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Influence of Beam Shape on *in-vitro* Cellular Transformations in Human Skin Fibroblasts

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ABSTRACT

A variety of strategies have been utilised for prevention and treatment of chronic wounds such as leg ulcers, diabetic foot ulcers and pressure sores. Low Level Laser Therapy (LLLT) has been reported to be an invaluable tool in the enhancement of wound healing through stimulating cell proliferation, accelerating collagen synthesis and increasing ATP synthesis in mitochondria to name but a few. This study focused on an *in-vitro* analysis of the cellular responses induced by treatment with three different laser beam profiles. The promoted cellular alterations were measured by increase in cell viability, cell proliferation and cytotoxicity. The results obtained showed that when cells undergo laser irradiation some cellular processes are driven by the peak energy density rather than the energy of the laser beam.

INTRODUCTION

LLLT has been employed for its photostimulatory effect on slow-healing ulcers and wounds such as burns, chronic leg ulcers, pressure sores and diabetic foot ulcers. A number of monochromatic radiation sources such as gas state lasers (argon-ion at 488 nm, HeNe at 633 nm), solid state lasers and laser diodes have been identified as producing a beneficial biological effect known as "photostimulation" or "biostimulation"; a process reported to generally depend on wavelength and dose of laser irradiation. Although many researchers involved in LLLT studies tend to focus on investigating wavelength and dose dependent effects of low energy lasers, they seldom mention the laser beam shape used to treat biological samples. This leads to confusion as to how these fluence values are defined, and how they are achieved. For example most authors report laser dosage in terms of J/cm^2 or J/m^2 without specifying the manner in which these fluences were administered to the sample as far as light energy distribution or beam shape is concerned. This makes it impossible to repeat the findings, and difficult to compare one report with another. The present study was performed to determine photobiological effects arising from using a HeNe laser of different beam shapes. This was performed by evaluating changes in cellular processes including cell viability, proliferation and cytotoxicity resulting from laser treatment through investigating how using different beam shapes of various doses would affect wound healing of human skin fibroblasts *in-vitro*.

CELL CULTURES

Only *in-vitro* experiments were performed in this work. A monolayer of human skin fibroblasts which is approximately a few microns in depth was used in this study; the wounds could therefore only be measured according to their diameter and length across the culture plates. Wound healing as a result of irradiation with the three laser beam shapes was evaluated by analysing cellular changes in adenosine triphosphate (ATP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels.

Figure 1: A typical diabetic foot ulcer that can lead to limb amputation. LLLT finds application in improving the healing process for such wounds

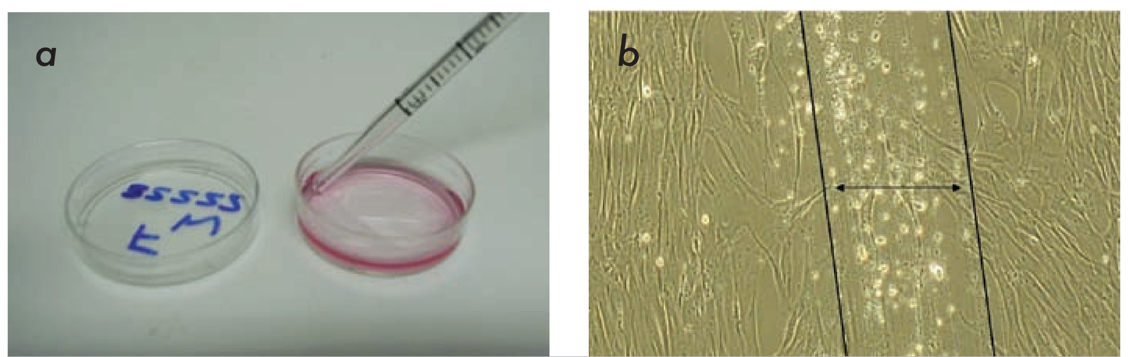


Figure 2: (a) Petri dish containing a monolayer of cells. The cells were then "wounded" with a pipette forming damage across a roughly 1-2mm band, as shown in (b). The monolayer either side of the scratch remained healthy

