Einfluss von PDT-Farbstoffen auf die migratorische Aktivität von humanen Fibroblasten (Abschlussbericht)

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Hintergrund - Der 3D-Kollagenmatrix-Migrationsassy

Der 3D-Kollagenmatrixmigrationsassay zeichnet sich dadurch aus, dass die Migration von Zellen, wie Lymphozyten/Leukozyten, Stammzellen und Tumorzellen in Zeitraffer gefilmt wird und nachfolgend die tatsächliche migratorische Aktivität der Zellen determiniert wird. Bedingt durch die Echtzeitanalyse lassen sich mit Hilfe des 3D-Kollagenmatrixmigrationsassay mehrere Parameter simultan ermitteln. Hierzu zählt nicht nur die migratorische Aktivität der Gesamtpopulation, sondern auch Parameter wie die Wanderungszeit (wie lange waren die Zellen migratorisch aktiv?), die Geschwindigkeit (wie schnell waren die Zellen?) sowie die Pausenzeiten und –längen (dieser Parameter erklärt sich von selbst. Charakteristisch für migrierende Zellen ist, dass sie nicht permanent migrieren machen, sondern auch Pausen „einlegen“). Bedingt dadurch, dass die Zellen in eine 3D-Kollagenmatrix eingebettet werden, sich somit in einer relativ physiologischen Umgebung befinden, die er *in vivo* Situation sehr nahe kommt, stellt dieser Assay ein sehr gutes *in vitro* Modell zur Analyse der Zellmigration dar. Ein weiterer Vorteil dieses Assays ist, dass weitere Komponenten zum Kollagengel hinzugegeben werden können. Neben löslichen Faktoren, wie Chemokinen, Zytokinen und Wachstumsfaktoren bzw. Antikörper und Inhibitoren, können auch unlösliche Komponenten, wie z.B. der PDT-Farbstoff der Firma *kryptronic technologies*, München, in das Kollagengel integriert werden und nachfolgend der Einfluss dieser Substanzen/Materialien auf die Migration von Zellen untersucht werden.

Die nachfolgende Liste gibt einen Überblick über die Literatur in denen 3D-Kollagenmatrix Migrationsassays zur Anwendung kam.

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Versuchs-Protokoll

Durch *kryptronic technologies*, München, wurden zur Verfügung gestellt der PDT-Farbstoff Toluidinblau-O in verschiedenen Konzentrationen, siehe „Report“. Die Untersuchungen wurden im nicht aktivierten und im aktivierten Zustand durchgeführt.
Als Laserquelle ist der BluLase 810nm eingesetzt worden.

Report

Two sets of tests were performed with human dermal fibroblasts derived from PromoCell (Heidelberg, Germany). The cells were harvested at the indicated date and passage number below. After centrifugation (3 minutes at 220g), the cells were resuspended in 1 ml PBS. Four aliquots were generated, each containing 60,000 cells. BluLase or PBS were added to the same amount, resulting in BluLase concentrations of 50% and 5% (v:v). Two of each samples were treated with laser-light (810 nm) for 1 minute at 0.3 Watt (PDT). After that (resulting in a total incubation time of 5 minutes). All samples were spun down and resuspended in 50 µl PBS. 100 µl collagen solution was added to each sample, resulting in a collagen concentration of 1.67 mg/ml. Locomotor behaviour was analyzed for a 10 hours period.

Set 1 (50% BluLase)
Set 2 (5% BluLase)

Results

At a concentration of 50% BluLase, the locomotor activity of the fibroblasts was significantly impaired, whereas 5% Blulase did not affect the part of locomoting cells (Figure 1). PDT treatment had no influence on the locomotory behaviour.

