Platinum conjugated to Graphen Oxide nanoplatform for drug delivery in antitumor therapy

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INTRODUCTION

Nanocarriers represent an interesting strategy to overcome the limitations of commonly used chemotherapeutics such as platinum-based drugs. Carriers can enhance drug accumulation in target cells while reducing the associated drug toxicity to normal cells.

Our study aim on the preparation and characterization of graphene oxide (GO)-based 2D nanoplatform, functionalized using highly branched, 8-arm polyethylene-glycol (PEG). The functionalization serves as an efficient tool decreasing the toxicity of the 2D GO flakes, and increasing loading capacity with cisplatinum (Pt) drug. Anticancer effect of GO-based 2D nanoplatform was evaluated in seven human tumour cell lines.

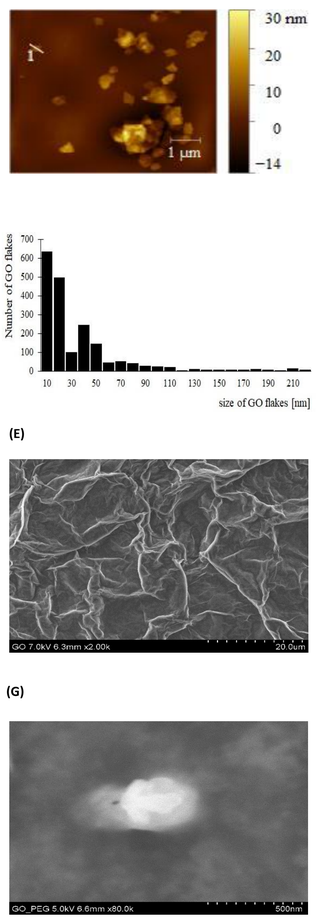
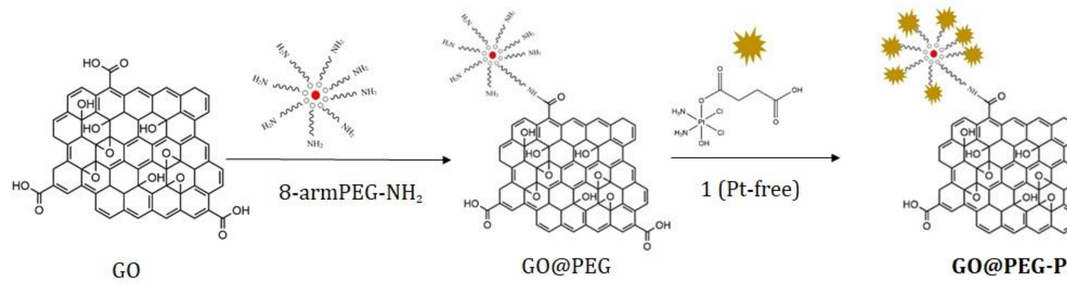


Figure 1. Characterization of nanoplatform. AFM image of PEGylated GO flakes (A), height profile (B) was determined for marked GO flake. Size distribution of GO flakes in supernatant (C) and a typical D a G bands of GO were showed by Raman spectrum (D). Scanning Electron Microscopy images (SEM) of GO in stock solution (E) and the presence of PEG in the sample with GO was demonstrated by IR spectra (F). Successfully PEGylated GO flakes were displayed by SEM (G). Stability testing of GO@PEG-Pt at 4°C and 23°C (n = 3) (H).

