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Preparation of Scaffold Using Rice Husk and Starch for Bone Regeneration

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Abstract: ***Bone scaffolds are used in the treatment of bone defects, various materials are widely used nowadays to produce osteoconductive tissues. The present study is framed to design and characterize a bone scaffold using rice husk and starch. With the aid of rice husk, porous scaffold is developed for bone tissue regeneration, which can provide a mechanical support in an ecofriendly and cost effective manner. The combination of rice husk and starch with a biodegradable polymer gives a good compressive strength and mechanical property. Characterization of rice husk and starch is performed by various techniques such as SEM, compressive mechanical test, porosity test, in vitro bioactivity test. Thus the blend of rice husk and starch with polymer is useful in forming an ideal scaffold intended for bone regeneration.***
Keywords: Rice husk, starch, scaffold, polymer, regeneration.

I. INTRODUCTION

The regeneration of bone is an emerging area and a significant interest has been shown in recent days to increase the life expectancy of the population. [24] Bone defects arise due to many factors such as trauma, osteoporosis, osteoarthritis and bone metastases. [27] Clinically the defects can be treated either by autografts or allografts. [31] The negative aspects of autograft include death of healthy tissue and donor morbidity issues. [8] However allograft also suffers from pathogen transmission, immune rejection and reduced bioactivity. [10] The shortcomings of these auto/allografts are overcome by implanting biocompatible materials (ie) scaffolds. [1]
Scaffold used for bone tissue engineering should satisfies the following needs, (1) easily processed and designed into 3D shape. [14] (2) Should be non-toxic and sterile. (3) Possess appropriate mechanical property to overcome stress environment for the bone in growth . [12] (4) Require suitable surface chemistry for cell attachment, proliferation and differentiation. [3] and (5) Presence of high porosity with interconnected network. [5] In recent years ,many researchers explores various materials such as synthetic and natural polymers, to design the scaffolds. Out of these, synthetic polymers are more easily processed and tunable. [24]The commonly used polymers for bone regeneration include poly (lactic-co-glycolic)acid (PLGA) Poly (Ɛ-caprolactone) (PCL), and polyvinyl alcohol (PVA). [17] FDA has approved these synthetic polymers due to its biocompatibility nature. [15] and aids in the enhancement of osteointegration and bone growth .[24]
Rice husk is a rich source of silica. [18] Silica based biomaterials shows a promising approach in skeletal therapies. [2] Ather Farroq khan et al reported that bioactivity nature of scaffold is enhanced due to the presence of silica and also revealed osteogenic differentiation, restoration of fracture site and production of growth factor has been improved. [4] Farnaz et al fabricated a three dimensional scaffold using rice husk composites, which has a highly porous structure with interconnected porosity. [11]
Starch , being a carbohydrate reserve in plants [16] in recent decade attracts a new era of interest in bone tissue engineering. Joao et al designed starch based scaffold which is bioresorbable and possess a porous matrix. [16] A.J Salgado et al fabricated a non - cytotoxic starch scaffold which allowed the ingrowth of new bone cells. [3]
The objective of the present work is to make use of renewable resources such as rice husk and starch for therapeutic purpose and to efficiently cure the bone defects. Our work is to develop a ideal scaffold for bone regeneration using rice husk, starch and PVA. Scaffold is fabricated using electrospinning and thermally induced phase separation technique. Various characterization studies such as morphology, x-ray diffraction, compressive strength and invitro studies are carried out in order to prove the fabricated product is a potent bone substitute.

II. MATERIALS AND METHODS

A. Materials

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PVA was purchased from CDH laboratory with a molecular weight of 125kDa. Native starch was obtained from Sigma Aldrich, India. Rice husk was procured from local rice mills, Kanchipuram, Chennai, India. Human osteoblastic (MG63) cell line was procured from the National Center for Cell Sciences (NCCS), Pune, India

B. Synthesis of rice husk

Rice husk is washed with distilled water and then dried in a hot air oven at about 110˚C for 1 day and acid leaching with 3% HCL and 10%H_2SO_4 for 2 h at a ratio of 55g/l . Then the leached husk is washed with distilled water and dried in a hot air oven at 110˚C for 1 day. [32] Processed husk were burned in a muffle furnace at about 650˚C for 4h, to form rice husk ash (RHA) which was white in color.