LASER BEAMS

Three beam shapes were used in the experiments reported in this work: a Gaussian beam (GB), a Truncated Gaussian (TGB) beam, and a Super-Gaussian beam (SGB). The use of these beam shapes allowed us to probe whether the laser beam interaction with the cells is driven by peak fluence, average fluence or total energy delivered. In all cases the beams were circularly symmetric, and therefore characterized by a radial distance parameter r. A Gaussian beam has an intensity function given by

$$I_g(r) = I_g \exp(-2(r/w_g)^2) \tag{1}$$

where w_g is the beam radius (1/e^2 value) and I_g is the peak intensity. Gaussian beams have the property that their peak intensity is double their average intensity, and that the beam size w_g is already the second moment radius of this field. In this application the output beam from the HeNe laser was very nearly TEM$_{00}$, and therefore already a Gaussian shape. A truncated Gaussian beam has an intensity function given by

$$I_t(r) = \begin{cases} I_t \exp(-2(r/w_t)^2), |r| \le r_0 \\ 0, |r| > r_0 \end{cases} \tag{2}$$

where w_t is the Gaussian beam radius and I_t is the peak intensity. In the limit that $w_t >> r_0$, the beam will approximate a flat-top distribution, and the average intensity will be identical to the peak intensity. This beam is generated by amplitude filtering. However, it is a useful approximation to a flat-top beam. A Super-Gaussian beam has an intensity function given by

$$I_s(r) = I_s \exp(-2(r/w_s)^{2p}) \tag{3}$$

where w_s is the beam radius (but not the second moment radius) and I_s is the peak intensity. In the limit of large p, the Super-Gaussian "edges" become steeper, the peak intensity is equal to the average intensity, and the beam becomes a perfect flat-top.

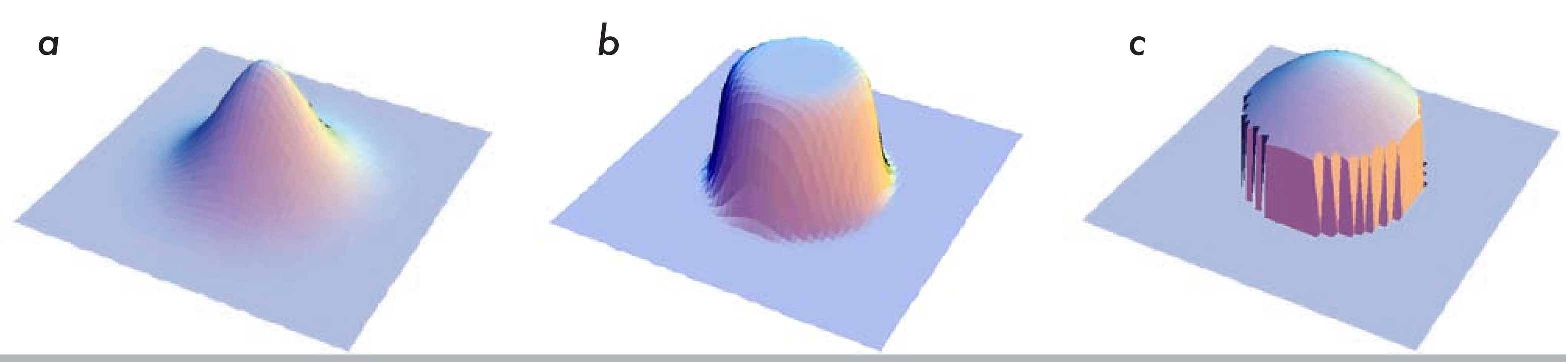


Figure 3: (a) Gaussian beam, (b) Super-Gaussian beam, (c) Truncated beam

RESULTS

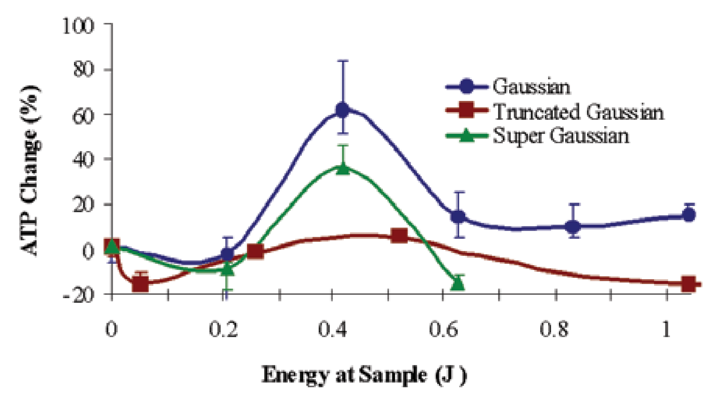


Figure 5: Wounded fibroblasts treated with the three laser beam shapes at doses 0.2–1J, depict a stimulatory effect i.e. an increase in ATP around 0.4J for both the GB and SGB beams with the TGB beam showing a lower response at this point

