# Graphenoid plates in glioma therapy

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## ABSTRACT

Gliomas are the most common primary brain tumors, glioblastoma multiforme (GBM) being the most aggressive subtype. Survival rates for the majority of malignant gliomas is bad, despite decades of advances in surgical, radiation, and chemical therapies, in contrast to improvements in many other cancers. New treatments are still badly needed.

In this research 3 forms of graphene oxide plates (GO), and 3 forms of reduced graphene oxide plates (rGO) were synthesised, described, and their influence on glioma IV cells was investigated, using U87, T98G and A172 cell lines as a model. Five concentrations of each of the plates: 1, 5, 10, 25, 50 μg/ml were tested. The morphology of cancer cells treated with GO and rGO was assessed using the light microscopy. In order to determine the cytotoxic effect of plates on U87, T98G and A172 cell line, the Cell Proliferation Assay (MTT) and and Anexin V/PI Test were conducted. Each of the plates type had a differential effect on U87, T98G and A172 cell line. There wasn't any interaction between the type of plates and cell effects. Graphenoids plates have potential in glioma therapy but only after individual adjustment.

***Keywords***: glioma, apoptosis, cancer, graphene oxide, reduced graphene oxide

## 1 INTRODUCTION

Glioblastoma multiforme (GBM) is a malignant primary tumour. The presence of necrotic areas and atypical vascularisation as well as the presence of atypical cells with nuclear pleomorphism and high proliferative ratio constitute the most important features of this type of cancer. Despite many years of research, glioma is still the most deadly form of human cancer. It is, therefore, necessary to seek innovative experimental therapies to treat this type of tumour [1].

Graphene and its derivates are a 2D carbon sheet with a honeycomb structure that exhibits extraordinary physical and electrical properties. One outstanding electrical feature of graphene is that its electron mobility derives from the presence of pi (π) electrons located above and below the graphene sheet [2].

Graphenoids (allotrop forms of graphene) have also biological activity; they are used in antibacterial, medicine regeneration and anticancer application. Inluences of graphene, graphene oxide and reduced graphen oxide on glioblastoma multiforme cells (GBM) were examinated. Choosen plates reduced cell viability and induced apoptosis via caspase 3 activation pathway [3, 4]. In this study we hypotesis that different form of graphene oxide and reduced graphene oxide will not have the same impact.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Graphenoid plates were synthesis by chemical methods desribed in Table 1. The powders were suspended in ultrapure water to prepare a 1.0 mg/ml stock solution. Immediately before to exposure to cells, hydrocolloids of graphenoids plate were sonicated for 30 min and diluted to concentrations 1, 5, 10, 25 and 50 μg/ml with supplemented Dulbecco's Modified Eagle's culture Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA).

| | Name | Method |
|---|---|---|
| GRAPHENE OXIDE | | |
| 1 | GO_US | modified Marcano method |
| 2 | GO_MII | modified Marcano method and sonicated (amplitude at 30%) |
| 3 | GO_MIV | modified Marcano method and sonicated (amplitude at 40%) |
| REDUCED GRAPHENE OXIDE | | |
| 4 | rGO_HTI | hydrothermal method (72h, 180°C |
| 5 | rGO_NA | reduced using sodium hypophosphite monohydrate |
| 6 | rGO_AS | reduced using ascorbic acid |

Table 1: Chemical methods of grafenoid plates synthesis.

## 2.2 Characterization of graphenoid plates

The shape and size of the plate were inspected using transmission electron microscopy (TEM) JEM-1220 microscope (JEOL, Tokyo, Japan). Zeta potential was measured using the Smoluchowski approximation with Nano-ZS90 Zetasizer (Malvern Instruments, Malvern, UK). Raman Spectroscopy was performed with Renishaw, inVia Raman microscope using Nd:YAG (532 nm, spot size 300 nm, energy 1mW). The resistance was measured in the Van der Pauw configuration using a Hall Effect measurement system (Ecopia HMS-5500) with 0.546 T permanent magnet.

## 2.3 Cell culture

A human Glioblastoma multiforem U87, T98G and A172 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, MA, USA) with addition of 10% fetal bovine serum (FBS, Thermo Fisher Scientific) and 1% penicillin and streptomycin (Thermo Fisher Scientific ) at 37°C in a humidified atmosphere of 5% $CO_2$ inside Incubator INCOMED153 (Memmert GmbH & Co, Germany).

## 2.4 Cell viability assay

Viability of U87, T98G and A172 cells was studied by colorimetric MTT assay where yellow soluble tetrazolium salt is converted to purple formazan crystals. After 24h cell culture, the medium was removed and replaced with medium containing plates at concentrations 1, 5, 10, 25 and 50 µg/ml and incubated for 24 h. 15 µg of MTT (5 mg/ml) were added per well and after 3 h a detergent (isopropanol with a drop of HCl) was added. Multi-well plate reader (Infinite® 200 PRO) i-control™ Software (Tecan Group Ltd., Männedorf, Germany) was used for performing the measurements at 570 nm. Cell viability was expressed as the percentage of the control group viability, which was 100%, and plotted as surviving fraction of cells relative to the no-treatment condition.

