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Evaluation of cell viability, adhesion, and morphology of Pc-12 cells on PLA-rGO composites

Sediqeh Jalali[1], Amin Dolatabadi[2], Maryam Poostchi[3], Aliasghar Behnamghader[4, *]

[1] Department of Biology, Payam Noor University, Tehran, Iran. Sediqeh.jalali@pnu.ac.ir

[2,3,4] Biomaterials Group, Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center (MERC) Tehran, Iran.

***Corresponding Author:**

a.dolatabadi@merc.ac.ir

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ABSTRACT

This study investigates the preparation and surface modification of polylactic acid (PLA) and reduced graphene oxide (rGO) composites at weight ratios of 0, 0.2%, 0.5%, 1%, 2%, and 3%. Before in vitro tests, surface of composites were modified using sodium hydroxide solution 1 M for one hour. Viability, adhesion, and morphology of PC-12 cells were analyzed directly on the composites' surfaces. The results indicated that the PLA/rGO composite incorporated 2% rGO showed the highest cell viability compared to the composites incorporating 0.5% and 3% rGo in first day of culture. By day 3, all the composite samples maintained acceptable cell viability; while the PLA/rGO 2% demonstrated maximum cell viability after 7 days, underscoring its effectiveness in promoting cell survival. Scanning electron microscopy (SEM) revealed significant surface porosity and roughness after surface modification, which corresponded with enhanced cell adhesion and spreading of PC-12 cells. These results underscore the beneficial impact of surface modification on the biological properties of PLA/rGO composites, indicating their promising potential in neural tissue

Keywords: Polylactic acid, Reduced graphene oxide, Neural tissue engineering, Cell viability

Introduction

The complicated relationship between cellular systems and biomaterials is an important factor to consider in the fields of tissue engineering and regenerative medicine. The viability, adhesion, and morphology of cells are instrumental in establishing not only the functionality but also the seamless integration of biomaterials within biological environments, which directly impacts their clinical applicability [1]. The PC-12 cell line, originating from a rat pheochromocytoma, has become an essential model in neurobiology research. This prominence is attributed to its exceptional capability to differentiate into neuron-like cells, making it an ideal candidate for investigating neuronal responses to a wide variety of substrates and physical conditions [2].

In recent years, advancements in materials science have enabled the development of innovative composites, particularly polylactic acid (PLA) enhanced with reduced graphene oxide (rGO). These composites are notable for their enhanced mechanical and biological properties, which elevate their potential for a range of biomedical applications [3]. PLA is noted for its biodegradability and biocompatibility, rendering it an exemplary substrate for cell culture. Furthermore, the inclusion of rGO not only reinforces the mechanical stability of the composite but also promotes cellular activities through its unique surface characteristics, creating an advantageous environment for cellular engagement. The synergistic combination of PLA and rGO offers significant opportunities for applications in nerve tissue engineering, particularly in the development of scaffolds aimed at optimizing cellular behavior [4].

The strategic incorporation of rGO into the PLA matrix is intended to enhance the electrical properties of the composite while simultaneously fostering a supportive environment that encourages the growth and differentiation of PC-12 cells [5].

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A comprehensive understanding of the influence of PLA-rGO composites on cell viability, adhesion, and morphology is essential for optimizing their utilization in biomedical applications [6]. This study aims to evaluate the interactions between PC-12 cells and PLA-rGO composites, thereby providing critical insights that could promote neuronal health and advance the development of innovative strategies within the field of tissue engineering.

Methods And Material

The reduced graphene oxide powder was obtained from previous research. Poly(lactic acid) (PLA) was purchased from Chemie Kas GmbH. Dichloromethane and sodium hydroxide, supplied by Merck, were utilized in this study. All materials were used as received without any additional processing.

Fabrication of Polylactic Acid/rGO Composite

The PLA/rGO composite was fabricated using the solvent casting method. Initially, 2.5 g of PLA granules were dissolved in 15 mL of dichloromethane (DCM) at room temperature. To achieve more uniform dispersion of the rGO powder in the solution, rGO at concentrations of 0.2%, 0.5%, 1%, 2%, and 3% (w/w) relative to PLA was dispersed in 4 mL of DCM and subsequently added to the PLA solution. The mixture was stirred continuously until the rGO was fully and uniformly distributed. The solution was then poured into flat glass molds to allow the solvent to evaporate. The resulting composite samples were prepared in dimensions of 7 × 7 × 0.3 mm and utilized in this study.

Surface treatment

After fabricating the composites with varying rGO ratios and preparing the sodium hydroxide solution, the samples underwent surface modification through immersion in the 1M NaOH solution for one hour at room temperature under continuous shaking. Following the treatment, the samples were rinsed thoroughly with deionized water for 5 minutes.

