

Full Length Research Paper

An evaluation of the effect of graphene oxide on *Saccharomyces cerevisiae*

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Graphene oxide (GO) has a wide range of applications. It has been identified to have significant potential for important biomedical and biochemical uses. Graphene and its derivatives are known to elicit toxic effects. *Saccharomyces cerevisiae* is a versatile yeast of importance with several uses, one of which is its application as a human probiotic. The interaction of GO with living systems is of interest and relevance. The effect of GO on the yeast *S.cerevisiae* was investigated. The yeast cell line was cultured in a liquid media composed of Yeast Peptose Dextrose and incubated at room temperature (25°C) for 72 h. Antibody RAD 17 was labelled with fluorescent dye fluorescein isothiocyanate (FITC) to detect cell structure change. 500, 750, 1000 and 1250 µg/ml GO aliquots were prepared from a stock solution and 96 microplate wells of *S. cerevisiae* were treated with various doses of GO for 24, 48 and 72 h. Microscopic analysis on treated yeast showed no apparent effect on the cell after exposure to GO.

Key words: Graphene oxide, *Saccharomyces cerevisiae*, yeast cell line, cell culture, incubation, toxicity, cell wall.

INTRODUCTION

Two-dimensional materials such as graphene oxide (GO) are currently a subject of investigation due to their

possession of properties that are fundamental in the production of innovative devices. Extensive use of GO in

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drug delivery and cancer therapy has been reported. Loading of more amount of anticancer drugs on GO, excellent drug release and enhanced photo thermal and photo acoustic effect, efficient and accelerated killing of tumor cells, inhibition of tumor growth due to sequential drug release, etc. have been achieved in cancer treatment. Hydrophobic drugs can be delivered to tumor cells via GO (Santhosh et al., 2017; Li et al., 2012). It has also been used in cancer therapy as a photo thermal agent with encouraging therapeutic outcomes due to its high, intrinsic near-infrared (NIR) absorbance for cancer treatment (Li et al., 2012). GO has a wide scope of potential as a raw material for several applications. Unique properties such as excellent aqueous processability, amphiphilicity, surface enhanced Raman scattering (SERS), fluorescence quenching ability, biocompatibility and solubility make GO a candidate for biological applications (Chung et al., 2013; Loh et al., 2010; Liu et al., 2008).

The oxide has antibacterial and anti-tuberculosis potential and showed glucose sensitivity, mutagenesis, scaffold generation, aromatic hydrocarbon extraction from food samples, and triggered growth in plants when treated with it (Santhosh et al., 2017).

It displays interesting optical characteristics such as effectively quenching the fluorescence of other fluorescent dyes (Liu et al., 2008) and fluorescing over a wide range of wavelength from near-infrared to ultraviolet (Loh et al., 2010). These make it a suitable material in fabricating fluorescence resonance energy transfer (FRET) sensors, in which an excitation on another molecule transfers nonradiatively to the oxide. It is used in immunosensing as an energy donor or acceptor (Lim et al., 2015).

Due to its excellent biocompatibility, flexible chemical modifications, ready cellular uptake and unique optical properties, GO has been explored for biological imaging. The protection of DNA from enzymatic digestion by the self-assembly of ssDNA on GO sheets was reported by Tang et al. (2010).

In vivo tumor uptake and photothermal therapy with PEGylated GO using xenograft tumor mouse models study was carried out for the first time by Yang et al. (2010) and it was observed that there is very high tumor uptake of the PEG-modified GO due to highly efficient tumor passive targeting of GO caused by enhanced permeability and retention (EPR) effect.

Graphene derivatives, including pristine graphene, GO, doped and chemically reduced GO (rGO) have been intensively studied for their widespread applications in biosensing and detection of biomolecules such as oligonucleotide, thrombin, adenosine triphosphate (ATP), amino acid, and dopamine graphene (Chang et al., 2010; Dong et al., 2010; Tang et al., 2009, 2011; Wang et al., 2009, 2010a, b).