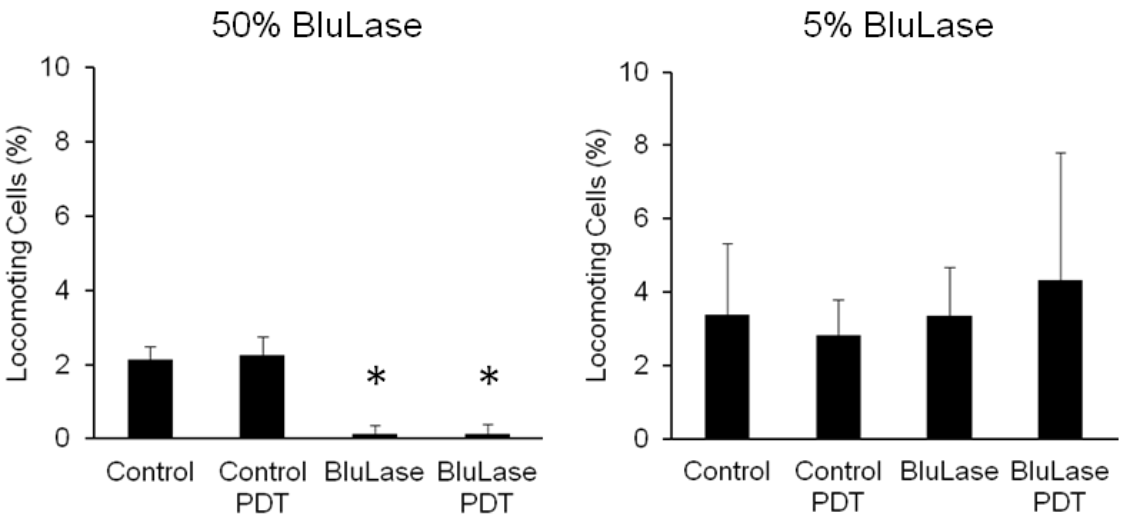

Figure 1: Percent Migrated shows the quantity of cells that are migrating each moment over time. The source video is divided into 15 minute time intervals, and for each interval, all cells visible during that interval are categorized as either migrating (meaning they moved more than a threshold radius of 3 μm) or stationary.

Concommitantly, the speed of locomotion was significantly reduced in those cells treated with 50% BluLase, but showed only a weak tendency to a reduced speed in those samples treated with 5% BluLase (Figure 2). Again, PDT treatment did not influence the speed of locomotion in neither control samples nor BluLase-treated samples.

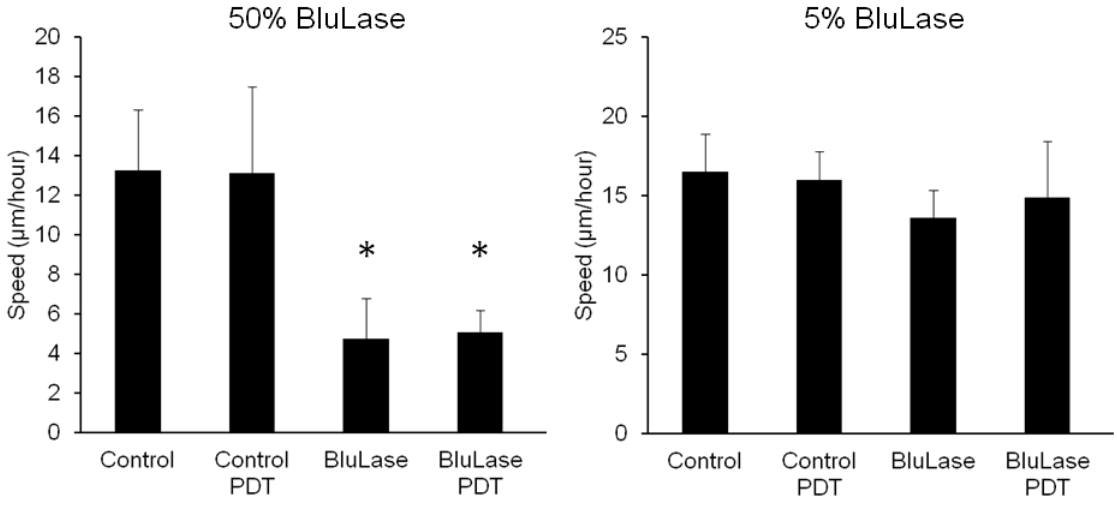

Figure 2: Total Path length of all cells divided by the total time cells were visible.

According to the reduced speed of locomotion, the distance of movement that 95% of the observed cells reached, was significantly reduced at 50% BluLase (Figure 3). Most

interestingly, even at 5% BluLase, those cells, which were not treated with PDT showed a significantly reduced 95% percentile radius, which is attributed to the reduced speed.

