Graphene substrates enhance optical transfection efficiency in pluripotent stem cells

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ABSTRACT
Studies directed at investigating the role of nanomaterial substrates with varying properties in tissue engineering research are essential. In this research arena, pluripotent stem cells are popular for their self renewing ability and are widely applicable as they can be specifically differentiated into different tissue cells. Availability of new biocompatible scaffold materials is a critical requirement in the tissue engineering research field. Due to its promotion of cell viability and cell proliferation, graphene has attracted much attention in this front. Remarkably, optical transfection has been previously demonstrated to successfully deliver transgenes into a host of mammalian cells and non-invasively drive pluripotent stem cell differentiation. In this work, we have capitalised upon the biocompatible properties of graphene merged with the non-invasive nature of optical transfection to significantly enhance pluripotent stem cell transfection efficiency.

EXPERIMENTAL SETUP
A near infrared (NIR) beam was originally emitted at a 1064 nm wavelength with a repetition rate of 80 MHz and pulse duration of 320 femtoseconds (fs), measured at the focus (Figure 1). A potassium titanyl phosphate (KTP) crystal was employed in frequency doubling a 1064 nm fs laser beam for all optical transfection experiments performed at 532 nm. The beam was reflected by three mirrors M1, M2 and M3 before being reflected by a dichroic mirror (DM) which transmitted white light from the Koehler illumination system. The white light and the laser light bounced off mirror M4 and was focused onto a tube lens (TL) and directed onto a sensor of a charge coupled device (CCD) camera.