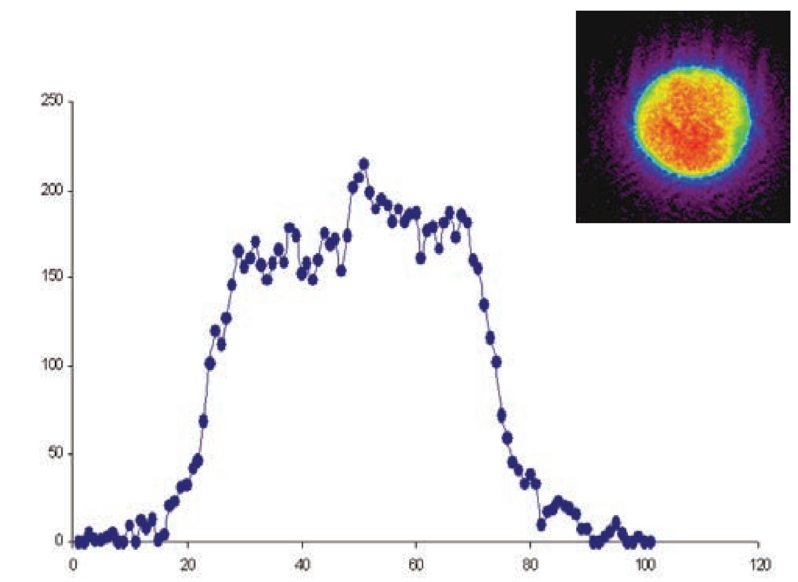


Figure 4: Intensity profile of the Super-Gaussian beam as measured at the exit of the multimode fibre, with corresponding intensity cross-section

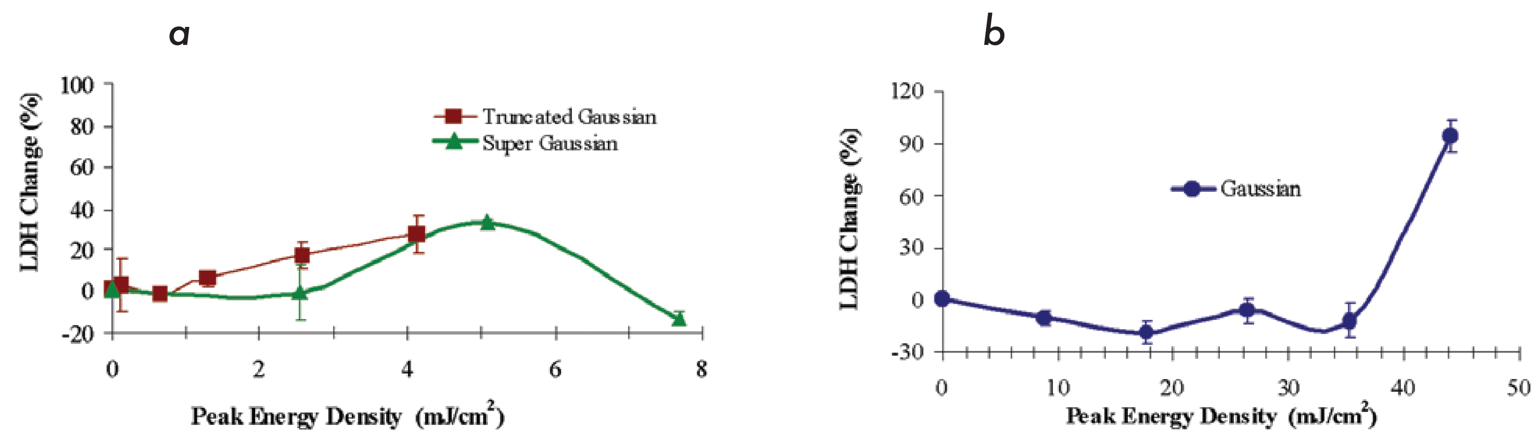
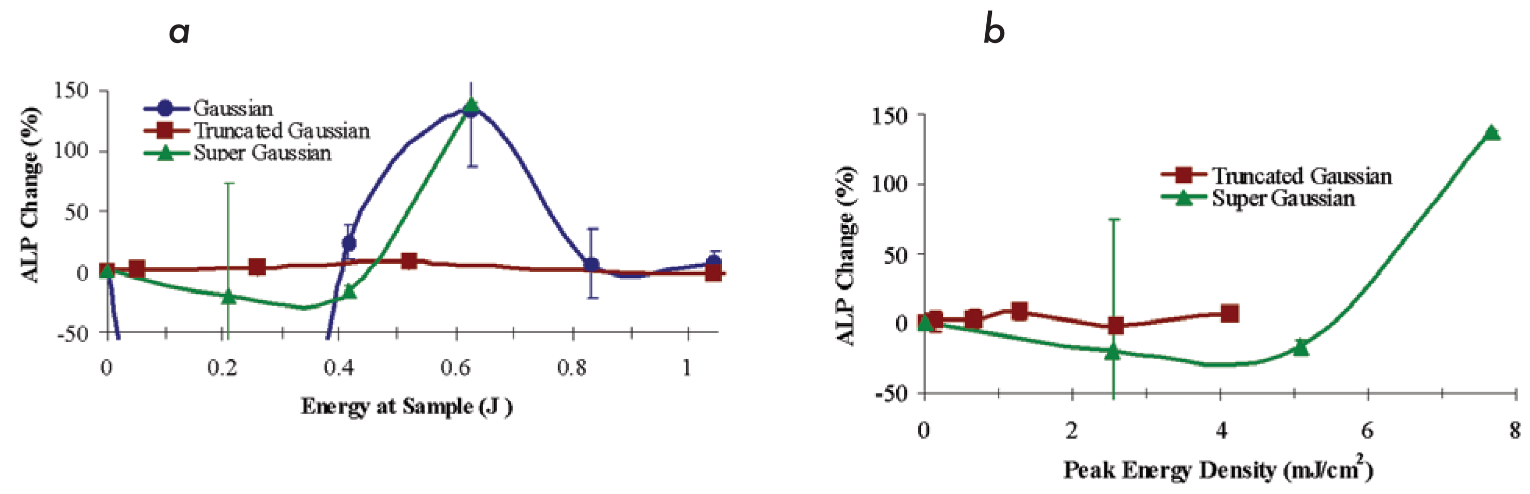


