JSMPM

JOURNALS OF SUSTAINABLE MATERIALS PROCESSING AND MANAGEMENT
e-ISSN: 2821-2843

Vol. 6 No. 1 (2026) 73-83
https://publisher.uthm.edu.my/ojs/index.php/jsmpm

Gelatin-Monosaccharide Functionalized Multi-Walled Carbon Nanotubes: Cytotoxicity and Cellular Uptake in MCF-7 Cells

Ahmed A. Haroun[1]*

[1] *Chemical Industries Research Institute, National Research Centre, 33 El Bohouth Str., Dokki, Giza, EGYPT*

*Corresponding Author: haroun68_2000@yahoo.com
DOI: https://doi.org/10.30880/jsmpm.2026.06.01.006

Article Info

Received: 8 February 2026
Accepted: 16 April 2026
Available online: 15 May 2026

Keywords

MWCNTs, hybrid materials, gelatin, monosccharides, in vitro cytotoxicity, MCF-7 cells

Abstract

This study aims to develop a tripartite hybrid material composed of multi-walled carbon nanotubes (MWCNTs), gelatin (gel), and a monosaccharide (2-glucosyloxyethyl methacrylate, GEMA) to achieve a synergistic interaction that integrates structural integrity and biological safety. The primary objective is to evaluate the potential in vitro cytotoxicity of these synthesized materials against the MCF7 breast cancer cell line, facilitating the development of safe bionanodevices for clinical applications. We prepare and characterize MWCNTs that have been functionalized with GEMA and coated with gel for the study. The cytotoxicity was carried out using breast carcinoma (MCF7) cell line via sulphorhodamine B (SRB) assay. This work also studied the effect of the functionalized MWCNTs on their physicochemical properties using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction patterns (XRD), scanning and transmission electron microscopes (SEM and TEM). The results showed that gelatin was successfully reacted with GEMA *via* their amino groups using Michael addition reaction. Besides, the functionalized MWCNTs exhibited low cytotoxic effects in comparison with the standard Doxorubicin®. These results suggest that gelatin-monosaccharide functionalized MWCNTs serve as a promising, low-toxicity platform for targeted drug delivery and imaging.

1. Introduction

The existing MWCNT functionalization methodologies encounter obstacles due to the intrinsic cytotoxicity and aggregation of pristine MWCNTs, their hydrophobic nature and inadequate dispersibility in biological systems, and the continual demand for enhanced biocompatibility and targeting strategies. Recent studies on the functionalization of multi-walled carbon nanotubes (MWCNTs) indicate enhanced dispersibility, biocompatibility, and cellular uptake; however, several critical aspects remain inadequately addressed in the existing literature. Specifically, the field lacks: (i) long-term stability of the functionalized CNTs under *in vitro* or *in vivo* conditions, including protein corona formation and enzymatic degradation; (ii) quantitative, tissue- and cell-type-specific targeting efficiency with appropriate controls and *in vivo* biodistribution data; (iii) comprehensive toxicity assessments across acute and chronic exposures, and immunotoxicity. To go from proof-of-concept to clinically useful CNT-based delivery systems, it is important to fill in these gaps [1,2].

Recent research on gelatin- and GEMA-functionalized MWCNTs presents a unique amalgamation of covalent surface stabilization, bioactivity, and modular functionality, distinguishing it from traditional coatings such as PEGylation, polydopamine, and purely non-covalent wrappers. Gelatin makes biocompatible, bioactive surfaces with cell-adhesion motifs, and GEMA makes methacrylate handles that let CNT surfaces bond permanently with covalent bonds. This dual functionalization creates a surface that is stable in complex biological media, can be adjusted for crosslink density and degradation, and can be used to attach targeting ligands or therapeutic cargos later. All of these features work together to make the product more stable and easier to disperse, allow for controlled interactions with biological systems, and create a flexible platform for targeted delivery and theranostics, while still being able to improve safety and translational relevance through defined scalable processing. Previous studies on MWCNTs have consistently shown that they could be used to carry drugs, genes, and imaging agents in large amounts, but they have also raised serious concerns about their safety and biocompatibility. Initial research demonstrated that pristine or unaltered MWCNTs displayed inadequate aqueous dispersibility and induced oxidative stress, inflammatory responses, and membrane damage upon interaction with biological systems, primarily attributable to their hydrophobic graphitic surface and propensity for aggregation. Chemical oxidation to add carboxyl or hydroxyl groups made the MWCNTs a little easier to spread, but they still tended to clump together and were still toxic to cells at higher concentrations [3].

To tackle biocompatibility, later research utilized polymer or carbohydrate coatings to create hydrophilic shells encasing nanotubes. For instance, a developed lysinated MWCNTs functionalized with carbohydrates (galactose, mannose, and lactose) for targeted doxorubicin delivery to breast cancer cells [4]. These formulations enhanced drug loading and tumour selectivity through carbohydrate–lectin interactions; however, they necessitated intricate multistep syntheses and expensive coupling reagents, which constrained scalability. Li et al. [5] also talked about gelatin-functionalized CNTs loaded with cisplatin that could be released in response to changes in pH. However, their system used polydopamine-assisted surface grafting, which took a lot of time and used strong organic linkages, and it didn't give them much control over sugar-mediated targeting [6]. Both studies stressed that the field still needs better ways to make things that are easier to make, have a balanced hydrophilicity, and have a combination of bioactive (protein + carbohydrate) functionalization.