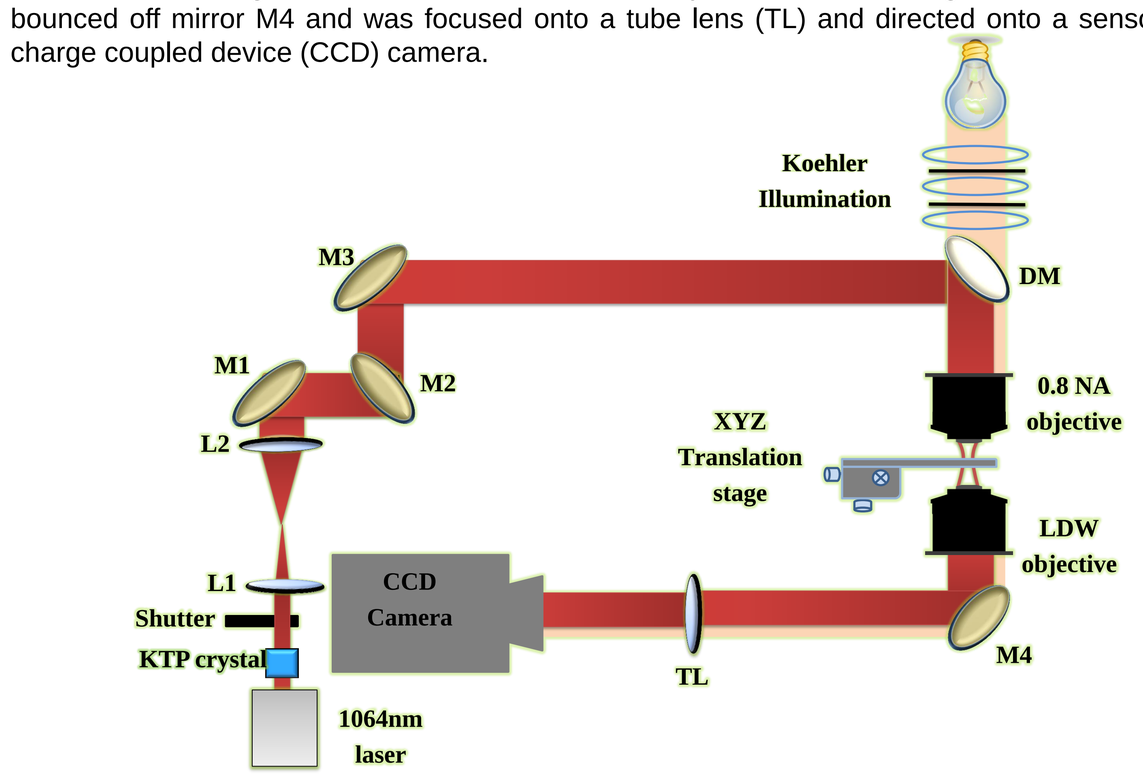

Figure 1 Arrangement of photo-translocation set up. The NIR Gaussian beam is emitted by a Fianium Femtosecond laser where it travels through an electronic shutter before being expanded by a two lens telescope to fit into the back aperture of a 60X objective lens.

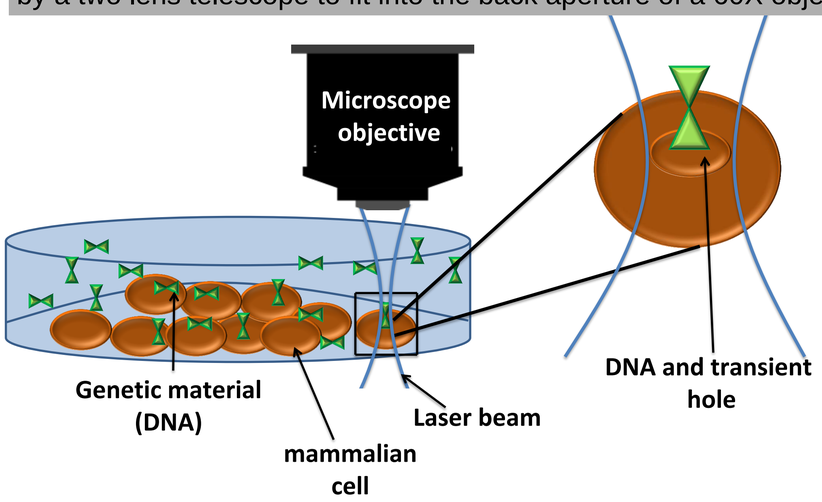

Figure 2 A graphical representation of the generation of a transient hole on the cell plasma membrane by fs laser beam irradiation. Through the transient hole, membrane impermeable materials are able to translocate into the cell.

EXPERIMENTAL RESULTS
Changes in cellular processes
The effects of growing CHO-K1 cells on graphene coated glass as opposed to neat glass was evaluated here. By monitoring mitochondrial activity through adenosine triphosphate (ATP) luminescence, the cell viability was determined. Cytosolic lactate dehydrogenase (LDH) was evaluated to determine the level of breakages on the cell membrane [1].

