

Chitosan Mediated Polymer Nano Composites Scaffold Synthesis for Tissue Engineering in Health Sciences

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Abstract— In this recent paper, polymer nanocomposites have received considerable interest because of their superior thermal and mechanical properties, as compared with the polymer itself. Biocompatible and biodegradable synthetic polymeric materials have been used by researchers to develop biological scaffolds. In recent years, significant development has been given to Chitosan composites for Tissue Engineering applications. In this study, preparing a Nanocomposite we have used chitosan with varying weight of Multiwalled Carbon Nanotubes (MWCNTs) and we synthesized scaffold by simple conventional technique for better affordability and cost effective yet qualitatively too. Carbon Nanotubes are electrically conductive, chemically and thermally stable, and exceptionally strong. Given this unique combination of properties there has been much interest in carbon nanotubes, and finding applications for them. One application where this combination of properties proves useful in the area of tissue regeneration, incorporating carbon nanotubes into scaffolds for tissue engineering. A combination of Fourier Transform Infrared Spectroscopy, X-Ray diffraction analysis, Scanning Electron Microscopy and Transmission Electron Microscopy results indicated that the MWCNTs was uniformly dispersed in chitosan matrix and there was a chemical interaction between chitosan and MWCNTs. Composite scaffold materials have greater cell proliferation, protein content, alkaline phosphatase, mineralization as compared to chitosan scaffold. Moreover, addition of the synthetic polymer Polycaprolactone in chitosan/MWCNTs lead to improve stabilized Scaffold product.

Key words: Chitosan, Multiwalled Carbon Nanotubes (MWCNTs), Polycaprolactone (PCL) Fourier transmission infrared spectroscopy (FTIR), X-ray Diffraction (XRD). Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM)

I. INTRODUCTION

Nanotechnology in Tissue engineering, an important emerging topic in biomedical engineering, has shown tremendous promise in creating biological alternatives for harvested tissues, implants, and prostheses [1]. Tissue engineering may be defined as the application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissues using biomaterials, cells, and factors alone or in combination [2]. Scaffold is one of the key components in the tissue engineering paradigm in which it can function as a template to allow new tissue growth and also provide temporary support while serving as a delivery vehicle for cells and/or bioactive molecules structure [3, 4]. The scaffold should be porous and permeable to permit the ingress of cells and nutrients and should exhibit the appropriate surface structure and chemistry for cell attachment. Ideal scaffold should

possess a suitable combination of physical properties to match those of the replaced tissue with good biocompatibility. Various synthetic alternatives such as metal, alloys, ceramics, polymers, and biocomposites have been researched as scaffold for bioapplications. Among those scaffold biomaterials, the polymers and polymer composites occupy significant position. Polymer materials are playing an increasingly important part in a diverse range of applications; polymer systems can be used for a variety of applications, such as drug delivery, diagnostics, tissue engineering and “smart” optical systems, switching surfaces and adhesives, and protective coatings that adapt to the environment, as well as biosensors, microelectromechanical systems, coatings, and textiles [5]. Nowadays, synthetic degradable polymers, such as Polycaprolactone (PCL) (fig-1), polyglycolic acid, polyvinyl alcohol (PVA), and polylactic acid (PLA), have been evaluated extensively as scaffold biomaterials [6]. However, the results of research show that their mechanical properties and biocompatibility are unsatisfactory for the tissue engineering of load-bearing bone. Several strategies for improving the mechanical properties (compression and flexural) of polymeric scaffolds have been reported, with a focus towards developing nanomaterials reinforced polymeric composites [6].

Nanomaterials, which are materials with basic structural units, grains, particles, fibers, or other constituent components smaller than 100 nm in at least one dimension, have evoked a great amount of attention [7]. Nanomaterials have special mechanical, electrical magnetic, optical, chemical, and other biological properties because of their high aspect ratio and surface area. Among nanomaterials, the nanotube material attracted wide attention of researchers. Nanotubes, with structures that resemble tiny drinking straws, large inner volumes can be filled with sundry chemicals and biomolecules, ranging in size from small molecules to proteins [8, 9]. After carbon nanotubes (CNTs) were discovered by Iijima in 1991, noncarbonic nanotubes were manufactured by different routes: in 2000, Rothschild et al. discovered Tungsten disulfide nanotubes (WSNTs) by vapor-solid growth method; Nath et al. composited MS_2 nanotube in 2001 by vapor phase method; Kinenkamp et al. synthesised ZnS nanotube by restoring sulfurization process [10–12]. Then a variety of noncarbonic nanotubes has been synthesized, such as boron nitride nanotubes (BNNTs) [13], Bi_2S_3 nanotube [14], NbS_2 nanotube [15], $NbSe_2$ nanotube [16], AlN nanotube [17], GaN nanotube [18], InP nanotube [19], SiO_2 nanotube [20], and ZnO nanotube [21]. CNTs, one of the most concerned nanomaterials, with unique electrical, mechanical, and surface properties, such as high aspect ratio, high strength-to-weight ratio, extraordinary mechanical properties (their axial elastic modulus and tensile strength were theoretically predicted to be as high as 1-2 TPa and 200 GPa, resp.), have held great interest with respect to biomaterials, particularly those to be positioned in

