

Fabrication, Characterisation, and Biocompatibility of Graphene-loaded Polymannose-Chitosan Scaffold for Potential Drug Screening Applications

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Abstract

Graphene-loaded polymannose-chitosan scaffolds have emerged as promising candidates for drug screening applications due to their unique properties. The combination of polymannose and chitosan, supplemented with graphene, offers a versatile platform with potential applications including drug screening. The aim of this study is to fabricate and characterize the biocompatible graphene oxide loaded polymannose chitosan scaffold (PM-Chi-GO) for potential drug screening applications. The scaffold was meticulously prepared by combining oxidized polymannose with chitosan hydrochloride and graphene oxide, employing gelation techniques. Characterization involved Fourier Transform Infrared Spectroscopy (FTIR) for functional group analysis and Scanning Electron Microscopy (SEM) for morphological studies. The biocompatibility of the scaffold was assessed using Peripheral Blood Mononuclear Cells (PBMCs). FTIR analysis revealed distinctive peaks at 3296, 1624, 1528, 1389, 1060, and 808 cm^{-1} , corresponding to specific functional groups within the scaffold. SEM displayed a porous morphological structure. Biocompatibility testing with PBMCs demonstrated favorable responses, confirming the scaffold's potential for in vitro drug screening applications. The synthesized PM-Chi-GO is characterized by its unique structural and biocompatible properties and holds significant promise for future drug screening endeavors. This study establishes a foundation for the utilization of this scaffold in drug screening applications.

Keywords: Chitosan, Drug Screening, Graphene Oxide, Novel Technique, Polymannose, Scaffold.

Introduction

Chitosan(Chi) is a lipopolymer chitin extracted naturally as a linear polysaccharide. It is derived from crustacean exoskeleton [1]. This material serves as a carrier for drug delivery in the form of micro and nanoparticles. Its method of preparation allows for a controlled and versatile profile in drug delivery. It is a composite micro capsule that has a natural positive charge in it which can be used for the lyophilization and Gelation process [2]. The main extract of chitosan from sources are traditional, lactic fermentation, and various enzymatic hydrolysis of crustacean bio waste. The extraction method contributes to

the development of structural analysis and Physio chemical properties [3,4].

The need for graphene oxide (GO) and Reduced Graphene oxide(rGO) in drug delivery, biomedical, biosensing, and diagnostic purposes has boomed a lot. They have been reported to play a major role in cytotoxicity, biodistribution, and biotransformation [5–7]. The 3D scaffold of GO and rGO will give an enhanced perspective of preclinical studies and trials in the medicinal field. It contains a range of reactive oxygen functional groups and is used for chemical processes and they are

electrically insulating materials due to their disrupted bonding networks [8,9].

Graphene possesses superior mechanical, electrical, thermal, and optical properties and is processed by micromechanical exfoliation of epitaxial growth and chemical vapour deposition. It is a cost-effective material with a high yield and has a highly hydrophilic, ability to form stable aqueous colloids to facilitate the assembly of a macroscopic structure by a simple and cheap process [5,10,11].

The preparation of highly complex and diverse scaffolds can be achieved through the utilization of natural products in various organic synthesis and structural aspects [12]. The 3D-based scaffold is made with an industrial filament for cell proliferation and spheroid formation, creating a boom in the possibilities of cellular and molecular biology. The microenvironment creates the cells that adhesion to the extracellular matrix, cell-to-cell interaction, cell growth, and cell differentiation. The development of drugs in today's world needs the validation of a wide range of preclinical tests and protocols that are costly and time-consuming. These require both cell and animal experiments. The biocompatible materials used are polylactic acid (PLA), polyglycolic acid(PGA), polycaprolactone(PCL), and polyethylene glycol(PEG) [13].

Polymannose(PM) is an average-weighted molecular compound of 30-53 kDa by polycondensation of mannose using phosphorous acid as catalyst and characterized using polarimetry, NMR [13,14].

Natural-origin polymers have been used as matrices due to their application of biocompatibility and biodegradability. Membranes, micro or nanoparticles, scaffolds, and hydrogels are biomimetic strategies for tissue engineering and processing [15–17]. Polymeric composites are obtained from renewable materials that can be either parallel renewable or total renewable origin. They are non-toxic and easily degradable, their

fabrication will help in better morphology and clinical studies [18–21].

This study aims to fabricate, characterize, and assess the biocompatibility of a scaffold composed of graphene-loaded polymannose-chitosan. The ultimate goal is to explore its potential applications in drug screening.