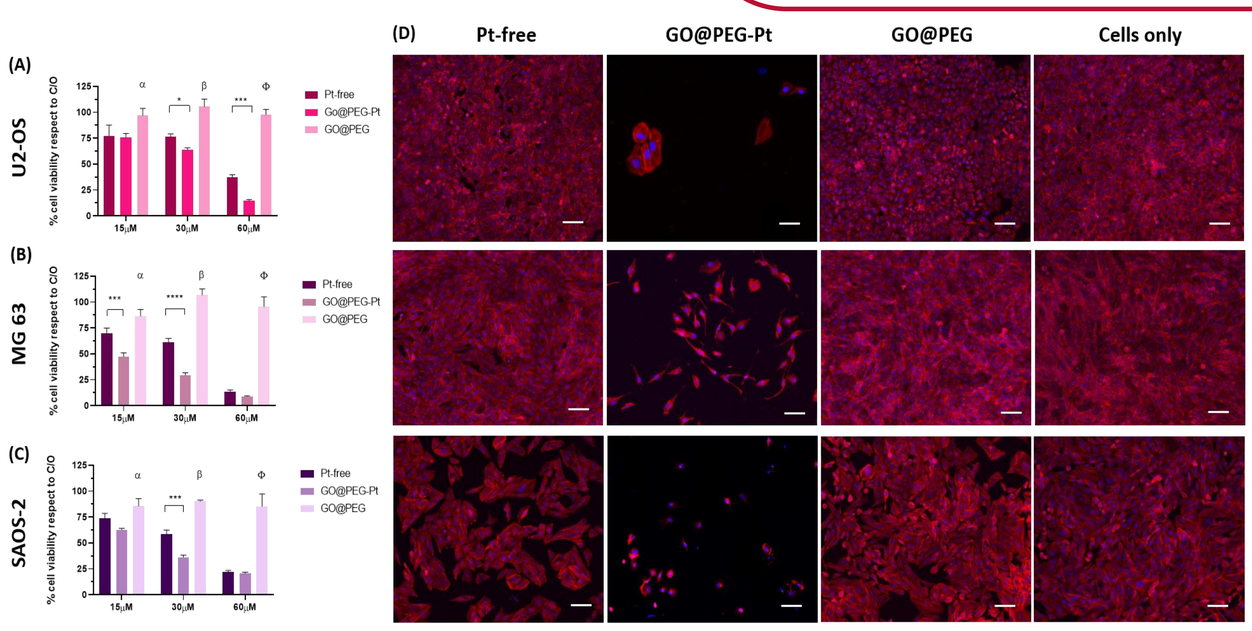


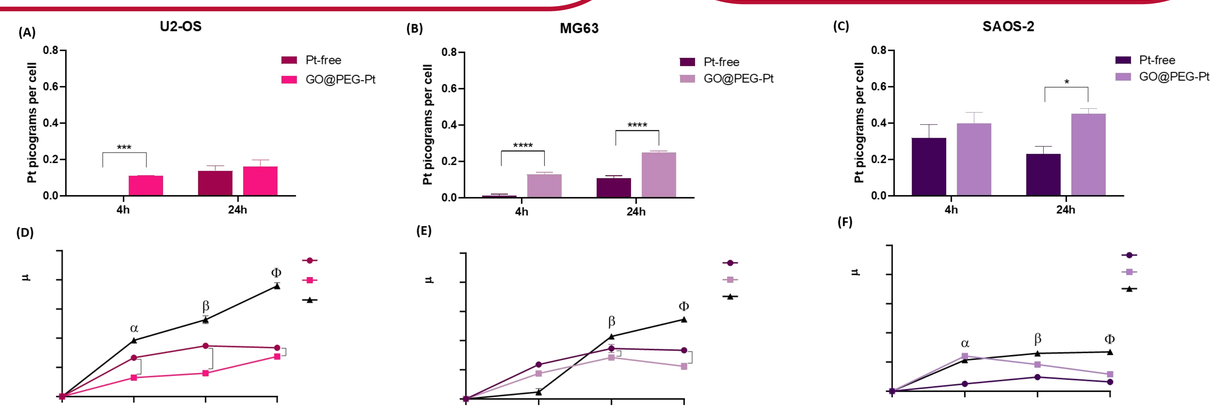
RESULTS

It was found that 85% of GO flakes reached an average lateral size of 130nm before PEGylation. GO@PEG-Pt showed high stability at two different temperatures (pH 7.4) and the Pt-loading efficiency of nanoplatform GO@PEG reached LE=64%. Unloaded GO@PEG showed no significant toxicity, and the GO@PEG-Pt bioactivity showed an apparent dose-dependent decrease in cell metabolic activity in all osteosarcoma cell lines tested in both GO@PEG-Pt compared to Pt-only cells (Fig. 2A-D). These overall results demonstrated that Pt retains its effect even after loading onto GO@PEG nanoplatform. GO@PEG nanoplatform enhanced the Pt internalization in all the cell lines tested after 24 hours, with the greatest differences compared to Pt-free in osteosarcoma (Fig.3A-C). A further investigation was performed to verify if the proposed GO@PEG nanoplatform delivery system could have a key role also in the inhibition of the cell migration/invasiveness. The slight but significant inhibition of MG63 migration, confirmed the aggressiveness and the metastatic potential of osteosarcoma cells to conventional chemotherapeutic drugs, including high-dose platinum-based drugs. On these bases, our results are suggesting an improvement of the Pt drug action on the migration's inhibition of osteosarcoma, especially in MG63 when loaded on the GO-based nanoplatform.

MATERIAL and METHODS

Nano-delivery system based on GO@PEG with bound cisplatinum was characterized and displayed by AFM, SEM, AAS, Raman, UV-VIS and FTIR spectroscopy (Fig.1A-H). The biological activity, including studies of cellular uptake, viability, morphology and migration, was studied in human tumour cell lines namely, gliomas (U87 and U118), breast cancer (MDA-MB-231 and MDA-MB-468) and osteosarcoma (MG63, U2 OS and SAOS-2). The nanoplatform GO@PEG was investigated at three different concentrations (i.e. 1.0 µg/ml, 2.0 µg/ml, 4.0 µg/ml) that correspond to the concentrations of GO@PEG nanoplatforms loaded respectively with 15 µM, 30 µM and 60 µM of Pt, respectively. We present results only for osteosarcoma cell lines where the most significant effect was observed.





