



Combined effect of cellulose nanocrystal and reduced graphene oxide into poly-lactic acid matrix nanocomposite as a scaffold and its anti-bacterial activity



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ABSTRACT

In the present study, cellulose nanocrystals (CNCs) and reduced graphene oxide (rGO) were successfully synthesized via acid hydrolysis and modified Hummer's method, respectively. Further, the synthesized CNCs and rGO were incorporated into poly-lactic acid (PLA) matrix using solution casting method utilizing different weight (wt.) % of CNCs (nanofiller) and rGO. The successful synthesis of various nanoformulations were confirmed by several characterization techniques including Transmission Electron Microscopy (TEM), Field-Emission Scanning Electron Microscopy (FE-SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis. Hydrophilicity measurement of the film was done by wettability analysis. The mechanical property evaluation of scaffold showed considerable increased tensile strength of PLA/CNC/rGO nanocomposite upto 23%, with increase in elongation at break (ϵ_b) indicating the ductile behavior of nanocomposite as compare to pristine PLA. The distinct anti-bacterial efficacy of PLA/CNC/rGO nanocomposite film was found against both Gram positive *Staphylococcus aureus* (*S. aureus*) and Gram negative *Escherichia coli* (*E. coli*) bacterial strains respectively. Furthermore the *in-vitro* cell based cytotoxicity assay showed negligible cytotoxicity of fibroblast cell line (NIH-3T3) upon treatment with nanocomposite film. Therefore, the as fabricated nanocomposite film possesses considerable potential in biomedical as well as in food packaging applications.

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1. Introduction

In recent years, biodegradable materials have fascinated substantial research interest because of having great capability for fabricating green/environment friendly composites for industrial applications which are low in cost as compared to conventional composites [1,2]. Biodegradable materials have decreased the problems of disposability of synthetic materials which is having low impact on environment because they are biodegradable and are derived from renewable resources [3]. PLA is obtained from L and D-lactic acid, which can be extracted from fermentation of renewable resources like corn, starch and potato and is nowadays, becoming more and more favored biodegradable polymer having great potential to produce high value products at low price [4,5]. Due to its high mechanical strength, easy

processability and biocompatibility in comparison to other polymers, it has been extensively used in barrier, packaging, biomedical and tissue engineering applications [6]. Nonetheless, there are still some drawbacks with this polyester like relatively low barrier to oxygen, water vapor, excessive brittleness and low resistance. Nanocomposite is a new innovative and promising field, in which nanofillers are added into the polymer matrix to escalate the ductile properties of some of the biopolymers which are used in those applications where flexibility is an essential issue such as films [10]. Although, many works have been already reported on the development of hybrid PLA composites [7–9]. The reinforcement of nanofillers such as CNCs and rGO has been recommended to significantly enhance the properties of PLA. The outcome of different studies shows that the performance of CNC composites in terms of barrier, packaging and bio-medical applications has been improved considerably [10,11]. The so-called cellulose nanocrystals (CNCs) or cellulose nanowhiskers can be obtained by acid hydrolysis of microcrystalline cellulose (commercially available used for the separation of microfibrils); the microfibrils undergoes transverse cleavage resulting in separation of amorphous and

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crystalline regions [12–14]. High specific strength, biocompatibility, biodegradability, low weight, abundance, barrier properties and reinforcing capability make CNCs an ideal contender for manufacturing polymer nanocomposites [15–18]. Nowadays, CNCs with PLA nanocomposites have been extensively studied and developed as a high performance materials for bio-medical applications via solution casting method [19,20]. One of the main complications that come across using unmodified CNCs is the high hydrophilicity of CNCs used as reinforcing agent which creates difficulty for the dispersion in non-polar media and limited its commercial viability as scaffold in tissue engineering. To solve this issue, the familiar alternatives are surface chemical modifications or to use surfactants [15], which are capable to coat the nanocrystals [21] and avoid the agglomeration of CNCs. Although, the modification of nanocrystals may bring or complicate further issues related to altering their bio-based and biodegradability character, price, and life cycle analysis when, for instance, used in food packaging. However, Solvent exchange method is one of the feasible solution to get well dispersed CNC within the PLA matrix. Another strategy followed in previous works consisted the use of unmodified CNCs in partially hydrated state instead of freeze-dried (because of the strong hydrogen bonding and the self-assembly property, it becomes difficult to again disperse unmodified CNC in the latter case) [22]. To get well dispersion of nanofiller into the polymer matrix one can also use the additionally functionalized silylated cellulose nanocrystals (SCNC) as a substitute that will result into increase of tensile modulus and tensile strength by 20% as compared to pure PLA [23,24]. One of the innovative research was reported for the PLA composites with CNC, which included multifunctional nanocomposites that are used for the packaging applications [25]. In order to make the nanocomposites multifunctional and antimicrobial (necessary for the materials used in food-packaging applications), silver nanoparticles (Ag) were incorporated with the surfactant modified (s-CNC) into the PLA composites [26]. Efforts were made to get the dispersion of CNC into PLA matrix in which the hydrated state of CNCs were freeze dried and dispersion of CNC in organic solvent was optimized [27]. Subsequently, another strategy i.e. use of one more nanofiller into the PLA matrix was explored.

