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SciMedicine Journal

Vol. 2, No. 1, March, 2020

Liver Tissue Regeneration using Nano Silver impregnated Sodium Alginate/PVA Composite Nanofibres

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Received 04 January 2020; Accepted 26 February 2020

Abstract

Liver regeneration is a highly organized tissue regrowth process and is the most important reaction of the injured liver. The present study endeavors towards the preparation and characterization of nanoporous Sodium Alginate (SA)/ Poly Vinyl Alcohol (PVA) composite, nanofibrous scaffolds coated with silver (Ag) nanoparticles for hepatocellular regeneration. Chitosan based Silver nanoparticles possess high antibacterial activity has been preferred in the scaffold preparation to improve the antibacterial properties. The structural characterization of Ag Nanoparticles revealed the amorphous nature with an average particle size of 300 nm. Nanofibres (Scaffolds) were prepared by electrospinning SA/PVA solution at a voltage of 18-25 kV and Ag NPs were coated on it for antibacterial activity. Invitro studies denoted the growth of nitro compounds, amides and collagen which are the major constituents of liver tissue.

Keywords: Chitosan Based Silver Nanoparticles; Electrospinning; Sodium Alginate; Liver Tissue Regeneration and Antibacterial Activity.

1. Introduction

The liver is metabolically an active organ that requires high oxygen and nutrient. A healthy liver is only the visceral organ that possesses the capacity to regenerate. But a damaged liver cause's major life threatening as hepatocellular regeneration is not supported. Liver failure is a life threatening condition that occurs by various factors such as viruses, bacteria, fungi, parasites, alcohol, drug and obesity. The current treatment methods include medications and liver transplantation depending on the extent of the damage. So, the people with liver failure are put to medication or go towards hiring the liver. While these methods have their limitations such as effect of steroids & liver transplantation has many consequences and might lead to disease like idiopathic neonatal hepatitis, Cholangio carcinoma, alagilles syndrome (bile duct complication) and also at certain cases causes liver rejection after few years of liver transplant which might result in death [1]. These major drawbacks can be overcome by regeneration of liver tissue which is a better alternative for liver transplantation. Development in the field of tissue engineering has to look forward to developing the regeneration capabilities of the host tissues with bioactive scaffolds to overcome the above mentioned limitations.

Though there are enormous researches on various tissue regeneration like bones[2], skin [3], the work on liver tissue regeneration is limited. Hence this project work is dedicated to tissue regeneration of the most important organ – "the liver". The main focus of this work is to formulate the scaffolds with biocompatibility and antibacterial properties along with biodegradability for hepatocellular regeneration which will serves as the function of hepatocytes for liver. The hepatocyte regeneration is done by the combination of two biocompatible polymers, Sodium alginate (SA) which is non- toxic, biocompatibility, biodegradable natural polymer with its anionic polysaccharide reduces

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http://dx.doi.org/10.28991/SciMedJ-2020-0201-3

liver inflammation and sustain hepatic synthetic function was blended with polyvinyl alcohol (PVA). To achieve better interaction with the body fluids for the proper and faster regeneration of tissues, among the various proposed methods for the preparation of scaffolds [4], the Electrospinning process has opted. Thus the novelty of work lies in the fact of preparing nanoporous scaffold with high surface area to enhance the bioactivity and facilitated the regeneration of tissues in a better way. Additionally, it is essential to have antibacterial properties for the prepared scaffolds to have healthy tissue regeneration which is obtained by coating with chitosan mediated silver nanoparticles.

2. Materials and Methods

2.1. Materials

Chitosan from shrimp shells (HIMEDIA) with deacetylation 75-85% & molecular weight 190-310kDa, sodium alginate (SA) from brown green algae with molecular weight 80-120kDa (LOBA chemi), Poly Vinyl Alcohol (PVA) (LOBA chemi), 99% acetic acid (AVRA), 99.9% silver nitrate ($Ag(NO)_3$) (SDFCL) and 99.9% of Ethanol were used as received.