C. Fabrication of nano fibrous hybrid RHA/PVA/Starch

Electrospinning process is carried out in a ESPIN-NANO apparatus. The polymeric solution consists of PVA, RHA and starch (9wt%) in a distilled water. Solution is heated at 40˚C for 3h in a magnetic stirrer and then allows to cool at room temperature. The solution is loaded into a 5ml syringe; rotating collector is wrapped with aluminium foil which was connected to ground. Distance between spinneret and collector was 15cm and flow rate was set as 0.2 ml/hr. High voltage power supply is used in order to generate electric field (0-25kv) and fibres were collected on a Al foil. Fibre formation is confirmed using visual and microscopic observation.

D. Characterization using optical microscope and SEM

Fibre morphology was examined using optical microscope (Nikon eclipse-LV100) equipped with cross polarizer and camera.SEM analysis is carried in order to determine size and distribution,interconnectivity between the pores. It is observed using Vega TESCAN, JAPAN equipment at a accelerating voltage of 5-10kv. Samples were sputter coated with gold prior to analysis.

E. Preparation of PVA/RHA/Starch hybrid foams

Scaffold is formed by using thermally induced phase separation method (TIPS). [11] PVA, RHA and starch were dissolved in distilled water and stirred for 3h. Homogenized solution was stored in a -18˚C freezer and kept in a deep freezer at -80˚C for 3 days. Foam obtained was then left at room temperature for 24h. The porosity of the scaffold was measured by immersing in ethanol. V_1 denotes the volume of ethanol, V_2 indicates the total volume of ethanol and V_3 denotes the remaining volume of ethanol after removing scaffold.

$$\text{Porosity} = (V_1\text{-}V_2/V_2\text{-}V_3) \times 100\%$$

F. Compression mechanical test

Mechanical properties of the foam tested by compression experiments using Instron universal test machine. Load cell of 1000N used with a cross head velocity of 0.001mm/min. Foam has a diameter of 28mm. Compressive strength is calculated by plotting stress vs strain values. [15]

G. MTT Assay

MG63 cells were grown in culture flasks containing DMEM supplemented with 10% FBS in a 95% air and 5% CO_2 in a humidified atmosphere at 37˚C. Cell viability was assessed by MTT method. Human osteoblastic cells (MG63) at a concentration of 10,000 cells were seeded to the 96 well cell culture plate. Briefly, 10 mg of scaffold rice husk and starch were weighed and soaked in 500 µl of the DMEM medium for 24 h. The supernatant termed as conditioned medium was taken at different volumes (10µl, 20µl, 50µl and 100µl) and made upto 1 ml with medium and added to the wells and incubated for 24 h. The media were removed and 100µl of 0.5% MTT solution was added and incubated for another 4 h at 37 ˚C. DMSO was used to dissolve the formed formazan crystals, and the optical densities (OD) were determined using the spectrophotometer (BioTEkmicroplate reader, USA) at 570 nm.

III. RESULTS AND DISCUSSION

A. XRD and SEM analysis of rice husk

Rice husk ash (RHA) after sintering at 650˚C is shown in Figure 1(a). X-ray diffraction studies revealed the phase of RHA, powder is amorphous and the peak value is observed at θ=24.7˚, [32] is shown in Figure 1(b). SEM image of RHA shown in Figure 1(c) and 1(d) confirms the porosity nature, which is essential for bone regeneration. Presence of interconnected porosity enhances the

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diffusion rate and facilitates vascularisation thus improves oxygen and nutrient supply. [11] The diameter is in the range of 1.62µm-2.56µm.