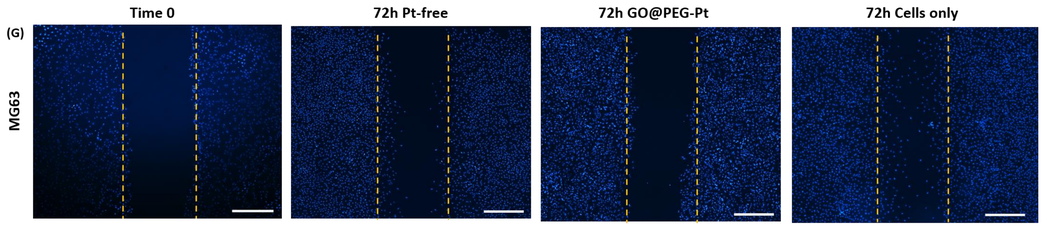


Figure 2. Cell viability and morphological analysis in osteosarcoma cell lines. MTT assay was performed after 72 hours of cell culture. The data show the percentage of viable cells compared to cells only as the control, and the mean ± standard error of the mean are presented. The graphs show the viability of U2-OS cell line (A), of MG63 cell line (B), of SAOS-2 cell line (C), and the respective morphology images of the cells cultured for 72 hours in the presence of the 30mM concentration. Phalloidin in red stains for actin filaments and DAPI in blue stains for cell nuclei (D). Scale bars: 100 µm.

Figure 3. Human Osteosarcoma cell lines. ICP-OES on U2-OS (A), MG63 (B) and SAOS-2 (C). Scratch test on U2-OS (C), MG63 (D) and SAOS-2 (E). Significant differences between Pt-free and GO@PEG-Pt are reported in the graph as follows: * p-value ≤ 0.05, *** p-value ≤ 0.001, **** p-value ≤ 0.0001. Representative DAPI staining of scratch test on MG63 cells (G). Scale bars 500µm. Cell nuclei in blue.

CONCLUSION

The most promising result achieved was related to the significant decrease in cell viability in the GO@PEG-Pt group compared to the Pt-free group, demonstrating that GO@PEG nanoplatforms are a promising Pt carrier for osteosarcoma treatment at a concentration of 30 µM. The obtained results confirmed that GO@PEG-based nanoplatform is a promising nano-delivery system in accordance with other works showing the absence of toxicity strictly related to GO@PEG even *in vivo* with higher concentrations and therefore, GO@PEG nanoplatforms could be administered with no side effects to patients.

ACKNOWLEDGEMENTS

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