Analysis and Characterization Methods

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM, TESCAN MIRA3) was employed to observe the surface morphology, including the distribution of rGO particles within the composite and the impact of surface modification on morphology. Prior to imaging, the samples were mounted onto aluminum stubs and coated with platinum for 3 minutes to ensure conductivity. Images were randomly captured from the surface of the samples and analyzed using ImageJ software.

MTT Assay

Cell viability of human OE-MSCs was quantitatively assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Kiazist, Tehran, Iran). In this study, MTT test is employed to assess PC-12 cell survival and their metabolic activity while cultured on composite surface, directly. Prior to cell seeding, all samples were sterilized by washing with ethanol and exposure to UV light for 30 minutes. The MTT assay was conducted in a 96-well plate, with each composite cut to a 4 mm diameter and placed at the bottom of the wells. PC-12 cells were seeded onto the surface at a density of 1×10^4 cells per well in DMEM medium and cultured for 1, 3, and 7 days. At each designated time point, the culture medium was removed and replaced with 150 µL of fresh serum-free medium containing 20 µL of MTT reagent. The plate was then incubated at 37°C with 5% CO_2 for 4 hours. After incubation, 100 µL of DMSO was added to dissolve the formazan crystals, and the absorbance was measured at 570 nm using an ELISA plate reader. Cell viability was calculated using the following formula:

$$\%\ Cell\ viability = \frac{At - Ab}{Ac - Ab} \times 100 \quad (1)$$

Where:
A_t = Absorbance of the test sample
A_β = Absorbance of the blank
Ac = Absorbance of the control

Cell adhesion assay

For the cell adhesion assay, PC-12 cells at a density of 1×10^4 cells were cultured on the PLA and PLA/rGO 2% composites for 24 hours. The samples were then washed three times with PBS and fixed with 2.5% glutaraldehyde solution for 75 minutes at room temperature, followed by another PBS wash. For the dehydration process, the samples were sequentially immersed in ethanol solutions with increasing concentrations of 60%, 70%, 80%, and 90%, each for 5 minutes. To complete the dehydration, the samples were placed in 100% ethanol for 7 minutes. Finally, the samples were coated with a thin layer of gold using a sputter coater for 3 minutes. The cell morphology was then analyzed using scanning electron microscopy (SEM) provided by Cambridge, UK.

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Results and Discussion

Scanning Electron Microscopy (SEM)

As shown in Figure 1(A), the surfaces of PLA/rGO composites containing 2 wt% rGO modified with NaOH 1 M solution, exhibited notable morphological alterations compared to untreated samples**.** In alignment with previous research, surface modification with NaOH is anticipated to enhance surface roughness and induce microscopic cracks on the polymer surface. This etching process may lead to greater exposure of rGO particles on the composite surface, thereby potentially improving its electrical conductivity. Furthermore, the etching treatment can generate a porous microstructure, a phenomenon commonly observed in other polymer composites [7].

As expected, the surface modification process led to a significant increase in surface roughness, resulting in the formation of grooves, pits, and depressions of varying sizes and depths, which are clearly visible in Figure 1(B). The etched surface exhibited substantial alterations in topography compared to the relatively smooth surface of the unmodified PLA/rGO composite.

MTT assay

To evaluate the cytotoxicity of rGO in PLA/rGO composites, MTT assay was conducted on PC-12 cells. Based on the data presented in the table in Figure 1, on the first day, the composite containing 2% rGO demonstrated the highest cell viability compared to other samples. On the third day, all samples, except for the PLA sample, showed acceptable cell viability. By the seventh day, all samples exhibited favorable cell viability, with the sample with 2% rGO achieving the highest viability. These findings indicated that the sample with 2% rGO concentration demonstrated not only no toxicity, but also enhanced in cell viability.

Cell Adhesion

Considering that PC-12 cell culture was conducted on the scaffold, the anticipated cellular morphology includes proper adhesion of PC-12 cells to the composite surface, extension of cellular processes, and the formation of elongated dendrites. Moreover, the cells are expected to grow in colonies, with observable morphological variations influenced by the surface characteristics of the composite[8]. As anticipated, PC-12 cells successfully adhered to the scaffold, exhibiting proper growth and proliferation. This observation highlights the scaffold's excellent capability to support cell adhesion and spreading, which is crucial for tissue repair processes and tissue engineering applications.