This work elucidates the synthesis methodology, assesses the metabolic activity of the modified cells, and monitors the intracellular localization of the nanotubes. Contemporary investigations reveal that multi-walled carbon nanotubes (MWCNTs) are progressively being functionalized with ligands, including sugars, peptides, and proteins, to augment their biocompatibility and targeting accuracy [7-12]. Sugars and carbohydrates function as specific ligands for receptor-mediated endocytosis. For example, the functionalization of MWCNTs with carbohydrates such as mannose or galactose enables the targeting of specific receptors that are overexpressed in certain cellular populations, thereby effectively serving as a "molecular handle" to enhance [13]. Untreated MWCNTs frequently demonstrate cytotoxic characteristics attributable to their hydrophobic properties and propensity for aggregation, potentially resulting in oxidative stress and damage to cellular membranes [14]. The application of hydrophilic biopolymers such as gelatin or other proteins forms a "corona" that improves stability within physiological environments and mitigates direct contact between the carbon surface and cellular membranes [15]. The functionalization of MWCNTs with carboxylic or amine groups prior to the application of polymeric coatings such as chitosan or gelatin has been demonstrated to enhance dispersibility while preserving a significant drug-loading capacity without compromising structural integrity. The functionalization of multi-walled carbon nanotubes (MWCNTs) constitutes a pivotal procedure in ameliorating their biocompatibility and attenuating inherent toxicity for biomedical implementations. In this inquiry, we present the successful surface modification of MWCNTs utilizing a dual-functionalization strategy that incorporates gelatin, a biocompatible protein, along with a monosaccharide derivative. Furthermore, CNTs, specifically MWCNTs, have emerged as transformative materials within the realm of nanotechnology due to their distinctive high aspect ratio, remarkable mechanical strength, and superior electrical conductivity. Within the biomedical sector, their capacity to traverse cell membranes renders them exceptional candidates for nanoneedles utilized in drug and gene delivery systems [16]. They possess the potential to function as delivery vehicles for natural compounds and gene therapies, thereby establishing their suitability for therapeutic applications in regenerative medicine [17-19]. Conversely, notwithstanding their promise, the clinical utilization of pristine MWCNTs is profoundly impeded by their hydrophobicity and tendency to aggregate in aqueous environments, culminating in pronounced cytotoxicity and asbestos-like inflammatory responses in biological tissues. To surmount these obstacles, it is imperative to undertake surface functionalization to enhance dispersibility and safeguard the carbon surface from direct cellular adversities [20]. They exhibited poor solubility, low wettability and dispersability in organic solvents. This major drawback is particularly relevant to their compatibility with biological systems. The chemical functionalization results in some effects like: attachment of different functional groups, and polymer grafting that enhance the biocompatibility, and conductivity. Acrylic monomers and polymers bearing a monosaccharide residue have interesting behaviour to be used as useful candidate for the biomedical applications. Some preliminary studies have showed that CNTs are biologically active to specific cells and organs under certain conditions [21]. Further research is required to demonstrate the cytotoxic mechanisms which are affected by the

different parameters. Therefore, the nanotoxicity of CNTs needs continuing and extensive work, this will be required before clinical used of CNTs as bionanodevices. As a denatured variant of collagen, gelatin exhibits biodegradability, lack of immunogenicity, and encompasses a plethora of functional groups (including amino and carboxyl moieties) that promote facile conjugation with the surface of CNTs [22]. It functions as a stabilizing "soft" layer that emulates the extracellular matrix. Carbohydrates are integral to the processes of biological recognition. By covalently linking monosaccharide derivatives (such as glucose or galactose) to the multi-walled carbon nanotubes (MWCNTs), these nanotubular structures may effectively target specific cell-surface lectins, thereby amplifying cellular uptake via receptor-mediated endocytosis. This research focuses on the preparation and characterization of MWCNTs functionalized with GEMA and coated with gelatin. By utilizing the SRB assay, the study aims to evaluate the potential in vitro cytotoxicity of these prepared materials against the MCF7 breast carcinoma cell line, contributing to the ongoing effort to establish safe bionanodevices for clinical use.

2. Materials and Methods

2.1 Materials

Multi-walled carbon nanotubes (MWCNTs), carbon content 95%, diameters 6-9 nm×5 μm obtained from Aldrich. 2 Glucosyloxyethyl methacrylate (GEMA), a monomer having a pendant glucose moiety, was supplied as an aqueous solution of 5 w/v % by Aldrich. Edible Gelatin, Mw~40000 (Gel) was purchased from Davis Gelatin NZ Limited, Christchurch, NZ. All chemicals and other reagents were used as received without further purification. The other ones were used as received from local resources.

2.2 Methods

2.2.1 Oxidation and Purification of MWCNTs

Pristine MWCNTs (300 mg) were dispersed in mixed concentrated sulphuric and nitric acids (3:1, v/v) at ratio of 50 mL acid mixture per 10 mg of CNTs. The resulted mixture was then refluxed at 110°C overnight with continuous stirring to produce oxidized mutli-walled carbon nanotubes (MWCNTs-COOH). The samples were washed with ultrapure water until the filtrate is neutral (pH 7.0). The collected solid was dried under vacuum at 70°C for 12 hr. The resulted materials denoted MWCNTs-COOH and kept for further modification and analysis [23].

2.2.2 Functionalization of MWCNTs using Michael Addition Reaction

100 mg of gelatin (Gel) was dissolved in 50 mL deionized water and 100 mg of GEMA dissolved in 20 ml of deionized water was added. Then, pre-sonicated 100 mg of oxidized MWCNT in 30 ml deionized water was added to above mixture, stirred for 48 hr at 50°C. Subsequently, the pH value of reaction mixture was adjusted to about 8 by adding saturated sodium biocarbonate solution [24]. The solution was poured into acetone to remove the unreacted reactant, filtrates to collect solid, dialyzed for 5 days and freeze-dried to obtain the different composites, as shown in (Table 1).