## 2.5 Apoptosis/necrosis assay

Annexin V and propidium iodide (PI) staining for apoptosis/necrosis assaywas performed by the Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit, (Thermo Fisher Scientific) according to the manufacturer's protocol. via flow cytometry. The U87, T98G and A172 cells were seeded onto 6-well plates and cultivated overnight. The next day, the medium was replaced with fresh medium containing plates at concentrations 5 and 50 µg/mL and the cells were cultivated for 24. Then, the cells were washed in PBS and stained. Readings were performed with BD FACSCalibur™ cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) measuring the fluorescence emission at 530 and 575 nm using excitation at 488 nm.

## 2.6 Statistical analysis

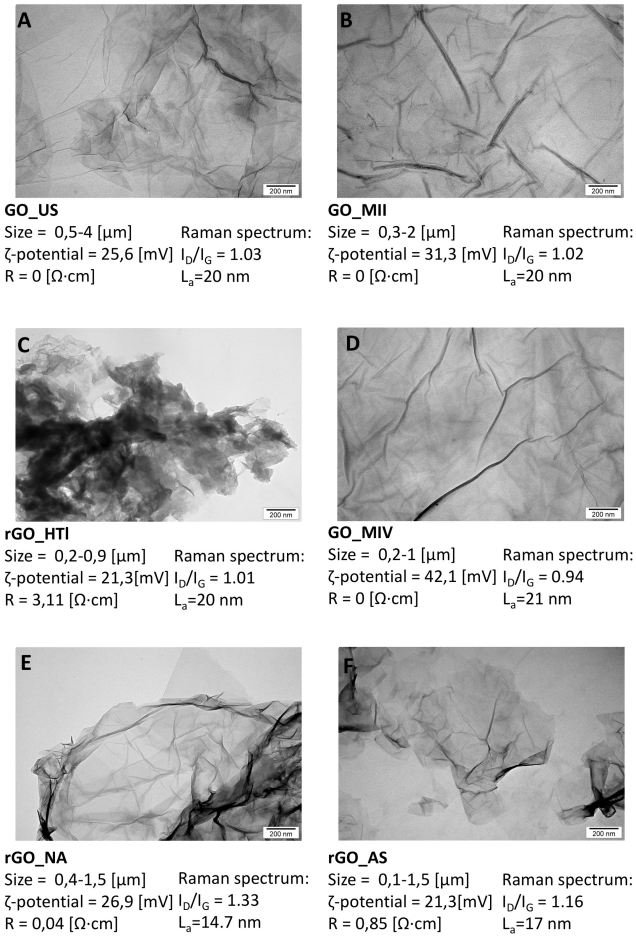
Data were analyzed using one-way analysis of variance and Bonferroni post hoc test, comparing to the control. Results are shown as mean values with standard deviations. Differences at $P<0.05$ were considered significant. All data were analyzed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA).

# 3. RESULTS AND DISCUSSION

The characteristics of graphenoid plates are presented in Figure 1.

The viability of the U87, T98G and A172 cells was examined using the MTT (Figure 1). We observed both dose dependent (T98G vs rGO_NA; A172 vs rGO_AS) and dose-independent (T98G vs GO_MIV; A172 vs GO_US) effect graphenoids plates on cell viabilty. The most toxic plates for U87 and T98G cell line was graphene oxide GO_MII which reduced the U87 cell vibalility by about 50% at the concentration of 25 and 50 µg/ml and T98G cell viability at the concetration 5, 10 and 25 µg/ml. GO_MII didn't inluence on viability of A172 cell line. For A172 cell line the most toxic was graphene oxide GO_US at the concentration at 1 and 25 µg/ml.

The percent of apoptotic cells (Figure 3) was the highest in U87 cell after GO_MIV treatment at concentration 50 µg/ml and in A172 cell after GO_MII treatment at concentration 50 µg/ml. In other groups was rather on the same level at 15-20%. The percent of necrotic cells was general low, the highest value was after U87 cell treatment by reduced graphene oxide (rGO_HTI) at concentration 5 µg/ml.




**GO_US**
Size = 0,5-4 [μm] Raman spectrum:
ζ-potential = 25,6 [mV] $I_D/I_G$ = 1.03
R = 0 [Ω·cm] $L_a$=20 nm

**GO_MII**
Size = 0,3-2 [μm] Raman spectrum:
ζ-potential = 31,3 [mV] $I_D/I_G$ = 1.02
R = 0 [Ω·cm] $L_a$=20 nm

**rGO_HTI**
Size = 0,2-0,9 [μm] Raman spectrum:
ζ-potential = 21,3[mV] $I_D/I_G$ = 1.01
R = 3,11 [Ω·cm] $L_a$=20 nm

**GO_MIV**
Size = 0,2-1 [μm] Raman spectrum:
ζ-potential = 42,1 [mV] $I_D/I_G$ = 0.94
R = 0 [Ω·cm] $L_a$=21 nm

**rGO_NA**
Size = 0,4-1,5 [μm] Raman spectrum:
ζ-potential = 26,9 [mV] $I_D/I_G$ = 1.33
R = 0,04 [Ω·cm] $L_a$=14.7 nm

**rGO_AS**
Size = 0,1-1,5 [μm] Raman spectrum:
ζ-potential = 21,3[mV] $I_D/I_G$ = 1.16
R = 0,85 [Ω·cm] $L_a$=17 nm

Figure 1: TEM images and characteristic of graphenoid plates (A, B, D – Graphene Oxide; C, E, F – reduced Graphene Oxide).