Several types of GO-based biosensors have been built

by making use of super-efficient fluorescence quenching ability of graphene; some novel fluorescence resonance energy transfer (FRET) based biosensors have been developed (Chang et al., 2010; Wang et al., 2010a). Field-effect transistor (FET) type GO-based biosensors are based on the unique electronic property of graphene (He et al., 2010). Controllable self-assembling of graphene-biomolecules allows for the building of highly ultrasensitive biosensors for detection of DNA and other molecules (Tang et al., 2011; Zhang et al., 2011; Wang et al., 2011b; Zeng et al., 2010). As a matrix for detection of molecules, graphene-based nanoplatfor for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been reported. GO-based novel biosensors via electrochemical principle have been built by using its huge surface area, good electrical conductivity, and excellent capability of loading various biomolecules via chemical or physical interactions (Wang, 2011a; Wan et al., 2011).

Macroscopic freestanding GO and rGO paper has been prepared from suspension by vacuum filtration technique, and these papers exhibit strong antibacterial effect (Hu et al., 2010).

Studies on the toxicity and biocompatibility of GO have shown potential accumulation of the oxide in the lung, liver and spleen resulting in acute liver injury and chronic inflammation of the lung, liver and spleen of mice (Ou et al., 2016; Jasim et al., 2015; Wen et al., 2015; Guo and Mei, 2014; Gurunathan and Kim, 2016).

Saccharomyces cerevisiae, commonly called brewers or baker's yeast has as many uses as GO. It is a source of B vitamins and minerals such as chromium and selenium which aid the metabolism of fats, proteins and carbohydrates (Yu-hui et al., 2016). It is widely used as a veterinary probiotic and have exhibited probiotic potential in humans with the strain *Saccharomyces boulardii* licensed for use as a human probiotic (Suarez and Guevara, 2018; Fakruddin et al., 2017; Moslehi-Jenabian et al., 2017; Romanio et al., 2017; Palma et al., 2015; Abu-Elala et al., 2013).

S. cerevisiae is used in the manufacture of nutritional yeast which is a rich source of folate used to enrich pastries. It aids the absorption of electrolytes in the body by degrading complexed phytates and has the ability to bind and degrade mycotoxins through fermentation (Shehu et al., 2016). Studies have shown that it reduces oxidative stress and maintains integrity of the mucosal barrier in mice and may be beneficial in gastrointestinal mucositis, a serious side effect of cancer therapy (Jumanah et al., 2017; Bastos et al., 2016; Ognjenovic et al., 2013). It is used in the formulation of cosmetics (Gaspar et al., 2008).

More studies on the standardization of GO and its biofunctionalization to clearly understand how cells respond to it are a requirement for the development of therapeutics. Toxicity and biocompatibility are also

Table 1. Samples of *Saccharomyces* cell line in microplate wells treated with varying concentrations of graphene oxide (GO).

GO concentration (µg/ml)	Incubation period (h)		
	24	48	72
0	A ₀	B ₀	C ₀
500	A ₁	B ₁	C ₁
750	A ₂	B ₂	C ₂
1000	A ₃	B ₃	C ₃
1250	A ₄	B ₄	C ₄

usually a concern for biomedical and other areas of application. Therefore, interaction of GO with living systems should be of relevance and stimulated the interest in this study. The versatility of the yeast, *S. cerevisiae* and the fact that GO is known to elicit toxicity calls for interest on the outcome of their interaction. The objective was to investigate the effect of GO on the yeast, *S. cerevisiae*.

MATERIALS AND METHODS

Materials

GO was obtained from Angstrom Materials (Dayton, Ohio, USA). Pierce® fluorescein isothiocyanate (FITC) Antibody Labeling Kit and anti-RAD17 were obtained from Thermo Scientific (Waltham, Massachusetts, USA). *Saccharomyces cerevisiae* cell line was obtained from ATCC. Nikon Eclipse Ti inverted microscope

Reagents

GO Solution: 0.5% aqueous solution of the oxide was prepared and stored as stock solution. Step down dilution of the stock solution was effected to obtain four aliquots of 500, 750, 1000 and 1250 µg/ml concentrations.

S. cerevisiae cell culture

S. cerevisiae cell line was cultured in an aqueous Yeast Peptone Dextrose (YPD) broth. The liquid media was autoclaved for 60 min to make it sterile. A colony of *S. cerevisiae* was introduced into the media and incubated at room temperature (25°C) for 72 h.

Labeling antibodies

A buffer solution composed of 0.67 M borate buffer and diethyl pyrocarbonate (DEPC) treated water with 1 mg of RAD17 was incubated and passed through purification resin in spin columns pre-treated with phosphate buffered solution (PBS) and stored in the dark at 4°C. Antibody RAD17 was labeled with fluorescent dye FITC to aid in the detection of any form of phenotypic expression in the DNA of the yeast. If DNA damage occurred, FITC labeled antibody RAD17 would fluoresce under the FITC filter in the form of foci.