Table 1 *Chemical composition of the prepared composites*

	Chemical composition (w/w %)		
Sample code	MWCNTs	Gel	GEMA
Gel/GEMA	0	50	50
MWCNTs-I	50	0	50
MWCNTs-II	50	25	25

2.2.3 Characterization

The IR spectra were recorded on a FTIR Perkin-Elmer spectroscopy, under the following conditions: scan resolution: 4 cm^{-1}, scan rate: 2 mm/sec, number of scans: 32, range: 400- 4000 cm^{-1}, and mode: transmission. The particle forms and morphologies were studied using a Transmission Electron Microscope (TEM, JEM-1230 Tokyo, Japan). Drops of the diluted formulations were placed on a carbon-coated copper grid and allowed to dry at room temperature for 10 minutes before testing. SEM images were also captured using the JXA-840A Electron Probe Micro Analyzer JEOL-SEM, Japan. Before being studied with the Polaron SEM Coating Instrument, the substrate was mounted on metal stubs and coated with gold-palladium with a deposit thickness of around 75 at vacuum

710-2 millibar and 2.4 kV cathodic voltage. The crushed powder sample was subjected to strong X-rays of wavelength 1.54060 A° (CuK) in a 2θ range 4°- 80° at a scan rate of 2°/min and in step size [2°Th.] utilizing PAnalytica Diffractometer System Empyrean, Netherland. The (2θ) values were immediately recorded, and the relative intensities of the diffraction peaks were determined using X-ray diffraction spectra.

2.2.4 In Vitro Cytotoxicity Examination using SRB Assay

Potential cytotoxicity of the prepared nanomaterials was tested using the method of Skehan *et al.* [25] as follows: the breast carcinoma (MCF7) cell line was plated separately in 96-multiwell plate (10 cells/well) for 24 hr before treatment with the prepared nanomaterials to allow attachment of cell to the wall of the plate. Different concentrations of the prepared nanomaterials under investigation (0, 1, 2.5, 5 and 10) µg/mL were prepared for each individual dose. Monolayer cells were incubated with the prepared materials for 48 hr in 5% carbon dioxide atmosphere at 37°C. After 48 hr, cells were fixed, washed and stained with Sulpho-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and the prepared nanomaterials concentration is plotted to get the survival curves of the used cell lines.

2.2.5 Statistical Analysis

The data obtained were subjected to standard analysis of variance procedure according to Snedecore and Cochran (1980), where the $LSD_{0.05\%}$ values were obtained at $P \leq 0.05\%$. All values are the mean of three replicates.

3. Results and Discussion

3.1 Physicochemical Characterization

Figure 1 displays the FTIR spectra of pure gelatin and Gel/GEMA. This study is used to confirm the chemical alteration of gelatin. The chemical structures of pure gelatin (Gel) and synthesized materials were compared using FTIR. The Gel spectrum displays unaltered gelatin, which is distinguished by the characteristic amide bands associated with protein structures. The large peak about 3400 cm^{-1} is due to N-H stretching and hydrogen bonding. It also overlaps with O-H stretching caused by leftover moisture. The peak at 2850 cm^{-1} represents the symmetric stretching vibrations of C-H groups in amino acid side chains. The signal at 1660 cm^{-1} is mostly attributable to C=O stretching vibrations of the peptide bond [26].