2.2. Methods

2.2.1. Preparation of Silver Nanoparticles

Chitosan (0.5 wt %) was dissolved in 2% acetic acid and stirred for 30 mins. Later the solution was centrifuged for 30mins at 4000 rpm to remove the undissloved particles. The resultant chitosan supernant (20ml) was dissolved in 5ml of 10mM $Ag(NO)_3$ and stirred continuously for 3hours at the speed of 400 rpm. Then the mixture was heated at 90˚C for 36hours under vigorous stirring. The colorless solution was gradually changed into a light yellow colored solution and finally becomes a yellowish brown viscous solution. The obtained viscous solution was dried at 70˚C and ground to a fine powder to obtain the silver nanoparticles (AgNPs) [5].

2.2.2. Preparation of SA/PVA Composite Nanofibres

SA/PVA composite nanofibres were prepared by dissolving PVA (10 wt %) and SA (2 wt %) separately in distilled water. Then both the solutions were homogeneously blended by stirring at 90˚C for 1hour. The blended solution was electrospun using the Espin-nano electrospinning apparatus. The solution was loaded in 2.5ml plastic syringe with 24G needle and feed rate was optimized at 0.5ml/hour with the tip to collector distance of 15cm and applied voltage about 25kV. The resultant SA/PVA bare fibre produced was collected by the cathode collector rotating drum covered by aluminium foil. Post to this, a solution containing 20mg of AgNPs dispersed in 5ml of ethanol through ultrasonication for 30mins was sprayed on the bare fibres. The resultant hybrid fibre was dried at room temperature for 12hours and stored in a desiccator.

2.3. Characterization Studies

The structural conformation of nanofibres and hepatocellular growth was examined using Fourier Transformed Infrared (FTIR) spectra under ATR conditioned, using a Nicolet iS5-6700 model with the wavenumber ranging from 4000cm^{-1} to 500 cm^{-1}. Rigaku MiniFlex XRD model using monochromatic copper radiation (Cuk_{α}) of wavelength λ=1.54Å was used to study the crystal structure of AgNPs. Particle size distributions for the AgNPs were measured using MALVERN particle size analyzer at ambient temperature. The size and shape of synthesized AgNPs were studied using Field Emission Scanning Electron Microscopy (FESEM) –FEI Quanta 200 Model SEM. Invitro studies were carried out by immersion of 1 cm × 2 cm hybrid scaffold in Stimulated Body Fluid (SBF) solution which was prepared by Kokubo's method [6].

3. Results and Discussion

3.1. Characterization of AgNPs

Figure 1 shows the X-ray diffractogram of synthesized Ag Nanoparticles. The 2Θ diffraction peak at 38.2˚, 44.56˚ and 64.59˚ corresponds to the diffraction planes of (111), (200) and (220) of AgNPs respectively (JCPDS No: 04-0783). The broad nature of the XRD patterns signifies the smaller particle size of AgNPs. The obtained XRD pattern clearly illustrated that the silver ions have been reduced to AgO by the stabilization of chitosan [7]. The surface morphology and the size of the developed AgNPs were analyzed using SEM. The SEM micrographs (Figure 3) displayed the spherical morphology with an average size of 300nm. The Amorphous nature of the NPs is inferred by the absence of grain boundary in the SEM image which is also supported by the lack of sharp crystalline peaks in the XRD pattern. The size distribution intensity of AgNPs obtained from PSA coincided well with the histogram obtained from SEM analysis. Additionally, the smaller size of the NPs has contributed to the high surface activity resulting in the formation of agglomerates which is also revealed from the "peak 3" in the PSA data.