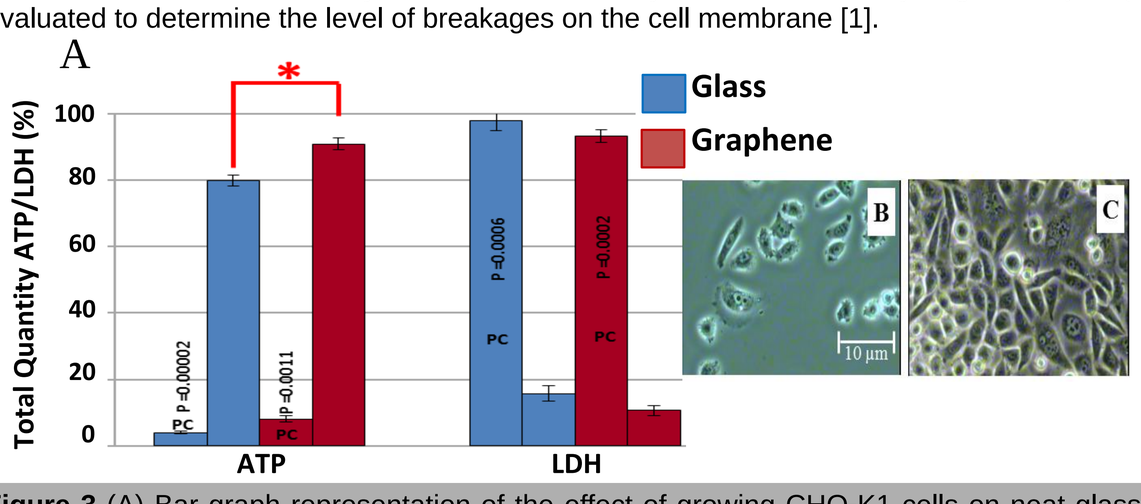

Figure 3 (A) Bar graph representation of the effect of growing CHO-K1 cells on neat glass and that coated with graphene, assessing the changes in ATP and LDH enzyme activity. (B) and (C) are images of CHO-K1 cells grown on glass and graphene coated glass respectively 36 hours after seeding [1].

Optical transfection efficiency of CHO-K1 on graphene
Figure 4 shows results obtained following laser transfections of CHO-K1 cells with different wavelengths. The results indicate an increase in transfection efficiencies for both 1064 nm and 532 nm laser pulses when cells were grown and optically transfected on graphene coated glass.

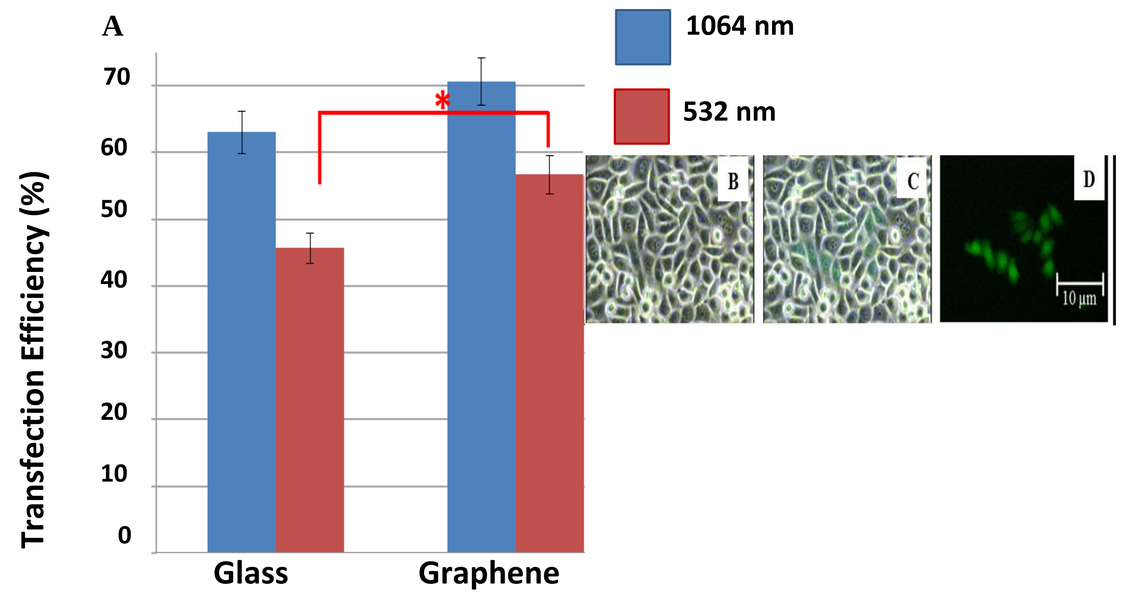

Figure 4 (A) Transfection efficiencies of CHO-K1 cells performed using 1064 nm and 532 nm laser pulses. (B), (C) and (D) are micrographs of CHO-K1 cells expressing green fluorescent protein genes as captured using brightfield, brightfield – fluorescent and fluorescent microscopy respectively [1].

Pluripotent stem cell optical transfection on graphene
Fs laser sources in the near infrared region have shown great transfection efficiencies of mouse embryonic stem (mES) cells without compromising the integrity of the cells [2-3]. When a green laser source at a 532 nm wavelength was employed, a decrease in transfection efficiency was observed. However, after optical transfection of mES grown on graphene coated glass, an increase in transfection efficiencies was observed (Figure 5) [1].