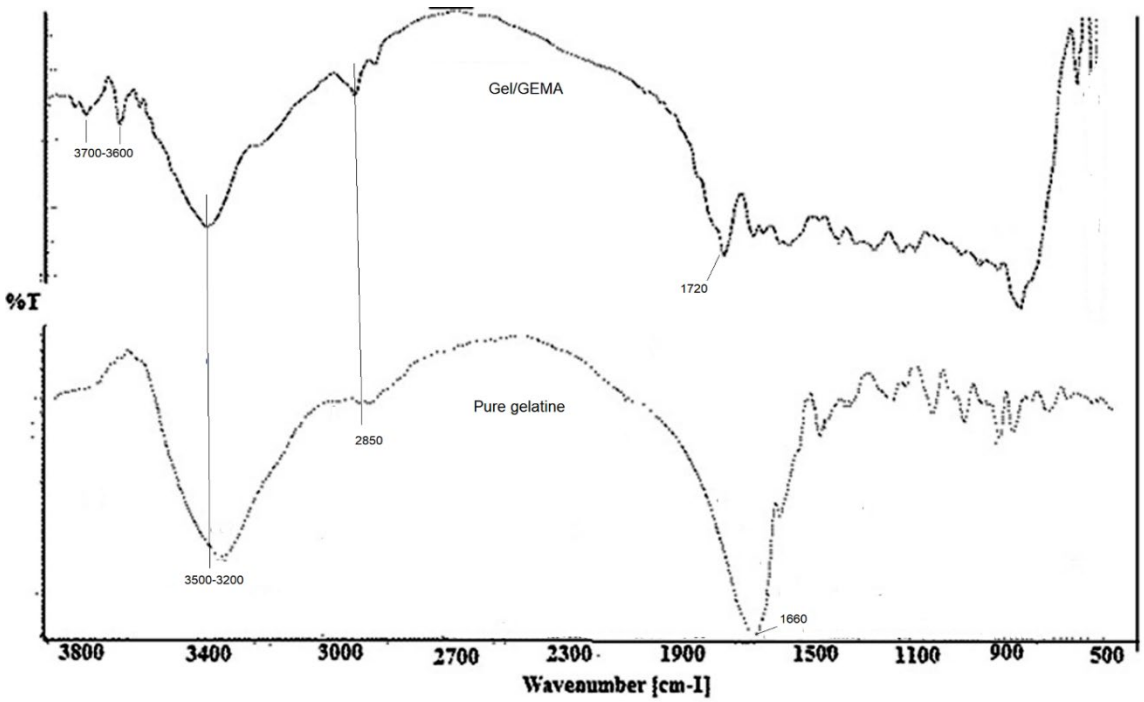


Fig. 1 *FTIR spectra of pure gelatin and Gel/GEMA materials*

J. of Sustainable Materials Processing and Management Vol. 6 No. 1 (2026) p. 73-83

In the spectrum for the Gel/GEMA material, there is a distinct absorption band in the 3400–3700 cm^{-1} This band is significantly different from that of pure gelatin, indicating that the Gel/GEMA composite possesses a high density of hydroxyl (O-H) groups. The increase in O-H groups is attributed to the presence of the pendant glucose moiety within the 2-glucosyloxyethyl methacrylate (GEMA) structure. While, In the pure gelatin spectrum, a distinctive peak appears at 1660 cm^{-1} which is assigned to the N-H bending vibration of the amino (-NH_2) groups. Notably, this specific absorption peak is absent (disappeared) in the Gel/GEMA spectrum. The disappearance of the amino group peak 1660 cm^{-1} and new one was appeared around 1720 cm^{-1} combined with the enhancement of the hydroxyl bands confirms a successful chemical reaction. Specifically, it demonstrates that the GEMA monomer reacted with the amino groups of the gelatin. The mechanism for this modification is identified as a Michael addition reaction, which effectively functionalizes the protein (gelatin) with the monosaccharide derivative (GEMA).

Figure 2 shows FTIR spectra of ox-MWCNTs, MWCNTs-I and MWCNT-II composites. It can be seen that in the spectrum, of ox-MWCNTs. the presence of oxygen-containing functional groups, specifically carboxylic groups (–COOH) at 1660 cm^{-1}, resulting from the oxidation process. In spectra of MWCNTs-I and MWCNTs-II, there is a prominent absorption bands in the 3400–3700 cm^{-1}. These bands correspond to O-H stretching vibrations, which are significantly more intense than in the pure ox-MWCNT spectrum. This increase confirms that the derivatives now possess numerous hydroxyl groups derived from the glucose moiety in the GEMA structure. On the other hand, a critical indicator of successful functionalization is the appearance of new peaks at 1720 cm^{-1} in both MWCNTs-I and II. These peaks are assigned to carbonyl ester bonds. The presence of these bonds proves that a new chemical bridge has formed between the ox-MWCNTs and the Gel/GEMA complex

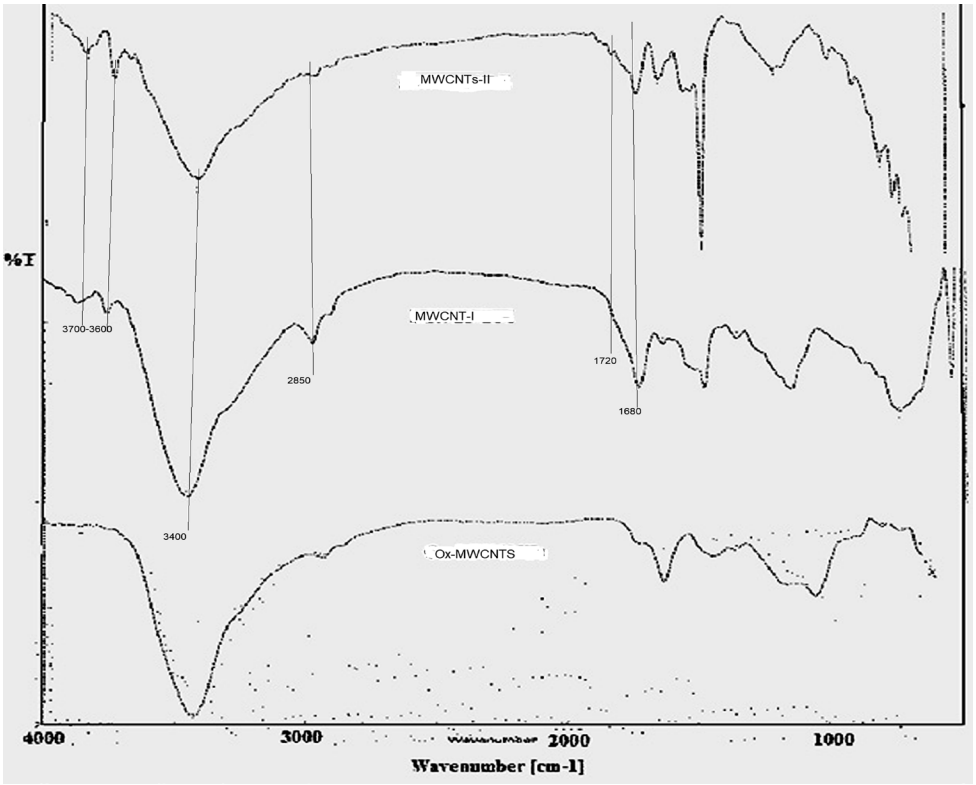


Fig. 2 *FTIR spectra of (a) Ox-MWCNTs; (b) MWCNTs-I; and (c) MWCNTs-II composites*

The study also uses XRD to understand how these chemical changes affect the physical structure of the nanotubes. Figure 3 shows XRD patterns of ox-MWCNTs and its derivatives I and II. As described in the previous works [27], in pristine MWCNTs, a significant diffraction peak appears at $2\theta = 26.1^{o}$ corresponds to (002) reflection planes or representing the interlayer spacing between graphite layers. In the derivatives (MWCNTs-I and II), this reflection peak either decreases in intensity or disappears entirely. This change indicates a loosening of the CNTs floss after treatment with GEMA and form less ordered MWCNTs. In other words, it suggests that the surfaces of the nanotubes have been successfully and completely coated with Gel and GEMA, leading to more amorphous surface structure. Furthermore, the 002 peak is broadening because of the finite number of layers and of the curvature of each tube. Besides, the average interlayer spacing decided from (002) peak is 3.5 and 3.43 Å in the case of ox MWCNTs and the prepared composites (MWCNTs-I and II), respectively. On the other hand, the broaden band (100) indicate the curved sheet of the ox-MWCNTs material [28], While, the disappearance of the

(100) peak and the appearance of new sharp peaks in the derivatives suggest that modifications have been introduced directly into the cylinder wall structure or have significantly lowered the packing density of the tubes.