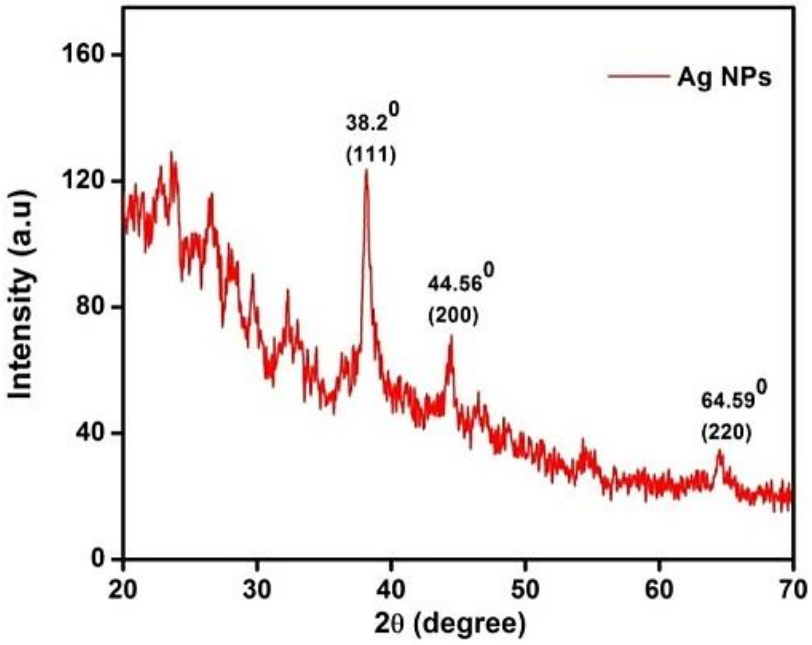

Figure. 1. XRD diffractogram of Chitosan mediated AgNPs

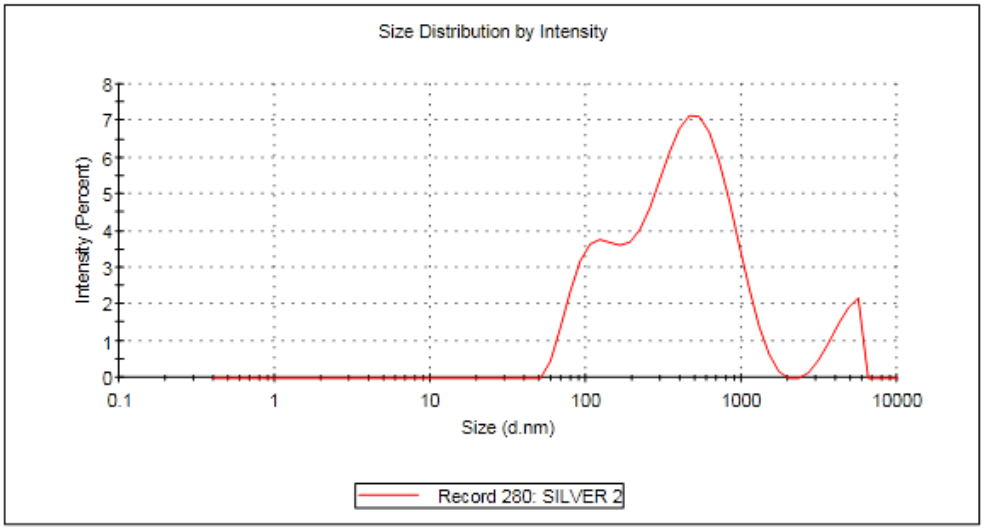

Figure 2. Particle size distribution PSA

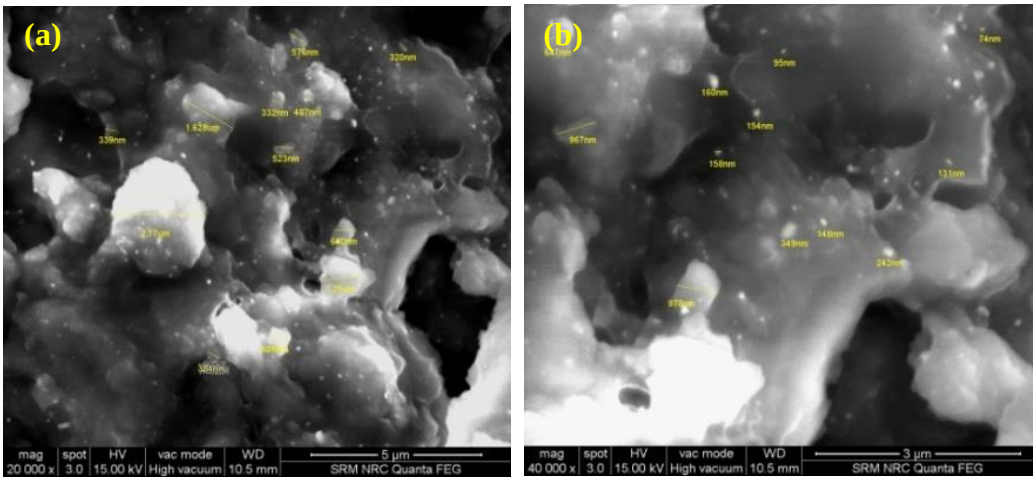

Figure 3. SEM Micrographs of AgNPs (a) 20kX (b) 40kX

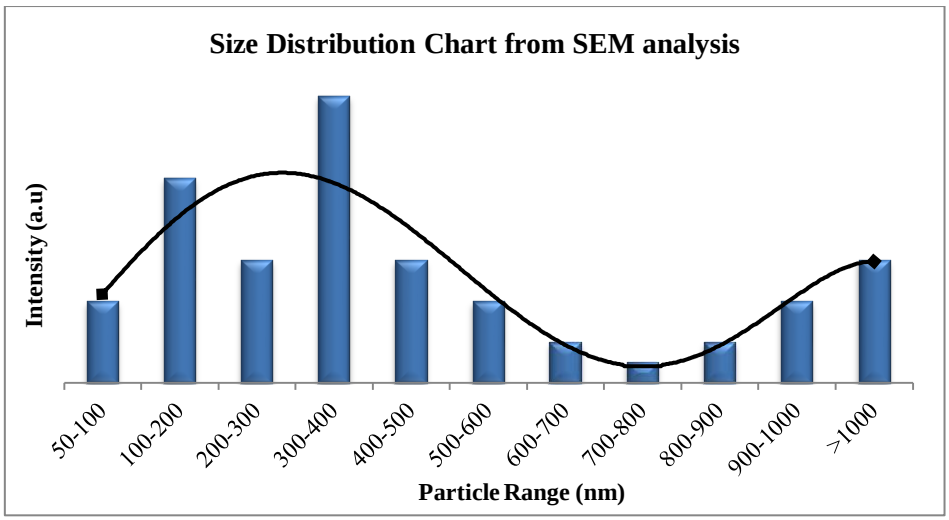

Figure 4. Histogram from SEM Analysis

3.2. Characterization of SA/PVA Composite Nanofibres

Structural analysis and presence of PVA, SA and Ag NPs in the nanofibres were confirmed through FTIR analysis. Comparative FTIR spectra of PVA, SA/PVA and AgNPs coated composite nanofibres were displayed in Figure 5 signify the changes in the spectrum with the addition of each component. In Figure 5a the broad band observed from 3100 cm^{-1} to 3500 cm^{-1} was assigned to O-H stretching which was due to the strong intramolecular and intermolecular hydrogen bonds and the band at 2920 cm^{-1} was due to C-H alkyl stretching found in PVA. The band at 1404 cm^{-1} was associated with (OH)-C-(OH) bending, the band at 1067 cm^{-1} was owing to(C-O stretching) and the peak at 847 cm^{-1} is due to (C-OH stretching) was assigned to PVA. In Figure 5b the band at 1576 cm^{-1} and 1406 cm^{-1} which are assigned to asymmetric and symmetric stretching peaks of the carboxylate salt group (COO^-). Besides, the peak at 1407 cm^{-1} (OH)-C-(OH) bending, 1067 cm^{-1} (C-O stretching), 1024 cm^{-1} (C-O-C stretching), 847 cm^{-1} (C-OH stretching) functional groups had been attributed to the presence of SA. The existence of AgNPs on the nanofibres was verified through the appearance of dip at 520 cm^{-1} correspondings to AgO [7].