In the present work to make nanocomposite multifunctional another nanofiller i.e. rGO was added into the polymer matrix which is the novelty of the research. It has already been proven that rGO has excellent biomedical properties [29,30]. For the better compatibility between nanofiller and polymer matrix, the freeze dried CNC, rGO and PLA were predisposed in chloroform (described in experimental Section 2.4) then added into each other by solution casting method. Nanocomposite films with different percentage of CNC and rGO having optimal mechanical, thermal, antimicrobial and biomedical properties were developed and characterized. Furthermore, films with different percentage of rGO were analyzed in order to evaluate the cytocompatibility on fibroblast cells. The antibacterial response of nanocomposites on Gram positive *Staphylococcus aureus* (*S.aureus*) and Gram negative *Escherichia coli* (*E.coli*) bacteria was also studied in this research.

More recently, a study only related to mechanical properties based on the addition of CNC nanofillers (0.1–0.4 wt.%) and graphene oxide (GO) (0.1–0.6 wt.%) individually into the PLA nanocomposites films prepared via solution casting method was demonstrated [28]. In that study, it was investigated that a small amount of CNCs and GO are required for the increment in mechanical properties of PLA nanocomposites but no other information regarding the preparation of nanocomposites (PLA/CNC/GO) and its properties like barrier, packaging and bio-medical properties were presented or demonstrated. Thus, the main aim of this work was to develop a nanocomposite film with CNC and rGO as a reinforcing nanofiller incorporated into PLA (polymer matrix) via solution

casting method and to explore its potential in packaging, barrier and medical applications.

2. Experimental section

2.1. Materials

Microcrystalline cellulose (MCC) with diameters of ca. 10 to 15 μm , sulphuric acid (H_2SO_4) with 98% purity, chloroform (CHCl_3) with A.R. grade, acetone A.R. and Agar–agar (culture media) were procured from Himedia Laboratory, Ltd. (Mumbai, India). Graphite powder with 98.5% purity was purchased from S K carbon Ltd. Faridabad, India. Hydrogen peroxide (H_2O_2) was provided by Loba Chemi Pvt. Ltd. Hydrochloric acid (HCl) of 35.4% purity, Perchloric acid (H_3PO_4) of 88% purity, Nitric acid (HNO_3) of (69–72%) purity, sodium hydroxide (NaOH) and Potassium permanganate (KMnO_4) of 99% purity were all purchased from Rankem RFCL Ltd., New Delhi, India. Poly lactic acid (with M_w of 150,000 D), an ion exchange resin (Dowex Marathon Mr 3 Hydrogen and hydroxide form) and hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$) were supplied by Sigma Aldrich, USA. PLA pellets were dehydrated in a vacuum oven at 98 °C for 3 h prior to use. The fibroblast cell line NIH-3T3 was procured from National Centre for Cell Science (NCCS), Pune, India. The Dulbecco's modified Eagle's medium (DMEM) along with 10% FBS (fetal bovine serum) and supplemented with 1% penicillin–streptomycin was procured from Sigma Aldrich, USA. The cell staining dyes rhodamine B (Rho B) and Hoechst 33342 were purchased from Life Technologies. Luria-Bertani (LB) and Nutrient broth (NB) medium were obtained from Merck India. All the reagents were of high quality and used without any further purification.

2.2. Synthesis of CNC

Cellulose nanocrystals (CNCs) were synthesized by following the previously reported method used by Cranston and Gray [30]. Initially, 10 g of MCC powder suspension was prepared by adding 100 mL of deionized water into it. Hydrolysis was carried out by the dropwise addition of sulphuric acid (64–65 (wt/wt%)) of 175 mL at 45 °C under constant stirring. The stirring was continued for 3 h. Immediately, after the hydrolysis process the suspension was diluted with 10- fold deionized cold water (4 °C) for quenching of the reaction. After 3–4 h, the suspension was centrifuged at 5000 rpm for 15 min to remove the acid and to concentrate the cellulose suspension. The precipitate obtained was rinsed and recentrifuged at 5000 rpm with deionized water until the pH became neutral. The precipitate was then dialyzed using dialysis membrane (Himedia cellulose dialysis membranes with 12–14 kDa molecular weight cut off) against deionized water for 4 days till the neutral pH was maintained. Dowex Marathon MR 3 Hydrogen and hydroxide form (Sigma), an ion exchange resin was added to the cellulose suspension for 48 h and it was removed by filtering the suspension through hardened ashless filter paper (Whatmann 541). An ultrasonic treatment at 60% output for 15 min in an ice bath through tip sonicator (Vibracell, 750) was repeatedly done on the suspension to obtain the nanocrystals. The resultant aqueous cellulose suspension was ca. 1% by weight and of ca. 20% yield. The CNC suspension was neutralized by the addition of 1% (v/v) of 0.25 mol l^{-1} NaOH [31]. The CNC suspension was then lyophilized. The freeze dried CNC obtained was stored in a refrigerator for further use.

2.3. Synthesis of rGO

The graphene oxide (GO) was prepared by the well-known Modified Hummer's method reported by Vinay and Chhatrie [32]. The rGO was synthesized by chemical reducing GO with the aid

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