate mini-incubators were used which were about 20 meters distant with several house wall between them. This guaranteed that there was no interference between the two cell samples.

Finally, the cells were collected and washed by several centrifugation steps at 190 x g. By adding a phorbol ester (phorbol-12-myristate-13-acetate, Sigma-Aldrich, Taufkirchen, Germany) to the reaction mixture, the functional neutrophils were stimulated to undergo an oxidative burst in which superoxide anion radicals were generated. The radicals caused a cleavage of the tetrazolium dye WST-1 (Sigma-Aldrich, Taufkirchen, Germany), which was also added to the reaction mixture. The cleavage of the dye was directly related to the amount of oxygen radicals, i.e. the more reactive radicals were present in the reaction mixture, the more pronounced was the cleavage of the dye and the change in optical density (= color). The optical density was recorded at t = 0 and definite time points with the Elisa reader (BioTek ELx808 with software Gen 5 version 3.00) and calculated with Microsoft Excel. A total of three experimental series with triplicate parallel wells was conducted.

STATISTICAL ANALYSIS

Statistical analysis was done using the parameter-free two-tailed Wilcoxon-Mann-Whitney rank sum test.

RESULTS

Viability of intestinal epithelial cells after exposure to oxidative stress

As shown in Figure 2, the negative cellular effects of oxidative stress (= 2 mM hydrogen peroxide in the culture medium) on the intestinal epithelial cells were significantly compensated by the action of the Regeneration Plate 3.0. After 24 hours, the percentage of surviving cells after exposure to the Regeneration Plate 3.0 for 8 hours was still 27.7 ± 5.7% (mean value ± standard deviation), while the viability of the untreated control cells was only 14.7 ± 2.9% (mean value ± standard deviation). If one compares both percentage values directly with one another, the portion of surviving cells under the influence of the Regeneration Plate 3.0 was almost twice as high as the one of the control cells. The difference between both experimental groups was statistically highly significant at the $p \leq 0.01$ level.

Superoxide anion radical generation of functional neutrophils

For the functional neutrophils representing inflammation-mediating cells, exposure to the Regeneration Plate 3.0 did not influence basal cell metabolism. When compared to the untreated control cells (100 ± 4.4%), the metabolism in the exposed cells was 95.7 ± 2.3% (mean values ± standard

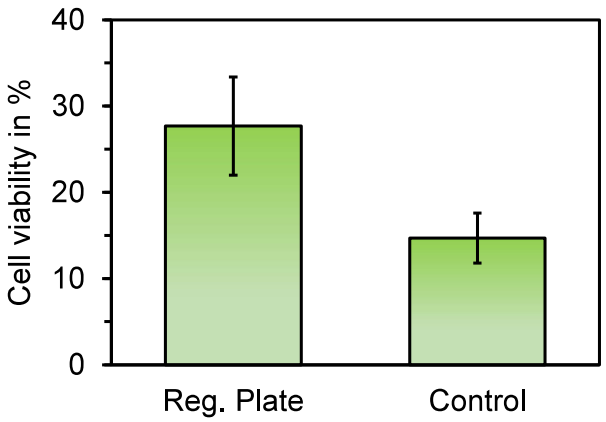

Figure 2: Presentation of cell viability data after a 24 hours exposure period of the intestinal epithelial cells to oxidative stress induced by the addition of 2 mM hydrogen peroxide to the culture medium. Cell viability is significantly higher for cells which were exposed to the Regeneration Plate 3.0 when compared with untreated control cells ($p \leq 0.01$; two-tailed Wilcoxon-Mann-Whitney rank sum test). Data represent mean values ± standard deviations of three experimental series with duplicate wells.

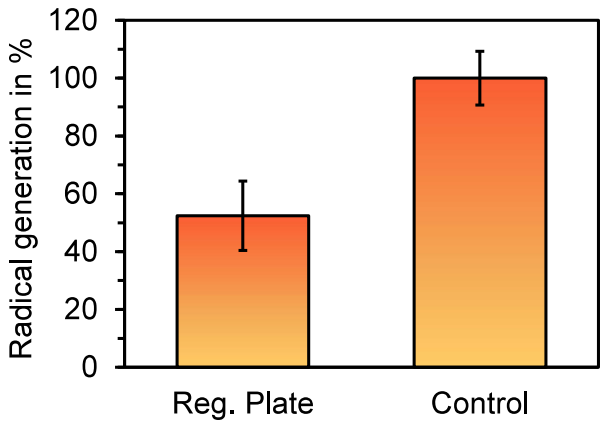

Figure 3: Presentation of superoxide anion radical generation after stimulation of functional neutrophils by a phorbol ester with and without exposure to the Regeneration Plate 3.0. The radical generation is significantly highly reduced for cells which were exposed to the Regeneration Plate 3.0 during their differentiation process when compared with untreated control cells ($p \leq 0.01$; two-tailed Wilcoxon-Mann-Whitney rank sum test). Data represent mean values ± standard deviations of three experimental series with triplicate wells.