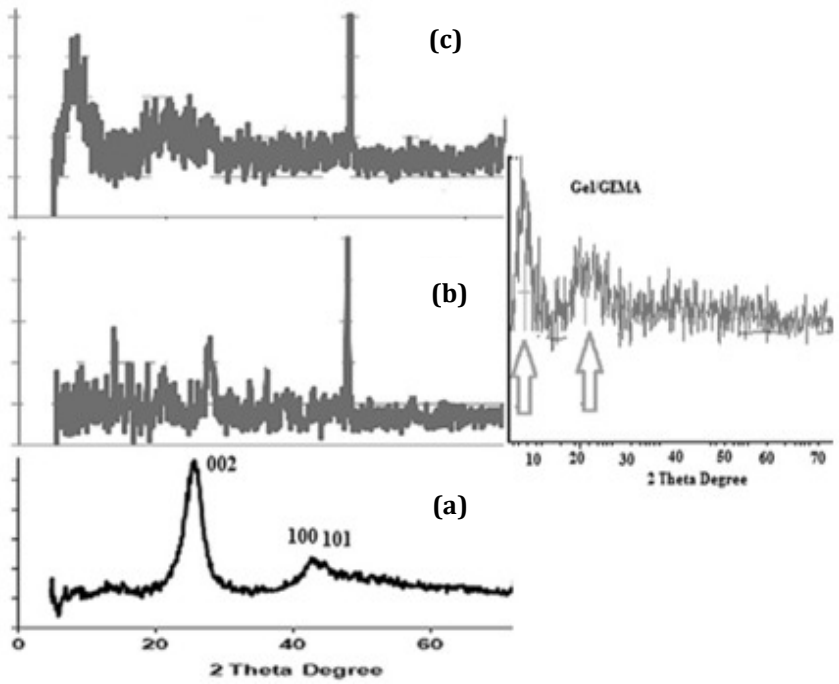
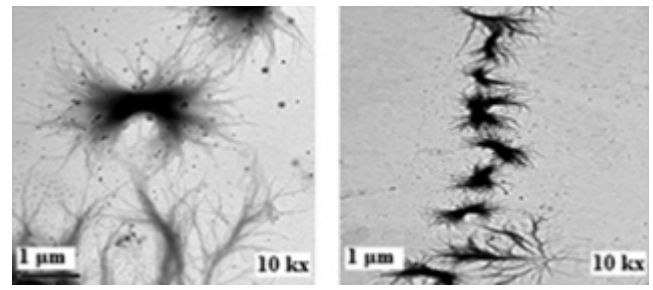
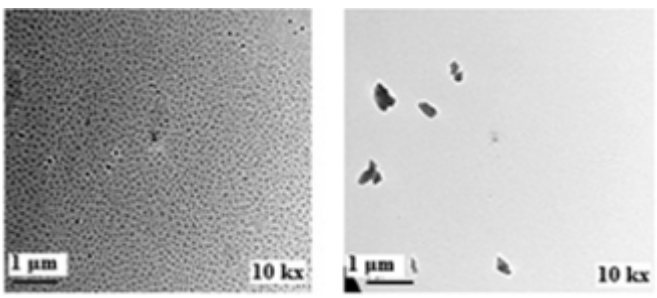


Fig. 3 *XRD patterns of (a) Ox-MWCNTs; (b) MWCNTs-I; and (c) MWCNTs-II composites*

The TEM analysis in Figure 4 provides a visual confirmation of the structural changes and how these hybrid materials behave in environments mimicking biological conditions. The Gel/GEMA composite (without nanotubes) exhibits spherical-like particles that tend to aggregate. This is typical for biopolymer complexes formed via Michael addition (Fig. 4a). In the MWCNTs-II composite, the nanotubes appear shorter, indicating they were cut during the oxidation process. The grafted polymers on the surface of the MWCNTs entangle with neighbouring tubes, creating a structure that resembles brush hair (Fig. 4b). After being placed in an aqueous medium for 24 h, the grafted and coated MWCNTs aggregate into a cluster form. This swelling behaviour is significant because it demonstrates how the material might interact with fluids inside the human body (Fig. 4c).

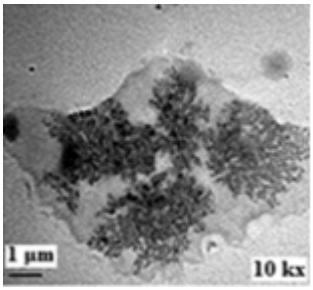


(a)



(b)

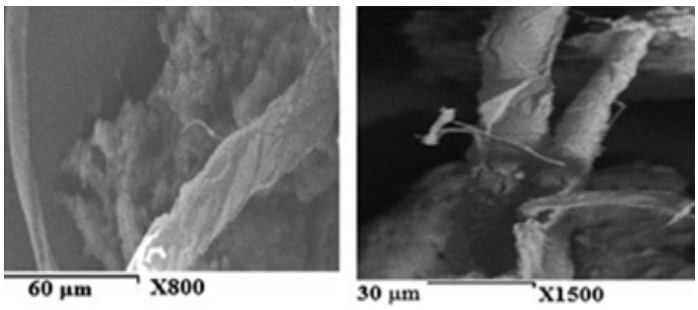
J. of Sustainable Materials Processing and Management Vol. 6 No. 1 (2026) p. 73-83