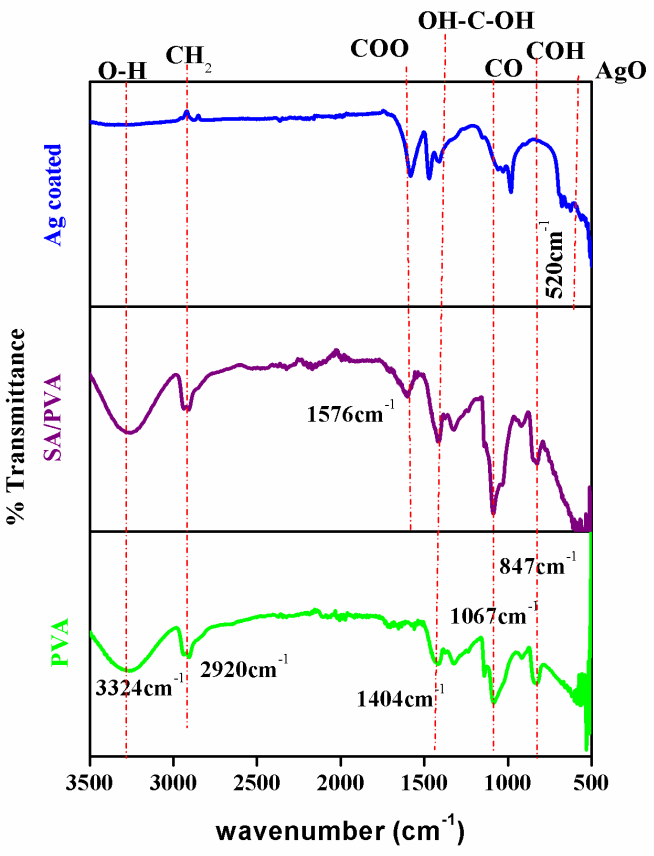

Figure 5. FTIR analysis of PVA, SA/PVA, Ag coated fibre

3.3. In vitro Studies of Ag Coated Composite Nanofibres

To examine the hepatocellular growth, the prepared scaffolds were subjected to in-vitro studies by immersion in SBF solution. The changes in the scaffolds were examined through FTIR analysis for every week was depicted in Figure 6. It is the evidence from the IR spectrum that after soaking in SBF solution, the initial characteristic of bands of AgNPs coated SA/PVA composite nanofibres were modified strongly because of the interaction between scaffolds and the SBF. Consequently, the spectra of these biomaterials reveal a new band which indicated the growth of liver tissues [8].

3.3.1. FTIR Analysis

On examining the FTIR spectra of composite nanofibres a bend appeared at 1631 cm^{-1} (stretching vibration) denotes the initial formation of nitro compounds starting from the first week. This band continues to show an increase in intensity during 2nd week indicated the growth constituents of hepatocytes. Later in the third week, the scaffolds reveal bands at 1596 cm^{-1} (amide I), 1414 cm^{-1} (lipids and proteins), 1260 cm^{-1}(phospholipids), 1020 cm^{-1} (carbohydrates) signifies the formation of amide complex and collagen which are the major constituents of the liver. Thus it can be inferred that these composite nanofibres are supportive for hepatocellular growth [9]. The positive effect of Ag particle and better surface activity of nanofibres had attributed the growth of essentials elements for liver tissue regeneration [10].