Materials and Methods

Polymannose Preparation

The preparation of polymannose involves subjecting mannose to phosphorous acid at 140°C under a vacuum. A Schiff base is formed through the interaction of the NH₂ (Amino) group and the CHO (Aldehyde) group. Subsequently, partial oxidation of polymannose is carried out in the presence of 20% Na₂IO₄ (Sodium meta periodate), followed by further dialysis and lyophilization.

Chi HCL Preparation

About 10g of chitosan was dissolved in 100 ml of 60% ethanol in hydrochloric acid (HCl) and continuously stirred for 6 hours. Subsequently, centrifugation was performed at 6000 rpm for 20 minutes to obtain a precipitate, which was then subjected to dialysis. The final product obtained is chitosan-HCl.

Preparation of PM-Chi- GO Scaffold

About 500 microliters of 5% chitosan hydrochloride acid (chi-HCL) were added to a 10% solution of oxidized polymannose with graphene oxide in PBS (Phosphate Buffered Saline) with a pH of 7.4. The scaffold was created employing the Gelation method.

The polymannose-chi and chi-PM loaded with GO underwent the lyophilization process. The characterization was conducted using SEM and FTIR, and the biocompatibility assay involved Flow cytometry.

Characterization Studies

The PM-Chi-GO scaffold's functional groups were examined through Fourier

transform infrared spectroscopy (FTIR) within the wavelength range of 3500 - 500 cm^{-1} . The size and structural morphology of PM-Chi-GO were analyzed using a Scanning Electron Microscope (SEM). Additionally, the hydrophilicity of the scaffolds was assessed by measuring the water contact angle.

Biocompatibility Assay

To assess the biocompatibility of the developed PM-Chi-GO, an annexin V PI assay was conducted. After obtaining approval from the institutional ethical committee, blood was collected from healthy donors. Equal volumes of blood were layered over histopaque and centrifuged in a gradient centrifuge at 2000 rpm for 30 minutes to isolate Peripheral Blood Mononuclear Cells (PBMCs). The buffy coat obtained post-centrifugation was cultured in RPMI media containing 10% Fetal Bovine Serum (FBS), 1% amino acid L-glutamine, and 1% Penstrep. Subsequently, the PM-Chi-GO scaffold is prepared by incubating approximately 2 mg of it in 1 ml of RPMI for 24 hours. About 200 microliters of the RPMI media were withdrawn from the scaffold, 100 μL of PBMCs were added and the incubation was carried out in triplicate for 12 hours. Cells without PM-Chi-GO served as the control. Following incubation, the cultured cells were collected and stained. The staining process involved adding 5 μL of Annexin V and 5 μL of propidium iodide, followed by incubation at room temperature for 15 minutes. Afterwards, 400 μL of 1X binding buffer was added to all tubes, and apoptosis was observed using BD

FACS Lyric flow cytometry. Result analysis was performed using FACSuite 4.1 software.

Statistical Analysis

Data from all experiments is obtained from all independent samples. All data were expressed as mean \pm standard deviation through SPSS 23.0 software. The positive test is used significantly. Differences that are considered were significant for $p < 0.05$

Result and Discussion

Figure 1 illustrates the PM-Chi-GO scaffold.

Characterization Studies

Figure 2 illustrates the FTIR spectrum of the PM-Chi-GO scaffold. Within the PM-Chi-GO scaffold, prominent peaks were observed at 3296, 1624, 1528, 1389, 1060, and 808 cm^{-1} . The peak at 3296 cm^{-1} is associated with O-H / C-H stretching, signifying the presence of alcohol and the alkyne group. The peak at 1624 cm^{-1} corresponds to C=C stretching, indicating the presence of an alkene group. The peak at 1528 cm^{-1} is attributed to N-O stretching, suggesting the presence of a nitro compound. The peak at 1389 cm^{-1} corresponds to C-H bending / O-H bending, indicating the presence of aldehyde and phenol groups. The peak at 1060 cm^{-1} corresponds to C-O stretching / S=O stretching, indicating the presence of primary alcohol and sulfoxide. The peak at 808 cm^{-1} corresponds to C-Cl stretching / C=C bending, indicating the presence of a halo compound and alkene group.