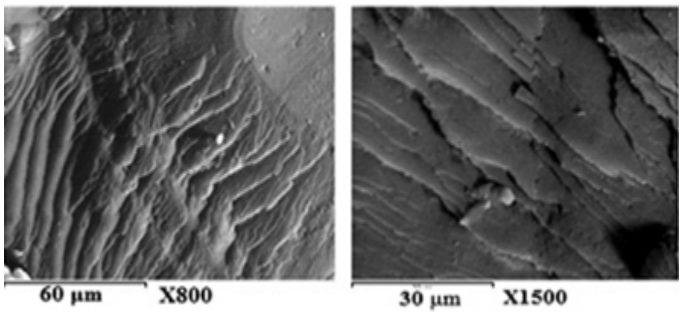
(c)

Fig. 4 *TEM images of the prepared composites (a) Gel/GEMA, and MWCNTs-II; (b) Before; and (c) After swelling in aqueous medium for 24 h*

To complement the internal view from TEM, Scanning Electron Microscopy (SEM) was used to look at the surface. Figure 5 shows SEM micrographs of MWCNTs derivatives I and II. The images show a clear increase in the diameter of the carbon tubes compared to pristine ones. This confirms that the GEMA grafting reaction successfully occurred across the entire surface of the nanotubes (Fig a). The tubes in this sample appear completely encapsulated. This confirms that the addition of gelatin creates a thick, protective coating around the GEMA-functionalized tubes (Fig 5 b). From FTIR, XRD and TEM morphologies data, it can be concluded that the grafting reaction with GEMA occurred over the entire surface of the MWCNTs (MWCNTs-I) and that they were completely encapsulated by gelatin in MWCNTs-II.



(a)



(b)

Fig. 5 *SEM micrographs of the composites (a) MWCNTs-I; and (b) MWCNTs-II*

Based on the data provided in Table 2, the differences between MWCNTs-I and MWCNTs-II highlight two distinct chemical strategies for modifying MWCNTs: direct surface grafting and multi-layer encapsulation. It can be concluded that the functionalization mechanisms can be carried out through two pathways. The first is covalent grafting, in which the GEMA is chemically bonded to the entire surface of the nanotubes. In polymer chemistry, this usually produces a polymer brush effect. The GEMA molecules are not just sitting on the surface; they are likely tethered via covalent bonds to functional sites on the MWCNTs, ensuring a robust interface between the inorganic tube and the organic polymer. While the other is adsorption and encapsulation ones. In this case, the gelatin acts as a primary coating layer that completely wraps the tubes before or alongside the GEMA. This

suggests a physical encapsulation or a core-shell structure, with the MWCNT as the core and the Gelatin/GEMA blend as the shell.

Table 2 *Differences in composite characteristics of MWCNTs-I and MWCNTs-II*

Feature	Differences between the prepared composites	
	MWCNTs-I	MWCNTs-II
Composition	50% MWCNTs + 50% GEMA	50% MWCNTs + 25% Gel + 25% GEMA
Surface coating	Grafting reaction with GEMA over the whole surface	Tubes are completely coated with a gelatin layer
Morphology (SEM)	Shows an increase in the diameter of grafted tubes.	Demonstrates full encapsulation by the biopolymer

3.2 In Vitro Cytotoxicity and Cellular Safety

The ultimate goal of these structural changes was to reduce toxicity. The SRB assay results shown in Figure 6 It was observed that both MWCNTs-I and II composites exhibited much lower toxicity toward MCF7 breast cancer cells than the standard drug Doxorubicin. In other words, while cell viability decreased as the concentration increased (5, 12, 25, and 50 µg/ml), the materials remained relatively non-toxic even at high doses. In other words, Doxorubicin kills more than 65% of cells at concentrations close to 50 µg/mL, but the MWCNT/GEMA and MWCNT/GEMA/Gel formulations kill much fewer cells.

A characteristic of MWCNT-based materials is the stabilization of cell death at around 35–40%. This plateau indicates a saturation threshold in cellular uptake or a controlled release mechanism, in contrast to the more linear toxicity observed with free Doxorubicin (Fig. 6 A). It can be concluded that as indicated in Fig. 6A, treatment of the cancer cells with various concentrations of the materials caused a dose-dependent decrease in cell viability relative to the control culture.

On the other hand, The IC_{50} values (the concentration required to kill 50% of the cells) were significantly higher than the standard, suggesting these materials are much safer for healthy cells (Fig 6 B). The high IC_{50} values of the composites relative to the standard drug suggest that these biopolymer-coated nanotubes are highly biocompatible, making them suitable candidates for future bionanodevice applications. In other words, both formulations exhibit much reduced cytotoxicity than pure Doxorubicin, with cell death plateauing at around 35-40%.

The plateauing effect indicates that these nanoparticle formulations may have a regulated release mechanism or a saturation point in cellular uptake when compared to the free medication. The fact that MWCNT formulations have higher IC_{50} values than free Dox is a favourable finding in drug delivery research [29, 46]. It implies that the carrier (MWCNTs/GEMA) masks the drug's toxicity, which may reduce side effects in healthy tissue.