Figure 6: (a) High levels of LDH were measured when the wounded fibroblasts were irradiated with the TGB and SGB at 5 mJ/cm^2. (b) When the samples were irradiated with a GB beam at peak energy densities greater than 35 mJ/cm^2, increase in cell damage was observed



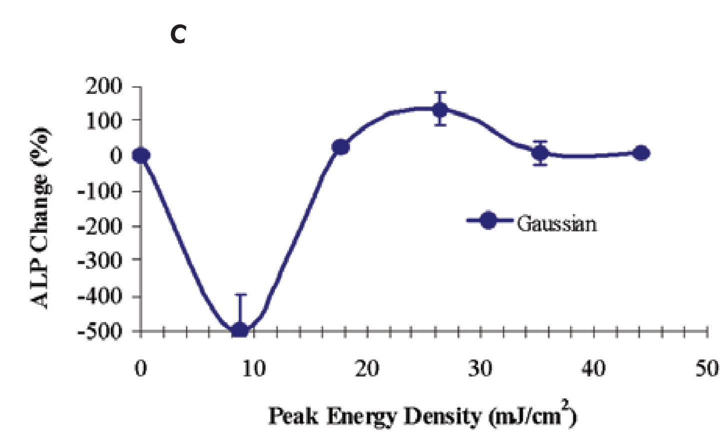


Figure 7: ALP was utilised as an indicator for wound healing. (a) Both the GB and SGB curves show an increased secretion of this enzyme around 0.6J, while the TGB remained unaltered. (b-c) ALP peak energy density curves for the TGB, SGB and GB show disagreeing data

CONCLUSION

Our conclusion thus far is that laser treatment with a Gaussian beam shape resulted in a marked increase in cell viability and proliferation of normal human skin fibroblasts (cells); this may lead to the promotion of *in-vitro* wound healing. We also observed that for some cellular transformation processes that occurred (increase in ALP and ATP) changes were related to the amount of energy delivered to sample at that point, but for others (increase in LDH) changes depended on peak energy density values of the different beams. This means that the way energy is delivered (beam shape) must be included in any discussion of changes in these cellular processes. Certainly this is important for the repeatability of results.