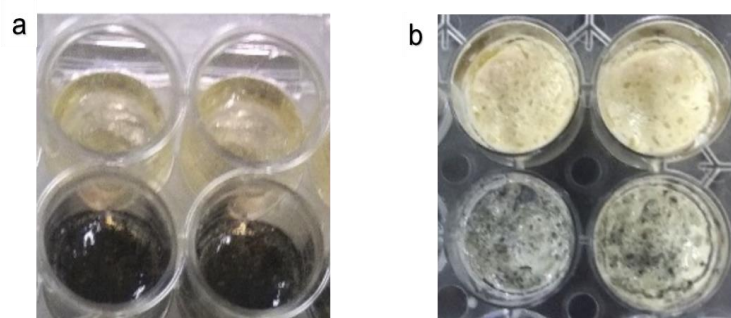


Figure 1. (a) PM-Chi-GO Hydrogel (b) PM-Chi-GO Lyophilized Scaffold

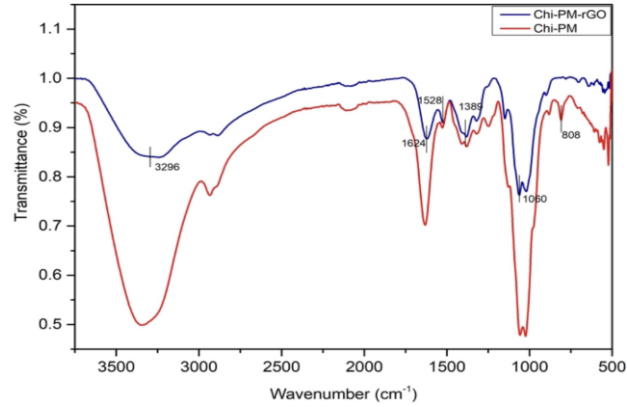


Figure 2. FTIR Spectra of PM-Chi-GO Scaffold

SEM analysis of the PM-Chi-GO scaffold revealed the porous morphology structure.

Figure 3 illustrates the SEM images of the scaffold.

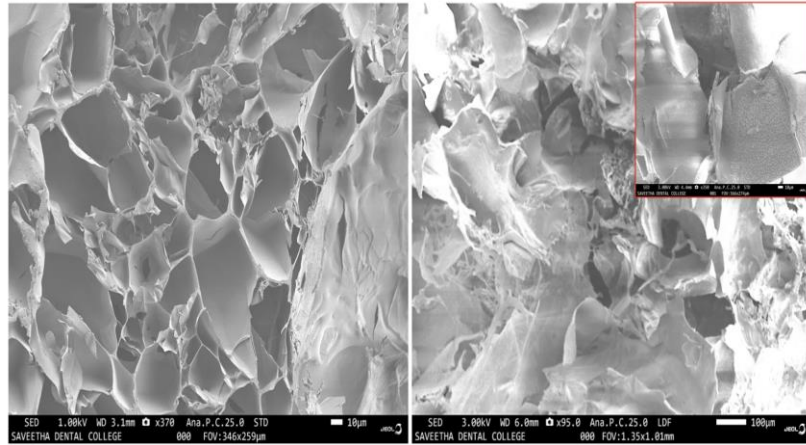


Figure 3. SEM of PM-chi-GO

The water contact angle measurement for the scaffold indicated an average angle of 38.36 degrees. This result implies that the scaffold exhibits moderate hydrophilicity, as the water droplet makes a contact angle of approximately 38.36 degrees on the surface. A

lower contact angle is generally associated with higher hydrophilicity, suggesting that the scaffold has a tendency to interact favorably with water. Figure 4 depicts the water contact angle of PM-chi-GO.

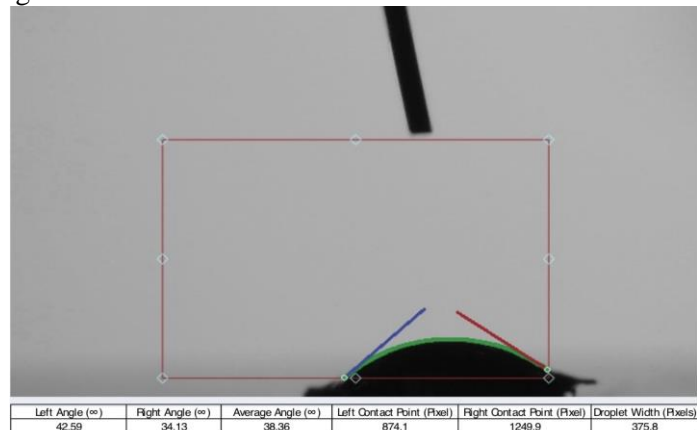


Figure 4. The Water Contact Angle of PM-chi-GO

Figure 5 illustrates the results obtained from the Annexin V PI assay conducted on the PM-Chi-GO scaffold. In the graph, Annexin V is plotted on the X-axis, while PI is plotted on the Y-axis. The Lower Left (LL) quadrant corresponds to viable cells, the Lower Right (LR) quadrant indicates cells in early apoptosis, the Upper Right (UR) quadrant represents cells in late apoptosis, and the Upper Left (UL) quadrant denotes cells in necrosis.