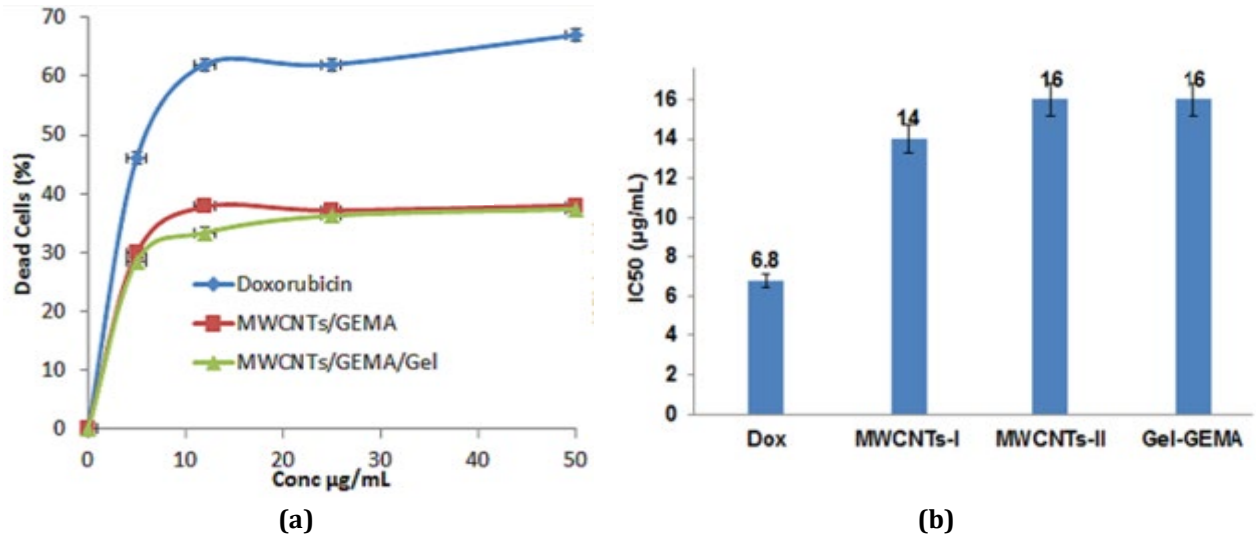


Fig. 6 *In vitro cytotoxicity study of the prepared materials using SRB assay (a) The parentage of the dead MFC7 cells at different concentrations; and (b) IC50 data profiles*

4. Conclusion

This research demonstrates the successful development of functionalized MWCNTs modified with GEMA and Gel. Spectroscopic analysis through FTIR and structural evaluation *via* XRD confirmed that the GEMA monomer and Gel reacted via a Michael addition reaction, effectively coating the surface of the nanotubes. Morphological assessments using SEM and TEM further validated the formation of these hybrid composites, showing a brush hair grafted structure and complete encapsulation of the tubes within the biopolymer matrix. Evidence from this study supports the conclusion that a two-pronged surface modification effectively transitions MWCNTs from toxic materials to biocompatible delivery platforms. By shielding the intrinsic toxicity of the carrier, these composites may offer a safer profile for healthy cells, establishing a foundation for their eventual use in clinical nanomedicine. Also, these results suggest that the combination of CNTs with biopolymers mitigates nanotoxicity, opening a promising new perspective for their use as bionanodevice in future biomedical applications.

Acknowledgement

The authors are thankful to National Research Centre, Giza, Egypt for supporting this work.

Conflict of Interest

Author declares that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The author confirms sole responsibility for the following: ***study conception and design***, ***data collection***, ***analysis and interpretation of results***, *and* ***manuscript preparation***.

References

[1] Thakur, C. K., Karthikeyan, C., Ashby Jr, C. R., Neupane, R., Singh, V., Babu, R. J., ... & Tiwari, A. K. (2024). Ligand-conjugated multiwalled carbon nanotubes for cancer targeted drug delivery. Frontiers in Pharmacology, 15, 1417399. https://doi.org/10.3389/fphar.2024.1417399

[2] Yang, M., & Zhang, M. (2019). Biodegradation of carbon nanotubes by macrophages. Frontiers in Materials, 6, 225. https://doi.org/10.3389/fmats.2019.00225

[3] Jain, A. K., Dubey, V., Mehra, N. K., Lodhi, N., Nahar, M., Mishra, D. K., & Jain, N. K. (2009). Carbohydrate-conjugated multiwalled carbon nanotubes: development and characterization. Nanomedicine: Nanotechnology, Biology and Medicine, 5(4), 432-442. https://doi.org/10.1016/j.nano.2009.03.001

[4] Thakur, C. K., Neupane, R., Karthikeyan, C., Ashby Jr, C. R., Babu, R. J., Boddu, S. H., ... & Moorthy, N. S. H. N. (2022). Lysinated multiwalled carbon nanotubes with carbohydrate ligands as an effective nanocarrier for targeted doxorubicin delivery to breast cancer cells. Molecules, 27(21), 7461. https://doi.org/10.3390/molecules27217461

[5] Li, R., Bao, Z., Wang, P., Deng, Y., Fan, J., Zhu, X., ... & Li, D. (2023). Gelatin-functionalized carbon nanotubes loaded with cisplatin for anti-cancer therapy. Polymers, 15(16), 3333. https://doi.org/10.3390/polym15163333

[6] Honmane, S. M., Charde, M. S., Salunkhe, S. S., Choudhari, P. B., & Nangare, S. N. (2022). Polydopamine surface-modified nanocarriers for improved anticancer activity: Current progress and future prospects. OpenNano, 7, 100059. https://doi.org/10.1016/j.onano.2022.100059

[7] Alam, F., Saikia, P., Kemisetti, D., Mandal, S., Rath, A. K., Ghosh, A., ... & Seksaria, S. (2025). Advancements in Carbon Nanotube-based Drug Delivery Systems: Innovations, Challenges, and Future Directions. Pharmaceutical Nanotechnology. https://doi.org/10.2174/0122117385379427250926093710

[8] Lohani, A. P., Elosta, M., Maksoud, M., & Murshid, N. (2025). Functionalized carbon nanotubes: emerging nanomaterials for enhanced cancer diagnosis and imaging. Molecules, 30(11), 2364. https://doi.org/10.3390/molecules30112364

[9] Haroun, A. A., Ayoob, F. A., & Masoud, R. A. (2023). Organic-inorganic composites based on multi-walled carbon nanotubes containing lysine/histidine. Egyptian Journal of Chemistry, 66(6), 499-510. https://doi.org/10.21608/ejchem.2023.212461.8004

[10] Haroun, A. A., Zaki, B. M., Shalash, M., & Morsy, R. A. (2019). Preparation and histological study of multi-walled carbon nanotubes bone graft in management of class II furcation defects in dogs. Open Access Macedonian Journal of Medical Sciences, 7(21), 3634. https://doi.org/10.3889/oamjms.2019.738