contact with bone such as prostheses for arthroplasty, plates or screws for fracture fixation, drug delivery systems, and scaffolding for bone regeneration, whose outstanding properties have sparked an abundance of research [22–31]. In recent years, some reports have showed that functionalized CNTs even can improve cell compatibility of matrix material, promote tissue regeneration, and inhibit the formation of glial scar and fibrous tissue [32, 33]. These results suggest that CNTs might hold great promise for synthesizing new kinds of multifunctional nanocomposites in biomedical applications and might be used as reinforcements to improve biological properties of polymer.

Chitin, a natural polymer, is the second most abundant organic resource on the earth next to cellulose [34]. It is an exoskeleton of crustacean, cuticle of insects, and cell wall of fungi. An estimated billion tons of chitins are synthesized every year in nature. Although chitin is structurally similar to cellulose, much less attention has been paid to chitin than cellulose, primarily due to its inertness. Therefore, it has remained an almost unutilized resource. Deacetylation of chitin yields chitosan, which is relatively reactive and can be produced in numerous forms, such as powder, paste, film, fiber, and more [36]. Several biomedical applications of chitosan have already been reported [34–38]. It has the potential to be used as artificial kidney membrane, absorbable sutures, hypocholesterolemic agents, drug delivery systems, and supports for immobilized enzymes. Besides, it has been claimed to accelerate the wound-healing process. Chitosan has some advantages due to its nontoxicity and biodegradability without damaging the environment. It is a biocompatible material that breaks down slowly to harmless products (amino sugars) that are absorbed completely in body [39]. Chitosan is a unique basic polysaccharide and generally is represented as a homopolymer. However, the deacetylation process is rarely complete and most commercial and laboratory products tend to be a copolymer of N-acetylglucosamine (NAG) and N-glucosamine repeat units. The ratio of two repeating units depends on the source and preparation of chitosan, but the glucosamine units predominate. The structure of chitosan is similar to that of cellulose, except at carbon-2, where the hydroxy group of cellulose is replaced by an amino group. Although the β-(1 → 4) anhydroglucosidic bond of chitosan is also present in cellulose, the characteristic properties are not shared by cellulose [40]. Figure 2. presents the structural similarities of chitin, chitosan, and cellulose.

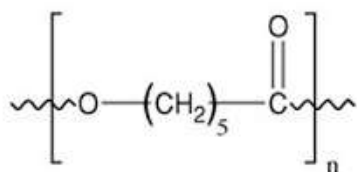


Fig. 1: Polycaprolactone (PCL) a synthetic Polymer

In this paper, we will present processing of polymer composites reinforced by Carbonnanotubes that potentially could be used as scaffolds in tissue engineering.

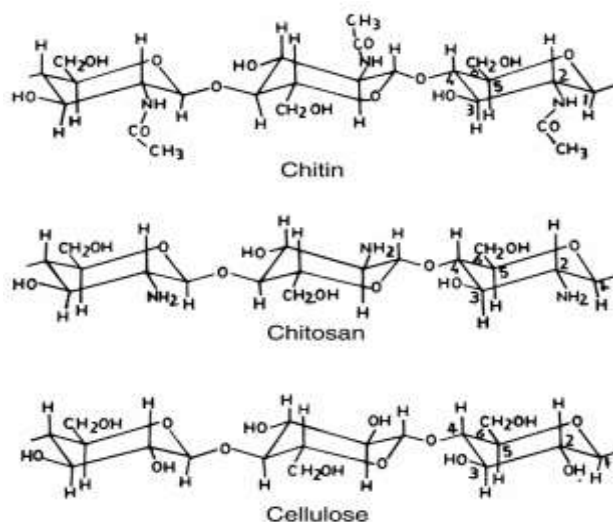


Fig. 2: Structure of of chitin, chitosan, and cellulose

II. MATERIALS

Multi-walled CNTs (90% purified) (MWCNTSs) was purchased from Cheap Tubes (USA, 10–20 nm diameter. Chitosan (CS) (Degree of Deacetylation = 95% determined by ¹H-NMR and Molecular Weight 13.45 × 10⁴ Da) was purchased from India Sea Foods, Kerela, India. Polycaprolactone(PCL) Polymer was purchased from Shenzhen Esun Industrial Co.Ltd. Acetic acid, Formic acid, Citric acid and other chemicals were used as analytical grade and purchased from Sigma–Aldrich Company.