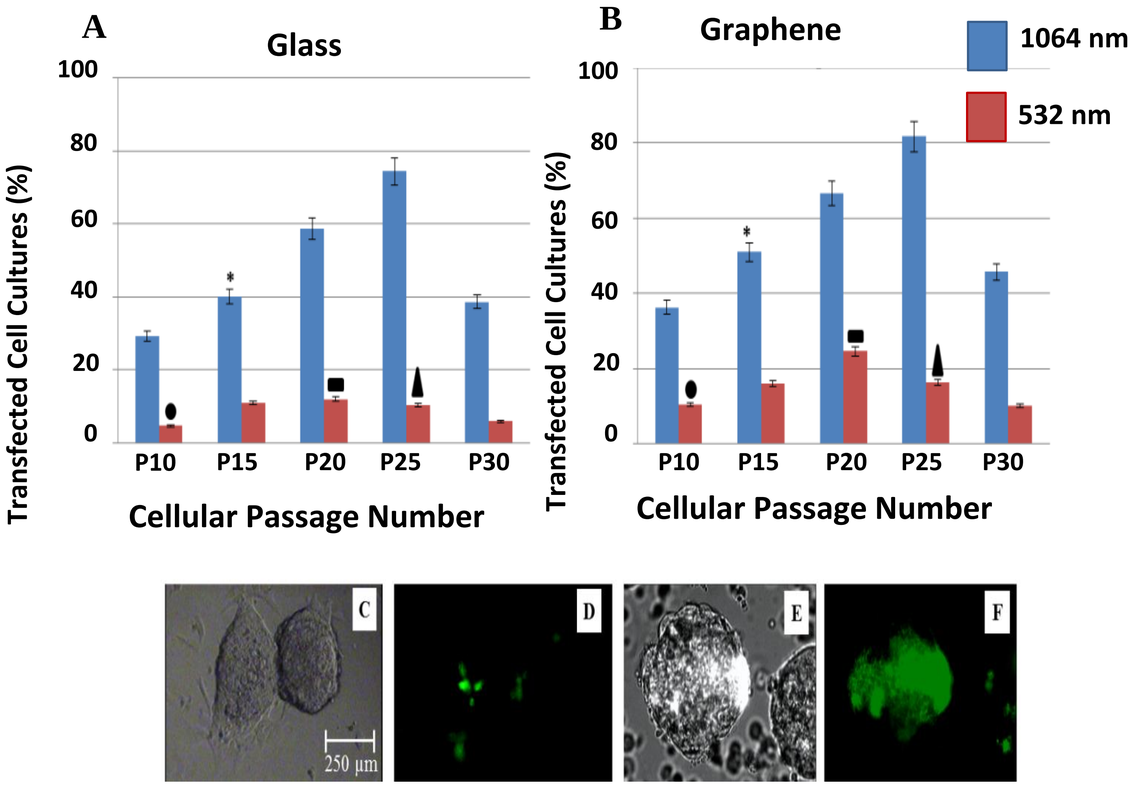

Figure 5 (A) and (B) show graphs depicting an enhancement of the optical transfection of pluripotent stem cells when grown on graphene coated glass using both 1064 and 532 nm laser pulses as opposed to those grown on neat glass. Symbols (●,*, ▬ and ▲) indicate that data sets are significantly different from each other. (C) and (E) show brightfield micrographs of ES-E14TG2a cells grown on neat glass and glass coated with graphene respectively. (D) and (F) show fluorescent counterpart micrographs of (C) and (E) [1].

CONCLUSION
In our study, growing and transfecting CHO-K1 and mES cells on graphene coated glass resulted in an improvement in optical transfection efficiencies. Future perspectives involve the testing of cytokines, growth factors and integrins to investigate the stimulation of extracellular matrix through graphene.

REFERENCES
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