Exposure to graphene oxide

Cultured *Saccharomyces* cell line was plated in three different well microplates labelled A, B and C. To determine the effect of the concentration of GO on the cell as a function of time, microplate wells were treated with 0, 500, 750, 1000 and 1250 µg/ml of GO and incubated for 24, 48, and 72 h. The wells were labeled A₀, A₁, A₂, A₃, A₄; B₀, B₁, B₂, B₃; B₄; C₀, C₁, C₂, C₃, C₄ for 24, 48 and 72 h incubation periods respectively (Table 1). Three replicates of microplate wells were used to determine the effect of the oxide on the cell line.

Microscopy

At the end of each incubation period, the media was aspirated after centrifuge; microscope slides were stained with samples and fixated by flame and washed in a 10% Triton X solution for 10 min. They were subsequently washed in PBS three times and viewed under microscope.

The procedure was replicated twice independently and viewed using a Nikon Eclipse Ti inverted microscope with and without FITC filter after exposure of the cell line to GO. This filter makes it possible to visualize any DNA damage that might have occurred (in the form of fluorescent foci) to *Saccharomyces* from exposure to GO.

Ethical considerations

All facets of the research were approved by Texas Southern and Augusta Universities, USA Ethical review Boards

RESULTS AND DISCUSSION

Table 2 shows the microscopic observation of the cell line following exposure to varying concentrations of GO. Samples were observed under a monochrome image device and viewed for any appearance of foci in the sample with an FITC filter. No foci were detected. The yeast shape and cell wall integrity were maintained despite the concentration of GO and length of incubation. The control cells, samples A₀, B₀, and C₀ looked similar as shown in Figure 1. The cells were oval-shaped with a clear outer cell wall and intact nucleus. However, looking at the result obtained after 72 h exposure (Figure 2), there is a slight morphological deformity of some of the

Table 2. The microscopic observation of the cell line following exposure to varying concentrations of GO.

Sample	Observation
A ₀	Cells are cylindrical with normal cell wall
A ₁	Cells shape and size intact
A ₂	Cells showed no change in shape and size
A ₃	No observable change in the shape and size of cells
A ₄	Cells remained cylindrical
B ₀	Cells looked the same, cylindrical
B ₁	Shape of cells remained the same
B ₂	No change was observed in cells
B ₃	Cells showed no sign of change
B ₄	Cell structure remained the same
C ₀	No observable change in cells
C ₁	No change was observed in the shape and size of the cells
C ₂	Cells looked the same
C ₃	No change was observed in cells
C ₄	Cells structure remained unchanged

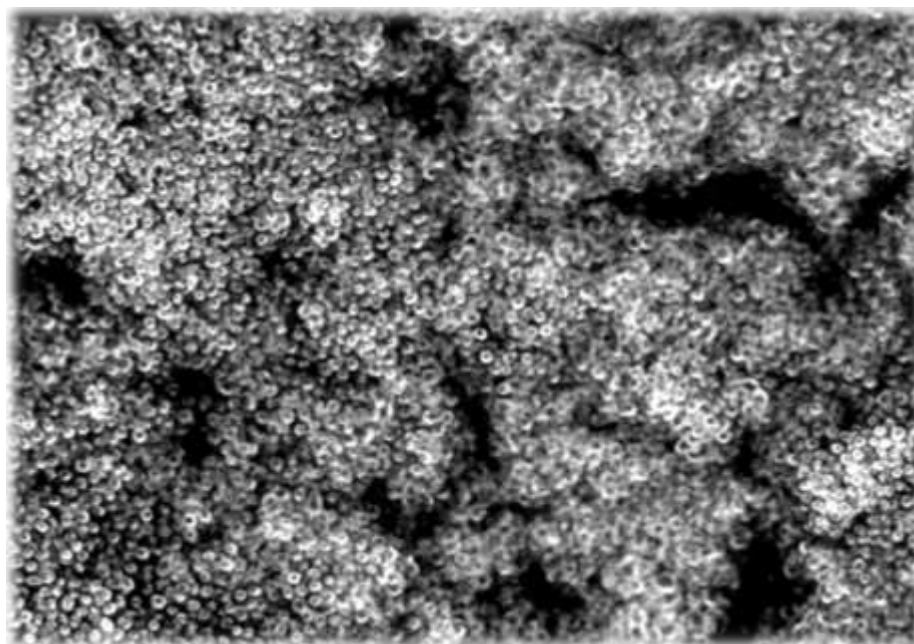


Figure 1. C₀ [Yeast Control after 72 h (40x Magnification)].