III. METHODS

Initially, 10 wt% Polycaprolactone (PCL) solution had prepared .With Glacial Acetic acid by 2% mixed mixture was stirred for 30 minutes at room temperature. Then Required amount of Chitosan powder was weighed and mixed to the PCL solution prepared before.It was stirred till it became Pellucid for nearly 2 hours .One control too was prepared with no Chitosan added to it.For making a Scaffold which is a porous like substance Sodium Hydroxide NaOH was prepared with 20wt% . Then this NaOH solution was poured slowly into the blends of Chitosan and PCL(Polycaprolactone) .Slowly Spongy like porous structures were started forming on the addition of NaOH which subsequently formed to Scaffold like structures .Then with Vaccum distillation technique whole solution was distilled and the product isolated was dried in Hot air oven and Scaffold samples collected which was composed of Chitosan-Polycaprolactone

Making it Nanocomposite traces of computed Carbon Nanotubes (1%-3%) specifically Mutiwallled Carbon Nanotubes(MWCNTSs) were added to the solution of PCL and Chitosan. Then the NaOH solution was poured slowly into the blends of Chitosan and PCL(Polycaprolactone) . In same way slowly Spongy like poruos structures were started forming on the subsequent addition of NaOH which subsequently formed to Scaffold like structures .Then with Vaccum distillation technique, whole solution was distilled and then the product isolated was dried in Hot air oven which was nothing but Scaffold samples, composed of Chitosan-Polycaprolactone-MWCNTSs.

The collected samples were characterized further with FTIR, XRD, SEM, TEM.

IV. RESULTS AND DISCUSSIONS

A. Isolated Scaffold(Chitosan-Polycaprolactone-MWCNTs)



Fig. 3: Isolated Scaffold(Chitosan-Polycaprolactone-MWCNTs)

In the above fig-3, the morphology of scaffold seems Porous. Spongy like networking structures which caused by the polymer components present in it .So It was standardized as a good quality scaffold from a external verification parameter only

B. Characterization

1) FTIR Spectrum

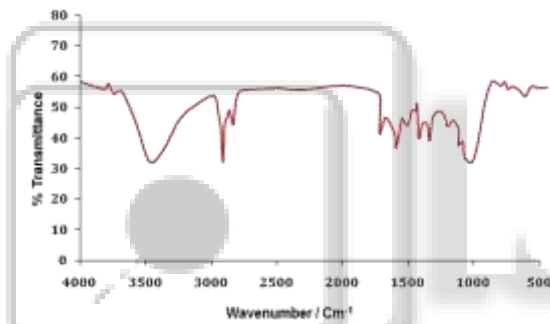


Fig. 4: FTIR spectra of MWCNTs+Chitosan+PCL

Figure-4 shows the FTIR spectra of MWCNTs-Chitosan-PCL. All the peaks, characteristic of MWCNTs, at 3500–1000 cm^{-1} (aromatic ring), appear in both spectra. To better understand the carboxylic acid-functionalized MWCNTs, the expanded FTIR spectra between 3000 and 1500 cm^{-1} was inspected. The FTIR spectra of the Chitosan, MWCNTs-Chitosan-PCL. These peaks were caused by C=O stretching vibrations and C-O stretching vibrations, respectively. The well-characterized absorption peaks for CS were at 1663 and 1545 cm^{-1} . They were caused by the carboxyl and amide groups of CS, respectively.