[11] Haroun, A. A., Ahmed, H. M., & Ahmed, E. F. (2019, August). Functionalized multi-walled carbon nanotubes as emerging carrier for biological applications. In Proceedings of the 5th World Congress on New Technologies. https://doi.org/10.11159/icnfa19.106

[12] Haroun, A. A., Ayoob, F. A., & Masoud, R. A. (2024). Sol-Gel Immobilization of Colistin Sulfate onto Double Walled Carbon Nanotubes: In Vitro Bone Bioactivity. Nano Biomedicine & Engineering, 16(3). http://doi.org/10.26599/NBE.2024.9290088

[13] Contreras-Torres, F. F., Salas-Trevino, D., Soto-Dominguez, A., & De Jesús García-Rivas, G. (2022). Carbon nanotubes in tumor-targeted chemotherapeutic formulations: A review of opportunities and challenges. ACS Applied Nano Materials, 5(7), 8649-8679. https://doi.org/10.1021/acsanm.2c01118

[14] AbouAitah, K., Abdelaziz, A. M., Higazy, I. M., Swiderska-Sroda, A., Hassan, A. M., Shaker, O. G., ... & Lojkowski, W. (2024). Functionalized carbon nanotubes for delivery of ferulic acid and diosgenin anticancer natural agents. ACS Applied Bio Materials, 7(2), 791-811. https://doi.org/10.1021/acsabm.3c00700

[15] Asghar, M. A., Yousuf, R. I., Shoaib, M. H., Asghar, M. A., & Mumtaz, N. (2021). A review on toxicity and challenges in transferability of surface-functionalized metallic nanoparticles from animal models to humans. Bio Integration, 2(2), 71. https://doi.org/10.15212/bioi-2020-0047

[16] Vardharajula, S., Ali, S. Z., Tiwari, P. M., Eroğlu, E., Vig, K., Dennis, V. A., & Singh, S. R. (2012). Functionalized carbon nanotubes: biomedical applications. International journal of nanomedicine, 5361-5374. https://doi.org/10.2147/IJN.S35832

[17] Saito, N., Haniu, H., Aoki, K., Nishimura, N., & Uemura, T. (2022). Future prospects for clinical applications of nanocarbons focusing on carbon nanotubes. Advanced Science, 9(24), 2201214. https://doi.org/10.1002/advs.202201214

[18] Liu, W., & Speranza, G. (2019). Functionalization of carbon nanomaterials for biomedical applications. C, 5(4), 72. https://doi.org/10.3390/c5040072

[19] Haroun, A. A., & Taie, H. A. (2014, March). Cytotoxicity and antioxidant activity of beta vulgaris extract released from grafted carbon nanotubes based nanocomposites. In Macromolecular Symposia (Vol. 337, No. 1, pp. 25-33). https://doi.org/10.1002/masy.201450303

[20] Yazdani, S., Biria, D., & Pazuki, G. (2025). Challenges and opportunities in the application of carbon nanotubes as membrane channels to improve mass transfer to cells. RSC advances, 15(30), 24624-24638. https://doi.org/10.1039/D5RA02939B

[21] Thirumalaisamy, R., Nimithap, S., Selvankumar, T., Manikandan, R., & Vadivel, P. (2026). Emerging Roles of Carbon Nanotubes in Cancer Therapy, Diagnosis and Targeted Drug Delivery: Current Insights and Toxicity Considerations. Chemical Physics Impact, 101003. https://doi.org/10.1016/j.chphi.2026.101003

[22] Yasmin, R., Shah, M., Khan, S. A., & Ali, R. (2017). Gelatin nanoparticles: A potential candidate for medical applications. Nanotechnology Reviews, 6(2), 191-207. https://doi.org/10.1515/ntrev-2016-0009

[23] Haroun, A. A., & Masoud, R. A. (2021). Preparation and characterization of oxidized multiwalled carbon nanotubes-immobilized Aspergillus sp. laccase hybrid materials. Int Res J Multidiscip Tech, 3, 83-92. https://doi.org/10.34256/irjmt21410

[24] Wang, K., Yin, R., Tong, Z., Yu, Q., & Nie, J. (2011). Synthesis and characterization of water-soluble glucosyloxyethyl acrylate modified chitosan. International journal of biological macromolecules, 48(5), 753-757. https://doi.org/10.1016/j.ijbiomac.2011.02.018

[25] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., ... & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute, 82(13), 1107-1112. https://doi.org/10.1093/jnci/82.13.1107

[26] Van Den Bulcke, A. I., Bogdanov, B., De Rooze, N., Schacht, E. H., Cornelissen, M., & Berghmans, H. (2000). Structural and rheological properties of methacrylamide modified gelatin hydrogels. Biomacromolecules, 1(1), 31-38. https://doi.org/10.1021/bm990017d

[27] Jiang, M., Wang, K., Kennedy, J. F., Nie, J., Yu, Q., & Ma, G. (2010). Preparation and characterization of water-soluble chitosan derivative by Michael addition reaction. International journal of biological macromolecules, 47(5), 696-699. https://doi.org/10.1016/j.ijbiomac.2010.09.002

[28] Ahmed, D. S., Haider, A. J., & Mohammad, M. R. (2013). Comparesion of functionalization of multi-walled carbon nanotubes treated by oil olive and nitric acid and their characterization. Energy Procedia, 36, 1111-1118. https://doi.org/10.1016/j.egypro.2013.07.126

[29] Kciuk, M., Gielecińska, A., Mujwar, S., Kołat, D., Kałuzińska-Kołat, Ż., Celik, I., & Kontek, R. (2023). Doxorubicin—an agent with multiple mechanisms of anticancer activity. Cells, 12(4), 659. https://doi.org/10.3390/cells12040659