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Effect of Porous Activated Charcoal Reinforcement on Mechanical and *In-Vitro* Biological Properties of Polyvinyl Alcohol Composite Scaffolds

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The present work focused on developing an innovative composite material by reinforcing polymer matrix with highly porous activated charcoal. Polyvinyl alcohol-activated charcoal (PVA-AC) composite scaffolds were developed by varying the AC concentrations (0, 0.5, 1, 1.5, 2 and 2.5 wt%) in PVA matrix by freeze drying method. The developed scaffolds were characterized for their physicochemical, mechanical and *in-vitro* biological properties. In addition, the effect of AC on the attachment, proliferation and differentiation of osteoblast MG 63 cells was evaluated by scanning electron microscopy (SEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, alkaline phosphatase (ALP) activity assay and alizarin red stain-based (ARS) assay. All the PVA-AC composite scaffolds exhibited good bioactivity, hemocompatibility and protein adsorption properties. The scaffolds with high AC concentration (2.5 wt%) showed controlled drug release kinetics that are suitable for long term healing. The mechanical properties of all the PVA-AC composite scaffolds were improved when compared to the pure PVA scaffold. The high porosity, swelling degree and hydrophilicity of PVA-AC composite scaffolds facilitated cell attachment and proliferation. This is due to porous AC present in the sample that supported the osteoblast differentiation and formed mineralized nodules without the addition of any extra agents. From the above studies, it can be concluded that PVA-AC composite scaffolds are promising biomaterials for bone tissue engineering applications.

1. Introduction

Various synthetic and natural biomaterials for better repair and replacement of the injured bone tissues have been developed. An ideal biomaterial should be biocompatible, biodegradable, bioactive, porous and mechanically compatible with the natural bone. Synthetic polymers represent the largest group of biomaterials used in tissue engineering due to their tunable properties. Among these synthetic polymers, polyvinyl alcohol (PVA) has received particular attention because of its suitable physicochemical and viscoelastic properties. It is a bio-inert, biodegradable and biocompatible synthetic polymer widely used for various biomedical applications such as tissue engineering, wound dressing and drug delivery[1,2]. However, owing to its poor mechanical properties, it is not used as a bone implant. Thus, adding suitable and biocompatible reinforcement materials in the PVA matrix is an effective method to improve the properties of PVA. Recent studies have reported that the reinforcement of carbon into PVA matrix induces high mechanical and biological properties[3].

Carbon based biomaterials have attracted intensive attention because of their nanoscale dimensions, large surface area and good acceptance by the biological environment[4]. Beyond the biological aspects, the mechanical properties of these biomaterials are also comparable to that of natural bone[5]. Thus, carbon biomaterials such as carbon nanotubes and graphene are being used as reinforcement in the polymer matrix to improve mechanical properties. For example, researchers have obtained a 79% improvement in the tensile modulus and 47% improvement in the tensile yield strength of PVA matrix with 0.8% of carbon nanotubes[6]. Further, carbon nanotubes with hydroxyapatite enhance the osteoblast and mesenchymal stem cell adhesion and proliferation when being coated on titanium surface[7]. Similarly, use of graphene showed an improvement in biomimetic properties of the scaffolds in tissue engineering applications[8]. However, carbon nanomaterials have the tendency to aggregate because of their intrinsic van der Waal interactions resulting in the reduced dispersion. When aggregated, these nanomaterials have shown cytotoxicity with human erythrocyte and fibroblast cells. Likewise, Ding et al.[9] reported the toxicity of graphene oxide to primary human peripheral blood T

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lymphocytes. Its presence caused deoxyribonucleic acid (DNA) damage and T lymphocyte apoptosis. In a recent study, the shape and concentration dependent cytotoxic effects of carbon materials were revealed[10]. They showed that graphene induced an intense toxic response at low concentration levels when compared to carbon nanotubes. These cytotoxic effects pose serious challenges to human health and reveal a need to develop a biocompatible carbon based biocomposite scaffold having properties suitable for bone repair and regeneration.

Activated charcoal (AC), a quasi-graphitic form of carbon, is a highly porous biomaterial with large surface area to volume ratio[11]. Due to its irreversible adsorption of toxic metabolites, it is widely used to enhance cell growth and development in plant tissue culture[12]. The large surface area and powerful adsorption properties of AC fulfill the space requirement and help living cells to attach onto its surface. In a similar work, the enhancement of corneal cell growth on hydroxyapatite-coated porous carbon matrix was reported by Sandeman et al.[13] They showed the adsorption of inflammatory cytokines by porous carbon matrix, hence suppressing the excessive inflammation. Further in another recent study AC-extracellular matrix composite scaffolds facilitated the regeneration of damaged neural tissues by promoting neuronal differentiation[11]. However, to the best of our knowledge, no studies have reported the impact of activated charcoal on osteoblast cells. Thus, the present work focused on developing an innovative composite material by reinforcing polyvinyl alcohol with highly porous activated charcoal. The novelty of this work is to reinforce the polymer matrix with a highly porous material which enhances the mechanical properties of composite scaffolds without compromising its porosity. Also, the developed PVA-AC composite scaffold is favorable for cell growth and proliferation without the addition of any extra growth factor. The developed scaffolds were characterized, and their *in-vitro* biological and mechanical properties were evaluated. These PVA-AC composites showed extensive potential as a scaffold material for bone tissue engineering applications.