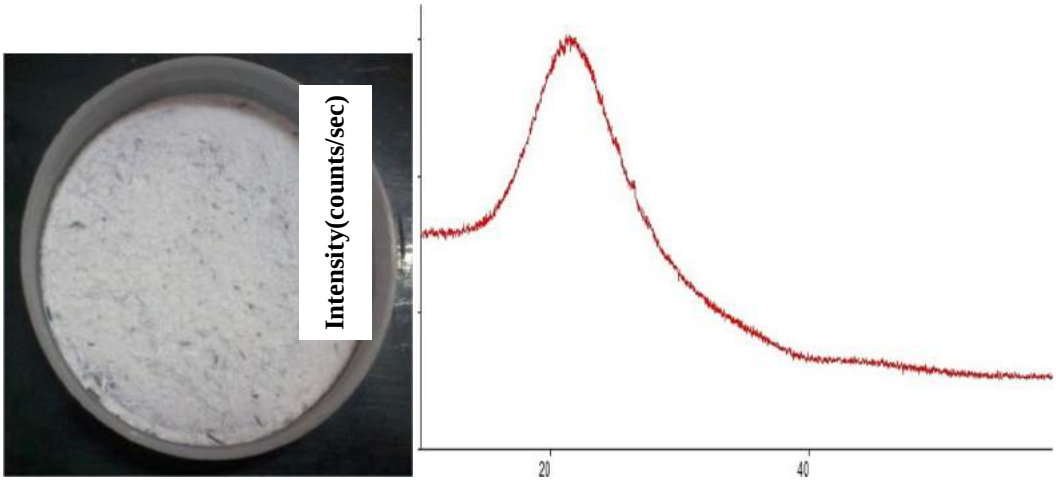
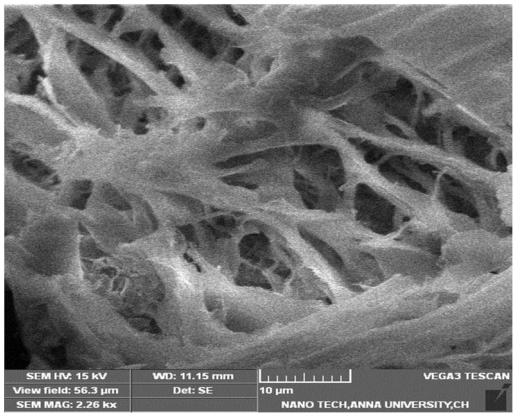


Fig 1(a) Rice husk ash

Fig1(b) XRD Analysis



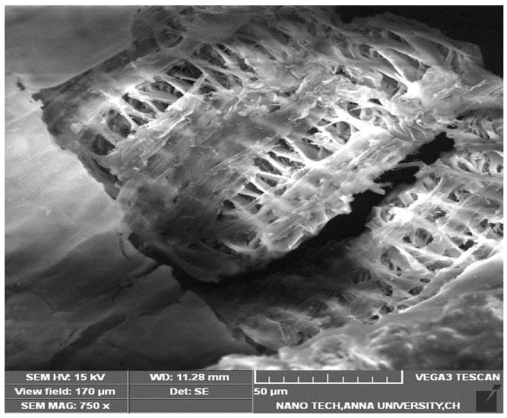


Fig1(c) SEM image of RHA at 10µm resolution

Fig1(d) SEM image of RHA at 5 µm resolution

B. Morphology characterization of nano fibrous hybrid RHA/PVA/Starch

Optical microscopic images of the RHA/PVA/Starch fibres are shown in Figure 2(a). It confirms the formation of nano fibres. Figure 2(b) illustrates the SEM image of PVA composite fibres (9wt%). The diameter of the fibres is in the range of 260nm-360nm. Orientation of fibres appears to be random and irregular. Electrospun of PVA, husk and starch results in bead free formation of nano fibres. Most of the fibre diameter is below 300nm.