cells. Bennis et al. (2004) observed deformity in the shape of *S. cerevisiae* cells induced by eugenol. In this present work the cells maintained their cell wall and there was no significant adverse effect on the cell and its structure under all magnifications regardless of dose and duration of exposure to GO. Zhu et al. (2017) reported a dose-dependent decrease in cell number relative to the control after 24 h of exposure of *S. cerevisiae* to 50 to

600 mg/ml of GO. The work by Yu et al. (2017) showed that GO concentration of 10 to 160 mg/ml inhibited the growth of *S. cerevisiae*.

Duration of exposure and increase in dosage of GO had no notable effect on the yeast cell. The incapability of GO to infiltrate the cell wall of *Saccharomyces* may have played a role in the non-observance of DNA damage. The nucleus and vacuoles within the cell were not

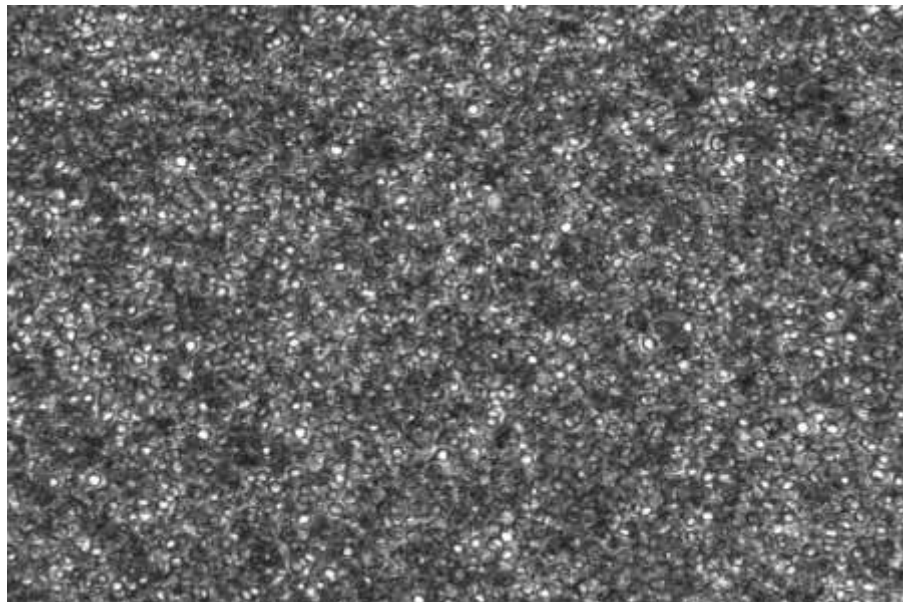


Figure 2. C₄ [Test Sample after 72 Hour Exposure (40x Magnification)].

affected by GO, eliminating any chance of toxic effects. Although RAD17 is a protein that is expressed in response to DNA damage in *Saccharomyces*, it may not be expressed as well as its other counterparts. Only the antibody RAD17 was labeled and this may have made it difficult to see any form of foci or reactive oxygen species that may have been induced in reaction to GO.

Graphene oxide had no perceived interaction with *S. cerevisiae*. This may have been due to the thick cell wall of the yeast which may have prevented the cell from undergoing any changes. In addition, the concentration of GO used, 500-1250 µg/ml may have been too weak to cause the expected toxic or adverse effect. GO concentrations used by Zhu et al. (2017) and Yu et al. (2017) were in the mg/ml range.

The protein RAD17 is one of the proteins expressed in response to DNA damage in the yeast *S. cerevisiae* (Paulovich et al., 1998). The antibody RAD17 was labeled with FITC, making any DNA damage visible under a FITC filter. There were no demonstrable foci seen with FITC, thus it can be inferred that RAD17 may not express well in response to reactive oxygen species.

Conclusion

Yeast showed no apparent effect on the cell after exposure to the various concentrations of GO used. Higher concentration in the mg/ml level may elicit toxicity.

To confirm the findings in this study other DNA damage sensors are to be investigated and GO concentration is to be increased to the mg/ml range.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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