deviations) and did not differ significantly from each other. However, the results were completely different after stimulating the functional neutrophils to undergo an oxidative burst with the generation of endogenous superoxide anion radicals. As shown in Figure 3, the cells influenced by the Regeneration Plate 3.0 only achieved an endogenous radical formation of 52.4 ± 12% (mean value ± standard deviation) in comparison to the control cells (set as 100 ± 9.4%). This difference between both experimental groups was statistically highly significant at the $p \leq 0.01$ level.

DISCUSSION

Oxygen possesses two contradictory properties for biological systems, which are primarily beneficial effects such as the generation of large amounts of adenosine-5-triphosphate (ATP) through oxidative phosphorylation, but on the other hand an excess of oxygen radicals can also cause potentially damaging effects [26,27]. Oxidative stress occurs when the balance between pro-oxidant and antioxidant situations in the organism causes an excess of reactive oxygen species and has been recognized to play a central role in the pathophysiology of many disorders [28].

In the present experimental setup we examined a situation in which an excess of reactive oxygen species comes from the cellular environment [29,30]. The influence of oxidative stress can be reduced by the recuperative and regenerative properties of a deep and undisturbed sleep and sleep dysfunction has also been related to a variety of gastrointestinal disorders [11,31].

The intestinal epithelium, which is only one cell layer thick, has two essential tasks. The first is to create a physical barrier between the contents of the intestinal lumen and the rest of our body. The second is to ensure an efficient absorption of essential nutrients from the gut lumen and to produce mucus, anti-microbial peptides and cytokines with both protective and immune-regulatory properties. Thus, a reduced barrier function due to oxidative stress may have far reaching consequences, not only for intestinal, but also for systemic health [32].

Against this background cultured intestinal cells were used as an in vitro model to examine the effect of the Regeneration Plate 3.0 on the effect of oxidative stress. For the experiments the IPEC-J2 cell line was chosen, because “the IPEC-J2 cell line is unique as it is derived from the small intestine and is neither transformed nor tumorigenic in nature. IPEC-J2 cells mimic the human physiology more closely than any other cell line of non-human origin” [33]. The advantage of the IPEC-J2 cell line as an in vitro model originates from its morphological and functional similarities with intestinal epithelial cells *in vivo* [34].

The results of this experimental study clearly demonstrate that exposure of the intestinal epithelial cells to the Regeneration Plate 3.0 improved the viability of the cells during oxidative stress in comparison to untreated control cells significantly. According to Kwolek- Mirek and Zadrag-Tecza [35] “viability is defined as a percentage of live cells in a whole population”. In addition, similar experiments (not shown here) have been conducted with other cell types such as liver cells (Hep G 2), lung cells (A-549), connective tissue fibroblasts (L-929) and kidney cells (MDCK). In all cell types the Regeneration Plate 3.0 reduced the influence of oxidative stress up to 18%. Therefore, it can be concluded that the reduction of oxidative stress is improved during a sleep period under the positive influence of the

Regeneration Plate 3.0. The improvement might be also related to a protective effect of the plate against environmental influences during sleep so that the body can undergo a better regeneration process.

Neutrophilic granulocytes (polymorphonuclear neutrophils, PMN) represent the largest group of leukocytes. They build the first line of defense against pathogenic microorganisms, fighting them by phagocytosis via release of antimicrobial molecules and generate reactive oxygen species by an oxidative burst [36]. Attracted by chemical substances such

as specific chemokines or cytokines which occur during the inflammatory process, these cells can migrate from the blood into the inflamed tissue and generate superoxide anion radicals in the tissue [37,38]. The radicals cause further tissue destruction (necrosis) in the inflamed tissue and might cause a progression of the inflammatory process with decelerated wound healing. For an overview of the role of neutrophils in health and disease, see [39,40].

We used an in vitro model which represents one facet of the inflammatory process to investigate whether the Regeneration Plate 3.0 was able to reduce endogenous superoxide anion radical generation to a higher extent than untreated control cells. The reduced radical generation of functional neutrophils is comparable to an anti-inflammatory effect in the tissue. The results show that exposure to the Regeneration Plate 3.0 caused a decreased endogenous radical generation by functional neutrophils by about 50%. This means that during sleep an anti-inflammatory action of the plate might take place and contributes to a better regeneration and health. However, the effect is within a range which should not markedly affect the innate immune system of the blood as a first defense against microbial pathogens *in vivo* [41].

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