In this study, the analysis revealed that 81.38% of cells were viable, characterized by negative staining for both annexin V and PI.

Cells in early apoptosis constituted 18.47%, where annexin V was positive, and PI was negative. A minimal 0.15% of cells were in late apoptosis, displaying positive staining for both annexin V and PI. Notably, no cells were found to undergo necrosis. These findings were deemed significant when compared to the control group.

From the figure, it was evident that the PM-Chi-GO scaffold has good biocompatibility as only a small fraction is identified in early apoptosis cells. This indicates that minimal cell death and low cytotoxicity.

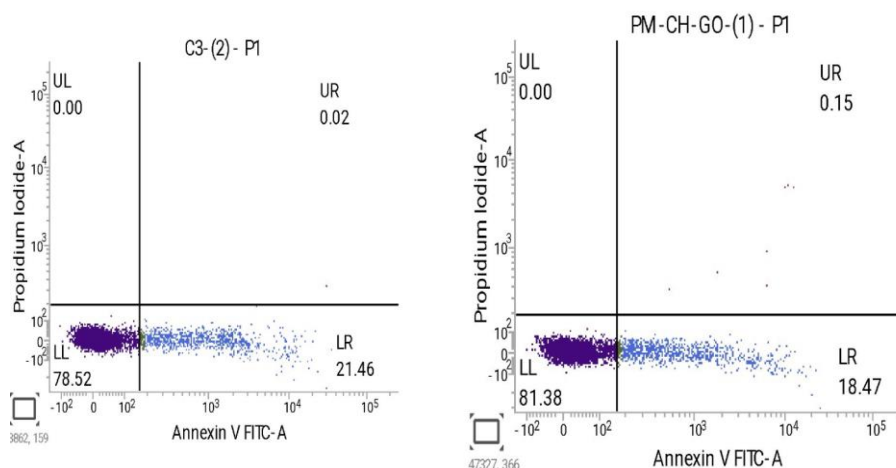


Figure 5. Annexin V-PI Assay of the PM-Chi-GO Scaffold

Discussion

Chitosan not only offers a unique combination of anti-bacterial properties, biocompatibility, and biodegradability but can also be easily fabricated into vesicles to control the release rate of drugs or improve the bioavailability of degradable materials. Biocompatibility is defined by the types of reactive elicited by a biomaterial intended for normal cellular activity [22]. These active groups make graphene oxide an excellent candidate for use as a drug carrier because they make it easy to immobilize molecules on its surface. Graphene oxide has good water and bio medium dispensability as well as good optical(absorption) and photothermal(conversion)properties [23].

These active groups are ideal for molecule immobilization on the graphene oxide surface and make it a powerful candidate as a drug carrier.

We have fabricated a Graphene oxide-loaded Polymannose-chi scaffold through Gelation. The presence of (-CHO) and amino group(-NH₂) group induce the Gelation. SEM results of the graphene oxide-loaded PM-chitosan scaffold showing the porous morphology[24]. Privileged structures are molecular scaffolds that, by the alteration of functional groups, can be used to produce effective and precise ligands for a variety of different biological targets. Furthermore, favored structures frequently have favorable drug-like characteristics, which generate additional drug-like compound libraries and

leads. The net result is the production of high-quality leads that provide a solid foundation for further development. [25]. The flow cytometry-based apoptotic assay revealed the biocompatibility of the PM-Chi-GO scaffold. The FTIR revealed the functional groups present in the PM-Chi-GO scaffold [26-29]. The PM-chi scaffold loaded with Graphene oxide is identified as biocompatible and non-toxic. In the future scaffold may be used as a substrate for 3-D cell culture and may potentially be used as a drug screening platform.

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Conclusion

In this study, we successfully fabricated the PM-Chi-GO scaffold, employing FTIR and SEM for characterization. The synthesized scaffold demonstrated biocompatibility with PBMCs. This scaffold holds promise as a substrate for 3D cell culture in future applications.

Conflicts of Interest

None

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