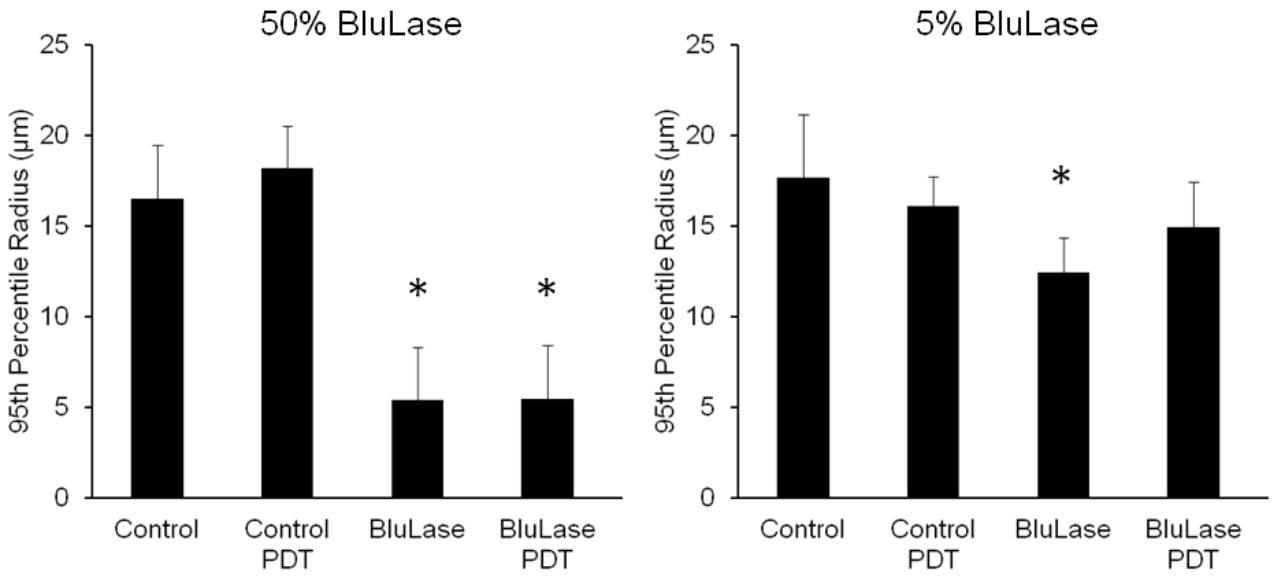

Figure 3: The Percentile Endpoint Radius metric indicates how far most of the observed cells (95%) moved within the observation time. Cell paths are sorted by their endpoint distance (the distance from their starting points to their starting points), and the radius that encompasses the shortest 95% of these cell path endpoint distances is calculated and plotted in the bar graph. The use of a percentile eliminates extreme outliers.

As a combined analysis of locomotor activity and speed, the average distance that each cell of the observed populations travelled, was significantly reduced at 50% BluLase (Figure 4), and showed a tendency to reduction at 5% BluLase without PDT treatment.

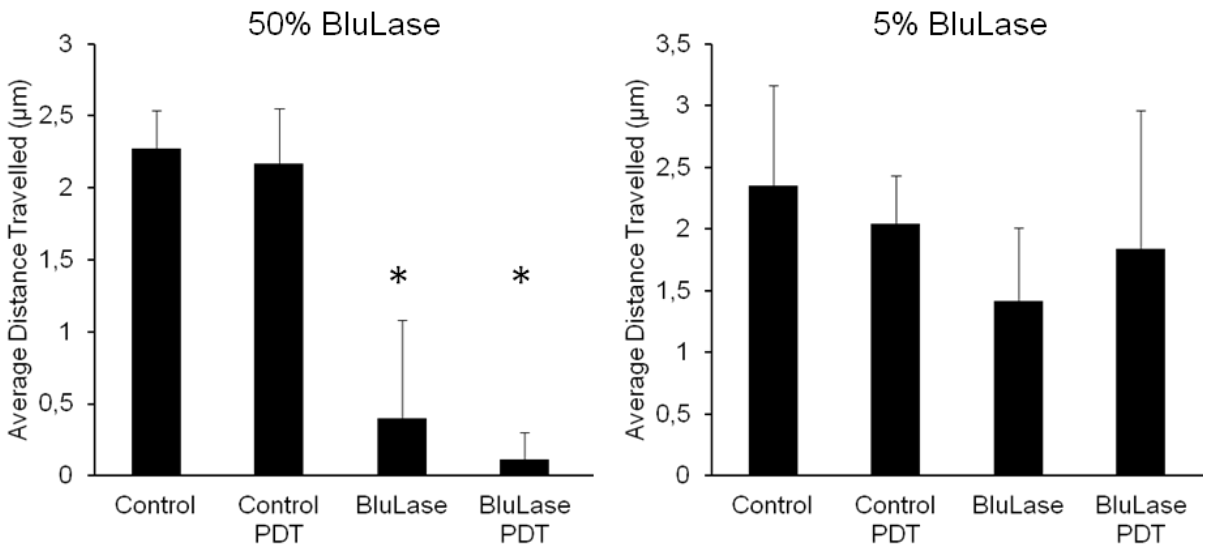

Figure 4: Average cell travel distance, with a 3.5 micron hysteresis threshold to eliminate noise.

Summary

High concentrations (50%) of BluLase affect the locomotory behaviour of human fibroblasts, whereas concentrations that are supposed to occur in physiological conditions (5% and below) have only weak influence on the locomotory behaviour. **PDT treatment does not influence locomotory behaviour.**

A detailed analysis of the individual cell behaviour (i.e. frequency and length of rest vs. active periods and further migrations parameters) will be delivered later on (videos, row data, final report).

All experiments were conducted and analyzed in good scientific practice.

Prof. Dr. Wolf-D. Grimm
-Studienleiter-