Graphene coated substrates for cell attachment and proliferation

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Statement of Purpose: Graphene has recently attracted tremendous interest in biomedical field [1]. However, the effects of graphene on cytotoxicity in cell systems have not yet established as a reliable and widely accepted results. Although there are few studies reported to investigate the effect of graphene on cells and bacteria, the effects of graphene substrate on cell attachment and proliferation need further theoretical and experimental investigations. In this paper, the proliferation and attachment of cells on graphene synthesized by chemical vapor deposition (CVD) on different substrates such as glass, stainless steel (S.S.), and Silicon wafer (SiO_2/Si stack) has been studied.

Methods: The growth of graphene films was realized on a copper (Cu) substrate (25 µm thick) in an alumina tube furnace system by the Chemical Vapor Deposition (CVD) process under the flow of methane (CH_4) and hydrogen (H_2) gases. More details about the fabrication procedure can be found in [2]. Murine osteoblast cells (25,000 per well) were seeded on different substrates in 24 well-plate. Normal growth medium (α-MEM, FBS and Pen strep) was used as a cell culture medium. Samples (n=3) were cut into small pieces (1cm X 1cm) and sterilized before the experiment. For cell proliferation and attachment study, cells were stained with *Live/Dead cell* assay at day 2 and day 5. Images taken by fluorescence microscopy were analyzed using ImageJ software. Statistical analysis was done based on $p<0.05$ as a significantly significant by ANOVA single factor method.

Results: Graphene characterized by Raman spectroscopy. Typical graphene peaks confirmed the presence of graphene layer on silicon substrate in Fig. 1(a). Other samples showed the same results. Studying cell images at day 2 and day 5 reveals different substrate behavior. While roughness is an important factor in cell attachment, plane glass is rough enough for cells to be attached on the surface. Coating the glass surface with graphene does not induce much effect on cell adhesion and proliferation rate. Obviously, based on the Fig.1(b) graphene does not have toxic effect on osteoblast cells. Plain glass and graphene coated glass did not show significant difference in the number of attached cell and cell area in both two time points in Fig.1(c).

According to fig.1(d) and (e) S.S. coated with graphene layer shows lower percentage of cell attachment but attached cells are more elongated and have larger area. At day 5 cells almost covered on the graphene coated S.S samples while cells adhesion on pure S.S samples was poor and cells started detaching during Live/Dead cell assay experiment (Fig.1(d)). Similarly, at day 2 less number of cells attached to pure silicon wafer in compare with the graphene coated silicon substrate (Fig.1(f) and (g)). Also, there is a significant difference between the cells area of two samples. Moreover, in day 5, similar to S.S. substrate, good cell adhesion and proliferation were observed for silicon substrate covered with graphene layers. In contrast, cells detached from silicon wafer. The arrows in fig 1 show the detachment of cell layer from silicon and S.S surface at day 5. Due to the cell detachment from the substrate, we were not able to quantify the data of S.S and silicon samples at day 5. Briefly, our results show although graphene layer does not induce any effect on cell adhesion at short time point (day 2) but it increase the average spread area of attached cells. In addition, graphene layers improved cell adhesion and proliferation at day 5.

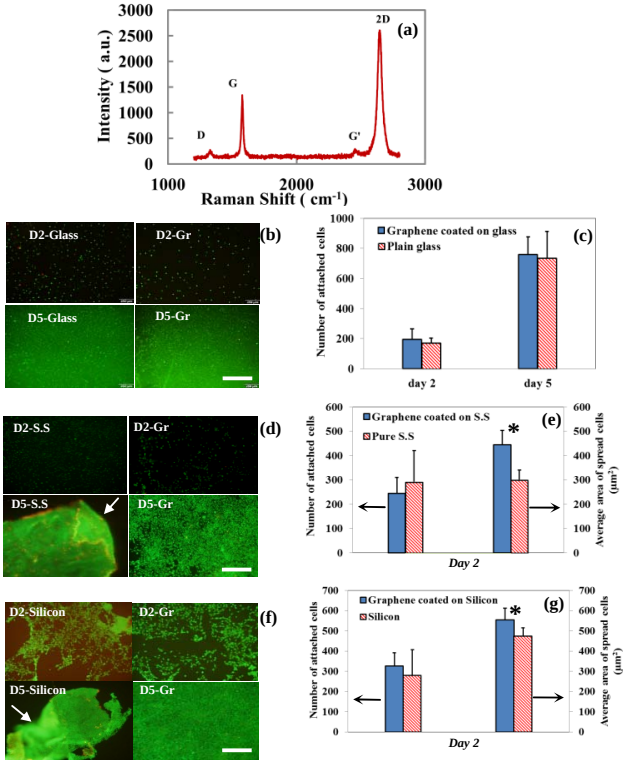

*Fig.1- (a) Raman spectrum of graphene coated layer. (b) Fluorescence image of cells on glass with/without graphene layer. (c) Number of attached cells for glass with/without graphene layer. (d) Fluorescence image of cells on Stainless steel with/without graphene layer. (e) Number of attached cells and cell area for S.S with/without graphene layer (day 2). (f) Fluorescence image of cells on silicon with/without graphene. (g) Number of attached cells and cell area for silicon with and without graphene layer (day 2). * denote the significantly different data. Scale bar=520 µm.*

Conclusions: In this study, we observed cells are viable on the graphene coated layers on three different substrates. In addition, graphene coated layers drastically improved cell attachment and proliferation on S.S. and silicon substrates. These results demonstrate that graphene is a promising candidate for next generation of biomaterials.

References:[1] Yang k. et al. *Small.* 2012;1-12.
[2] Gautam M. et al. *J. appl. phys.* 2012;064307.