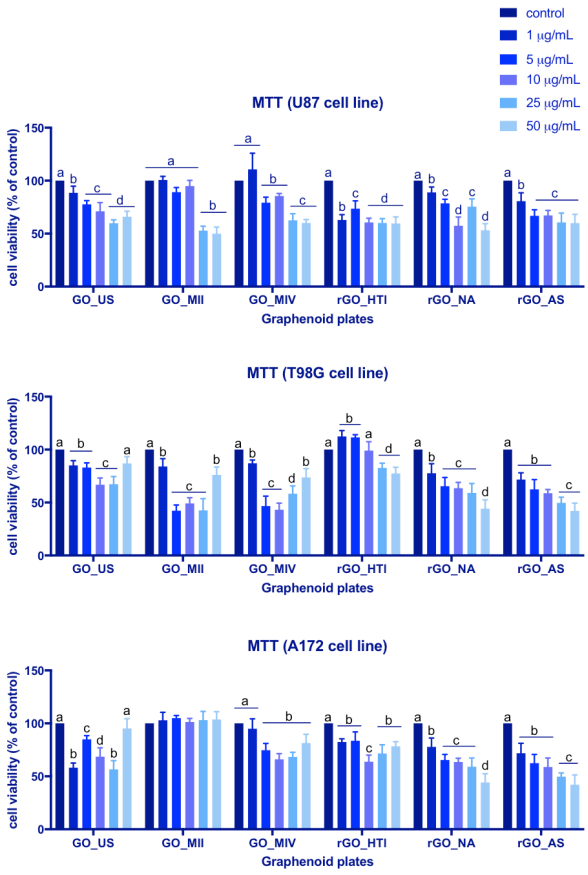



Figure 2: Viability of U87, T98G and A172 cells after GO_US, GO_MII, GO_MIV, rGO_HTI, rGO_NA and rGO_AS treatment at concentrations of 1, 5, 10, 25, 50 μg/ml.Values with different letters are significantly different, $P < 0.05$.

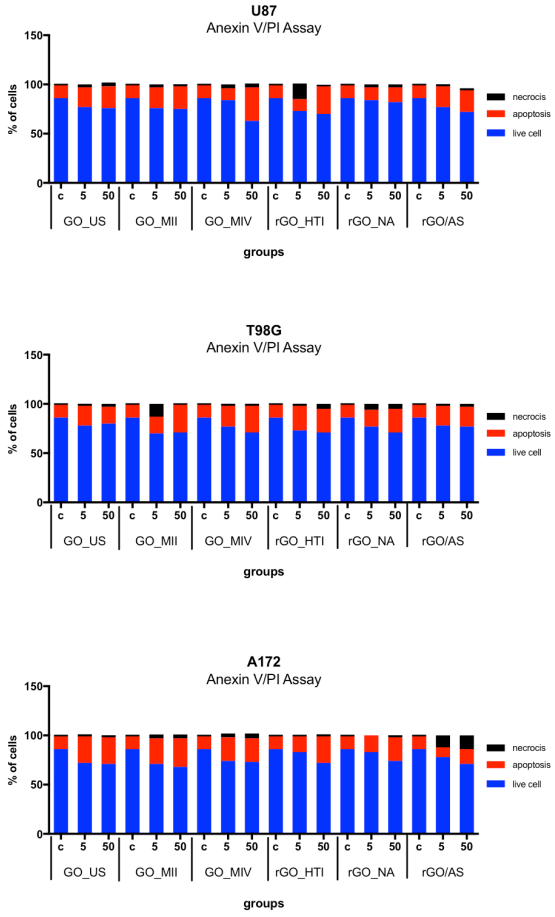



Figure 3: Analysis of apoptosis in U87, T98G and A172 cell lines after GO_US, GO_MII, GO_MIV, rGO_HTI, rGO_NA and rGO_AS treatment at concentrations of 5 and 50 µg/ml. Measurements were performed by Annexin V-Alexa Fluor® 488 and PI assay analyses.

## 4. CONCLUSION

Different form of graphene oxide and reduced graphene oxide may have various influence on tumour cells. The use of graphenoid plates in glioma therapy still requires many studies.

## 5. REFERENCES

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## 6. ACKNOWLEDGMENT

This work is supported by the National Centre for Research and Development (NCBiR) under grant LIDER/144/L6/14/NCBR/2015