2. Materials and Methods

2.1. Materials

PVA (hot water soluble and average molecular weight of 70,000–100,000), activated charcoal powder, antibiotic–antimycotic solution, phosphate buffer saline (PBS), 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit from HiMedia, India; the human osteoblast-like MG 63 cells (human osteosarcoma cell line) from the National Centre for Cell Science (NCCS, Pune, India); Dulbecco's Modified Eagle's medium (DMEM), trypsin-ethylenediaminetetraacetic acid (EDTA) solution, fetal bovine serum (FBS), alkaline phosphatase (ALP) assay kit and alizarin red stain-based (ARS) assay kit from Sigma-Aldrich, USA were procured and used in this research work. All reagents used in this research work were of analytical grade.

2.2. Preparation of PVA-AC composite scaffolds

The porous PVA-AC scaffolds were prepared by freeze drying method (Fig. 1). Briefly, 10% w/v of PVA was dissolved in distilled water and stirred at 90 °C for 3 h in a magnetic hot plate. Different concentrations of AC powder (0, 0.5, 1, 1.5, 2 and 2.5 wt% with respect to PVA) were added to the solution and stirred. The obtained PVA-AC composite solutions were frozen at –20 °C for 24 h before lyophilization. After 24 h of freezing, the samples were lyophilized (SCANVAC CoolSafe 55) at –55 °C for 48 h at 4×10^6 Pa in vacuum to obtain dry, porous PVA-AC composite scaffolds. Six scaffolds with different concentrations of AC powder were prepared and coded as PC 0, PC 0.5, PC 1, PC 1.5, PC 2 and PC 2.5, respectively.

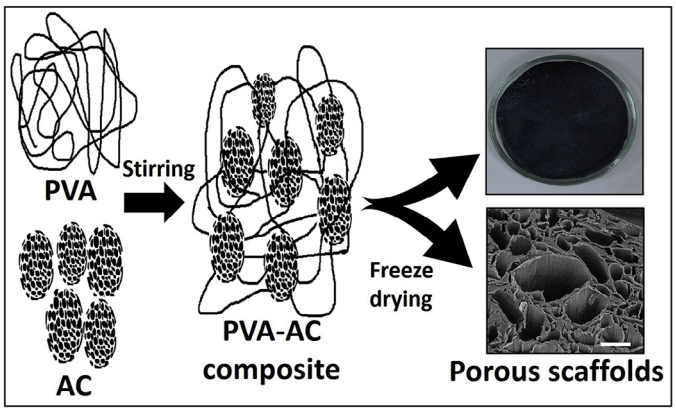

Fig. 1. Schematic representation of PVA-AC composite scaffold preparation.

2.3. Characterization

The cross-sectional morphology and porosity of the composite scaffolds were observed using a field emission scanning electron microscope (FESEM-FEI NovananoSEM 450). The samples were then gold sputter coated prior to SEM imaging to avoid imaging artifacts from electrical charging. Pore sizes of the scaffolds were measured from FESEM micrographs using ImageJ software. For each sample, 20 measurements of pore dimensions were taken. The porosity of the composite scaffolds was also measured using mercury intrusion porosimeter (MIP, Quantachrome, PM -33-13) by varying the pressure from 0.003 MPa to 200 MPa. The phase and crystallinity of the composite scaffolds were studied using X-ray diffraction (XRD, Rigaku Ultima IV Diffractometer, Japan) technique. The XRD over a scan range of 5°–60° was performed with a scan speed of 5°/min and step size of 0.05° using Cu$K\alpha$ radiation. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR, AlphaE, Bruker, USA) was performed to characterize the presence of functional groups in PVA-AC composites. Transmittance spectra within the range of 600–4000 cm^{-1} were obtained. Differential scanning calorimetry (DSC, Mettler-Toledo DSC 822) was performed to examine the effect of AC on polymer over a range of 50–250 °C at a heating rate of 10 °C min^{-1}. The effect of AC addition on the hydrophilicity of composite scaffolds was evaluated by measuring its contact angles (DSA25, Kruss, Germany) using distilled water at room temperature by the sessile drop method. The contact angle at ten different positions for each sample was measured. By the water displacement method, porosity of the scaffolds was measured. Scaffolds of known dry weight in water at room temperature was recorded. After 2 h, liquid impregnated scaffolds were taken out, and wet weight was recorded. The water volume was calculated by dividing the weight difference of scaffolds before and after immersion in water with the density of water. The porosity of the scaffolds (P) was calculated from the following equation[14]:

$$P = \frac{V_w}{V_s} \quad (1)$$

where V_w is the water volume and V_s is the scaffold volume after immersion in water.

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