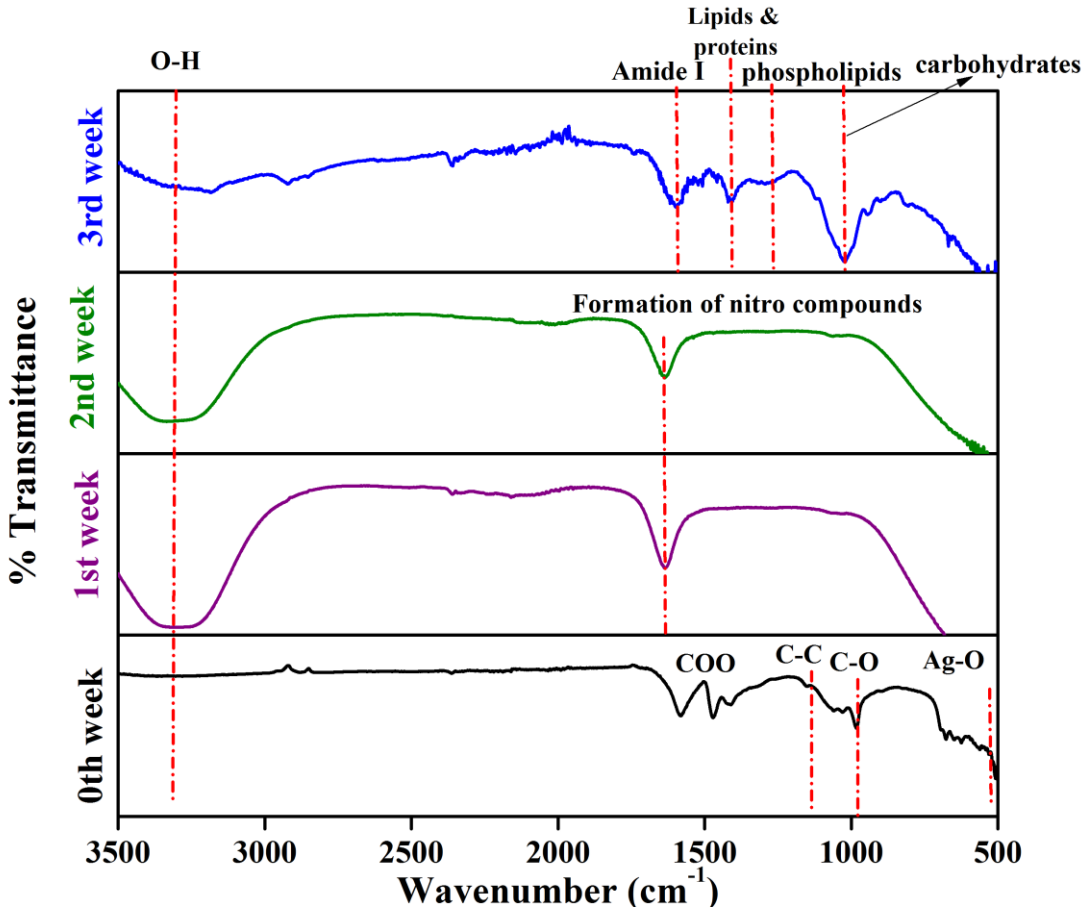

Figure 6. FTIR analysis of Ag coated fibre

4. Conclusion

Silver Nanoparticles have been synthesized using chitosan as a reducing agent. The structural characterization revealed the amorphous nature with an average particle size of 300nm. The prepared AgNPs were impregnated on the electrospun nanofibres for antibacterial activity. This composite nanofibre was subjected to Invitro studies. Thus the current study illustrated that composite nanofibres support the growth of hepatocellular regeneration. This approach may enable the use of SA/PVA scaffolds in case of liver tissue regeneration that can serve as a better alternative for liver transplantation.

5. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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