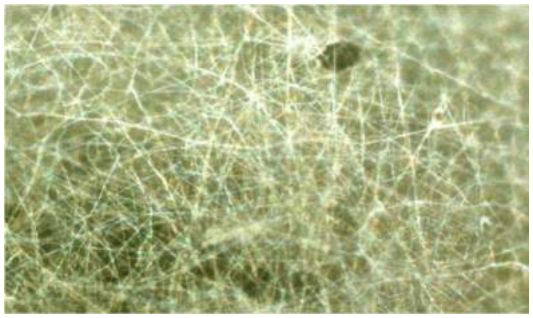


Fig 2(a) Optical microscope image of PVA Composites

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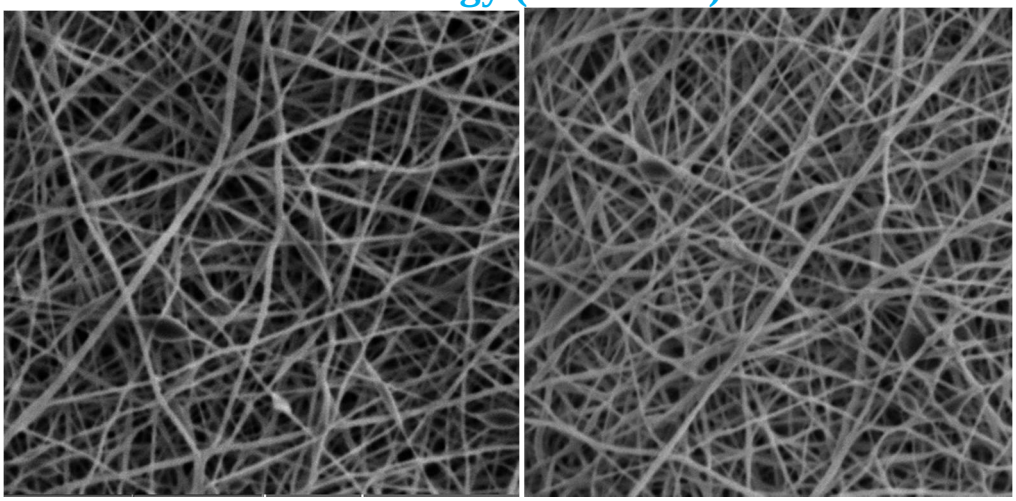

Fig 2(b) SEM image of PVA/husk /starch nanofibres under 5µm resolution

C. Porosity measurement of PVA/RHA/Starch hybrid foams

Scaffold formed using thermally induced phase separation technique is shown in Figure (3(a)). Porosity of the scaffold is measured by immersing scaffold in ethanol for 10 min. Shape of the scaffold is circular and the porosity of the scaffold is found to be 70%, which supports cell migration and proliferation and is intended for bone regeneration. [15]

D. Compression mechanical test analysis

When a load cell of 1000N is applied on a foam, compressive modulus was obtained at 527.25 N . Stress vs strain values of composite scaffold is shown in Figure (4). The compressive modulus of the PVA composite scaffold obtained is 0.875MPa, which is higher when compared to previous study based on PVA for bone tissue engineering applications. [19]

E. Invitro bioactivity test

All data were analyzed using GraphPad Prism 5.0 Software (GraphPad Software, La Jolla, USA). All the data are expressed as mean ± standard error of mean (SEM). Figure(5) shows the results of the 24 h MTT assay, expressed as absorbance levels. Optical density values reflects the possibility of cell growth and cell proliferation .OD values of samples remains similar when compared with positive control, which indicates that scaffolds have no negative effect on cell viability and its proliferation thus the materials possess good biocompatibility.

Fig 3 (a) Foam formed using TIPS technique Fig 3(b)Porosity Measurement of scaffold

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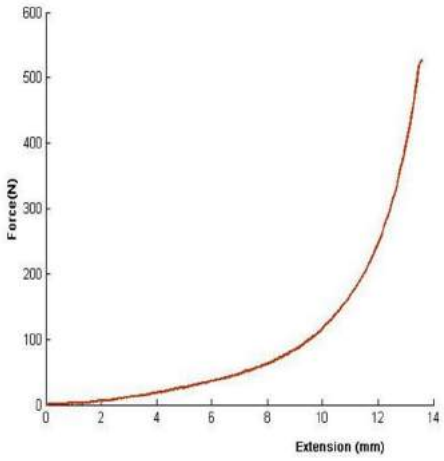
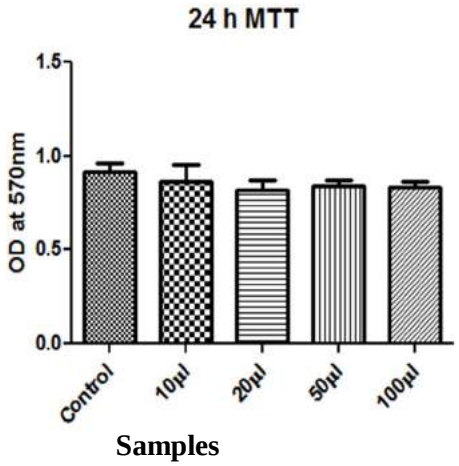


Fig(4)Compression mechanical test analysis



Fig(5) Cell viability tested by MTT

IV. CONCLUSIONS

The present study proved that the rice husk ash is rich in amorphous silica. Si plays a biologically significant role in bone regeneration and repair due to which bioactivity is improved. SEM image revealed the porous nature of rice husk ash, which is deemed suitable for cell migration and penetration.