C. X-Ray Diffraction Spectroscopy

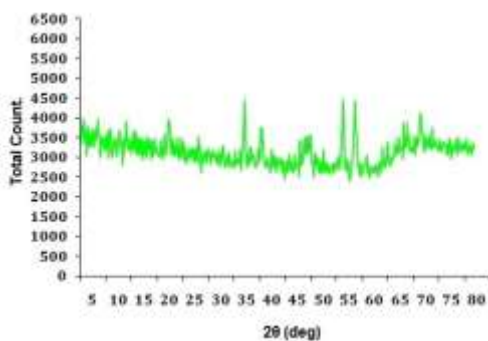


Fig. 5: X-RAY Diffraction of MWCNTs+Chitosan+PCL

This fig-5: shows that X-ray diffraction was used to examine the crystalline structures of pure MWCNTs-CHITOSAN-PCL because there is a peak at about at 35^oc, 55^oc and 60^oc. In the spectrum of MWCNTs-CHITOSAN-PCL was observed

D. Scanning Electron Microscopy

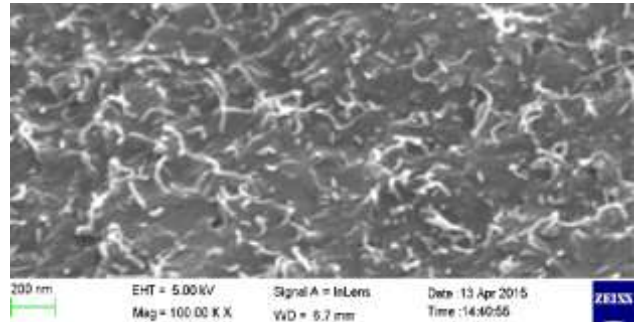


Fig. 6: SEM of Chitosan + PCL+ 3%CNT

The figure-6 shows the morphology of the MWCNTs-chitosan-PCL and polymer blends, since the mechanical properties depend on it. In general, good dispersion of MWCNTs in the matrix and strong interfacial adhesion between the two phases are required to obtain a composite material with satisfactory mechanical properties. Scanning electron microscopy was used to study the tensile fracture surfaces of composite samples of MWCNTs- (3 wt %)-chitosan-PCL blends, in which the major component forms the matrix and the minor component (MWCNTs) the dispersed phase.

E. Transmission Electron Microscopy

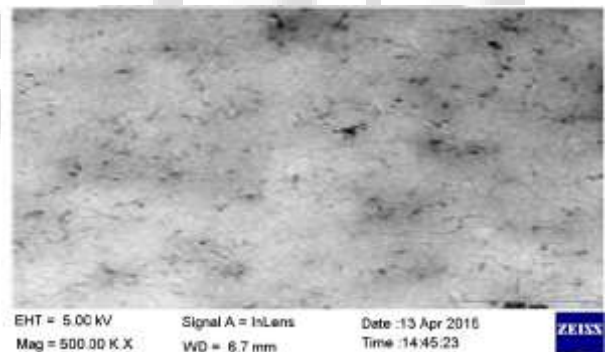


Fig. 7: TEM of Chitosan+PCL+1% CNTs (MW)

In the above figure no 7, less adequate amount of MWCNTs seems to be attached in the Nanocomposites matrix.

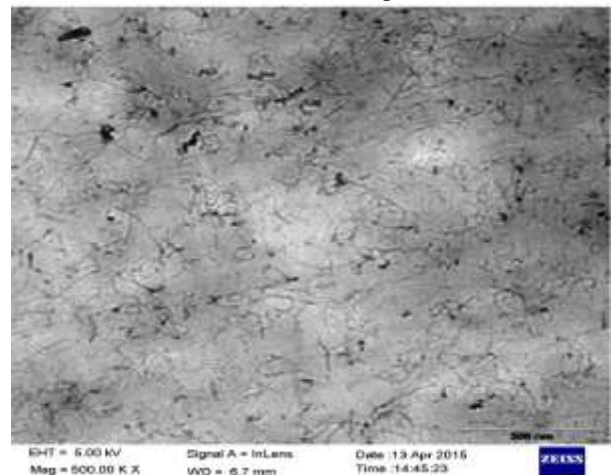


Fig. 8: TEM of Chitosan+PCL+3% CNTs (MW)

The Fig-8 shows that the TEM was used to give an indication of the attachment of chitosan and eventually chitosan to MWCNTs. TEM indicated MWCNTs clearly visible on the surface Nanocomposites matrix and had attached to the chitosan surface with adequate amount by interaction within components.

V. CONCLUSIONS

The novel Chitosan/PCL/MWCNTs Nanocomposites scaffold isolated by simple conventional method to mimic the function of extracellular matrix of tissues like in a living biomatrix. Techniques as Scanning electron Microscopy, Transmission electron Microscopy showed the successful integration of Carbon Nanotubes in the composite matrix by which we succeeded in making it a Nanocomposite. In conclusion, we conclude that MWCNTs/chitosan scaffold is a novel composites scaffold that will have great potential applications in the field of engineering. The improvement and application of these composites will depend on how effectively we can handle the challenges. The significant progress in the understanding of these composite systems within the past few years points towards a bright future.

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