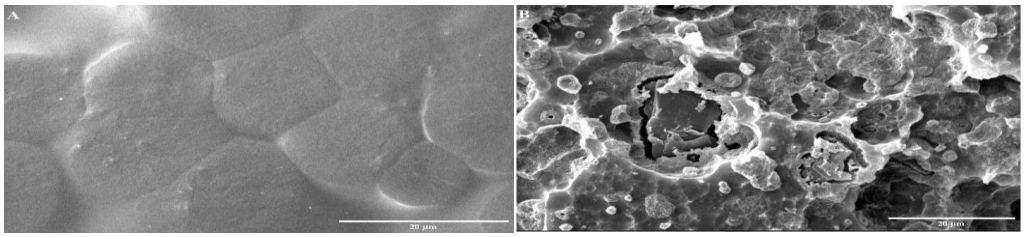

Figure 1. Unmodified (A) and modified (B) Surface of PLA/rGO 2% Composite

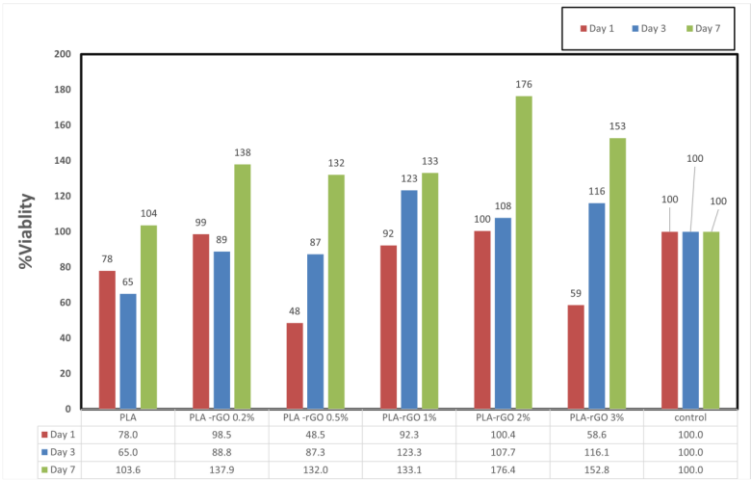

Figure 2. MTT assay of PLA/rGO composites for 1,3, and 7 days

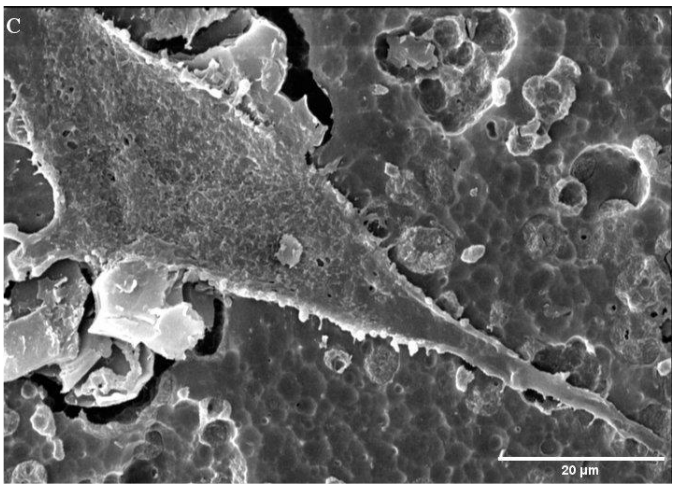

Figure 3. Morphology of PC-12 cells on surface-modified PLA/rGO composite with 2% rGO

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Conclusion

Biocompatibility is one of the most critical characteristics of composites used in tissue engineering. Previous studies have shown that the use of reduced graphene oxide (rGO) at higher concentrations can lead to cytotoxicity [9]. However, rGO is considered more suitable for tissue engineering applications due to its lower toxicity compared to graphene oxide (GO) and its other favorable biological properties.

This study investigates the synthesis and characterization of PLA/rGO composites with varying weight ratios (0.2%, 0.5%, 1%, 2%, and 3%) for potential applications in tissue engineering. A 1 M sodium hydroxide (NaOH) solution was used to perform chemical surface modification on the composites through immersion for one hour. Following this, MTT assays were conducted to assess the viability of PC-12 cells cultured on the scaffolds, with data collected on days 1, 3, and 7. SEM imaging was utilized to analyze morphological changes in the composites and the cultured cells.

Results indicated that the 2% rGO composite exhibited the highest cell viability on day 1, while the 3% and 0.5% samples showed slight cytotoxicity. By day 3, all samples (except the PLA control) demonstrated acceptable viability, with the 2% rGO composite maintaining superior cell viability on day 7. SEM analysis revealed enhanced surface porosity and roughness following modification, with morphological observations confirming effective cell adhesion and spreading on the scaffolds. Overall, this study highlights the potential of modified PLA/rGO composites in promoting cell viability and supporting tissue engineering applications.

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