Blend of starch and rice husk ash with biodegradable polymer has been explored by forming a nanofibers and scaffold formation. Average diameter of PVA composite nanofibers are in the range of 300nm hence high surface to volume ratio is achieved which aids cell behaviours such as migration, proliferation, differentiation. Porosity of the scaffold is found to be 70% so that it helps in tissue ingrowth and vascularsiation. Compressive strength of the scaffold is 0.87MPa so which is appropriate for load bearing applications. Thus the scaffold also exhibits cell viability behaviour which make it to be considered as a potential biomaterial for bone tissue engineering applications.

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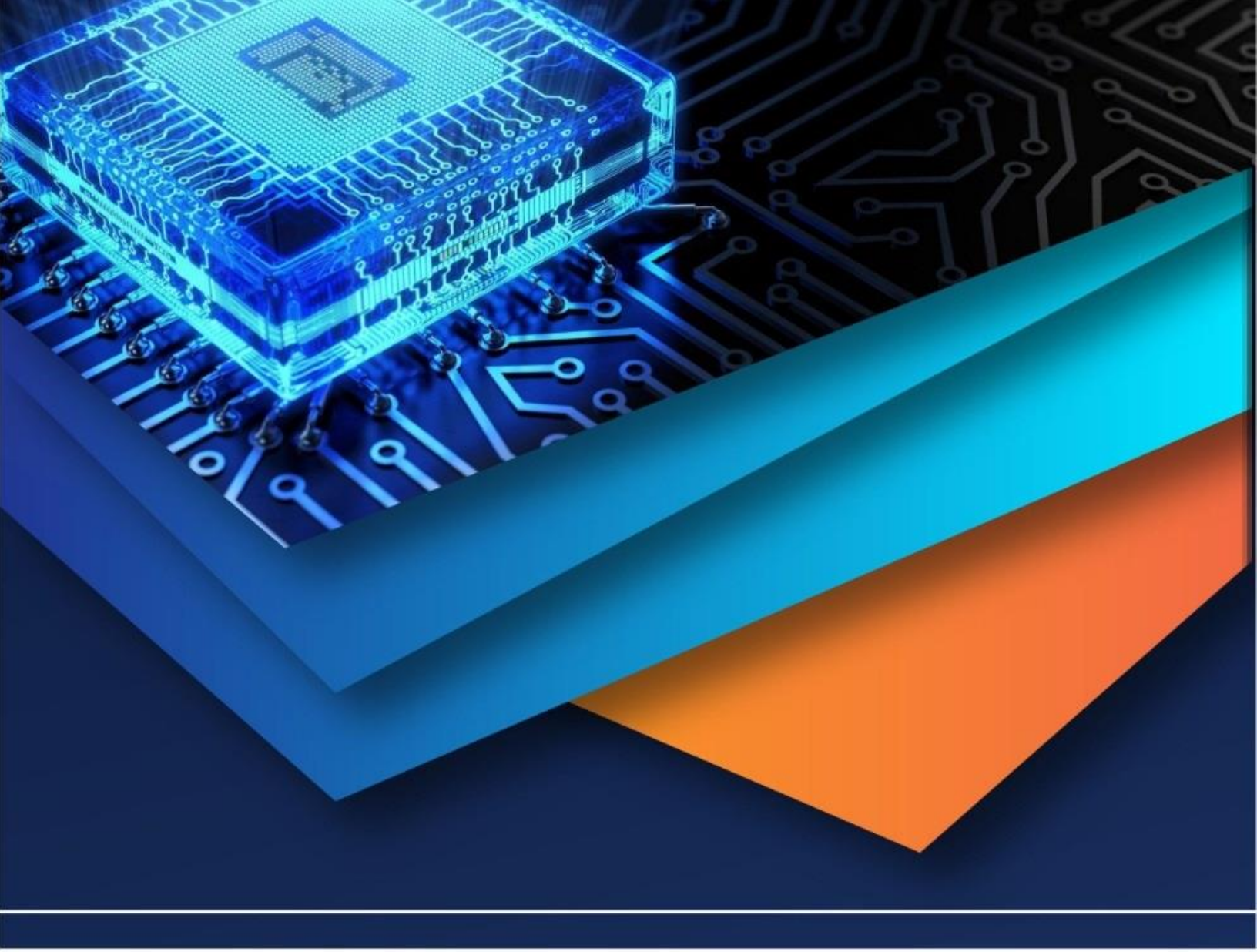

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