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Synthesis of PBAT/CNT Bone Scaffold

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Abstract:

Bone marrow produces blood cells and supports the immune system in our body but due to some reasons like diseases, injuries, medical treatment damages bone marrow. Traditional bone marrow regeneration methods, like transplantation, face limitations due to donor availability and immune rejection. Researchers are exploring bio scaffolds for functional tissue regeneration. The bio scaffolds are synthesized by using PBAT and CNT at different ratios. PBAT/CNT (0.5%) and PBAT/CNT (5%). This can be done in 4 steps (1) PBAT synthesis (2) g-PBAT synthesis (3) Amine CNT (4) Final Product PBAT/CNT. Characterized was done using FTIR, NMR, mechanical strength, DSC, & SEM. FTIR intensity at 1741 shows PBAT grafted to maleic acid and 3498 peak shows NH stretching, NMR explain about the structure. PBAT/CNT 3.5% got high tensile strength. DSC could calculate the melting point. SEM gives images of pore size, PBAT, is a biodegradable polymer through a process of hydrolysis, it is mimicking bone marrow's extracellular matrix, providing support, growth, and promoting biocompatibility. Carbon nanotubes (CNTs) are incorporated into PBAT offering high mechanical strength, electrical conductivity, and cell adhesion, making them ideal for tissue engineering applications. Biodegradability of PBAT prevents surgical removal, and the bio scaffold gradually breaks down over time, promoting regenerated tissue development.

Keywords; PBAT (polybutylene adipate terapthalate), CNT (carbon nano tubes), bio composite, bio degradable, mechanical strength, bio availability, cell differentiation, Osteoblast proliferation

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Introduction:

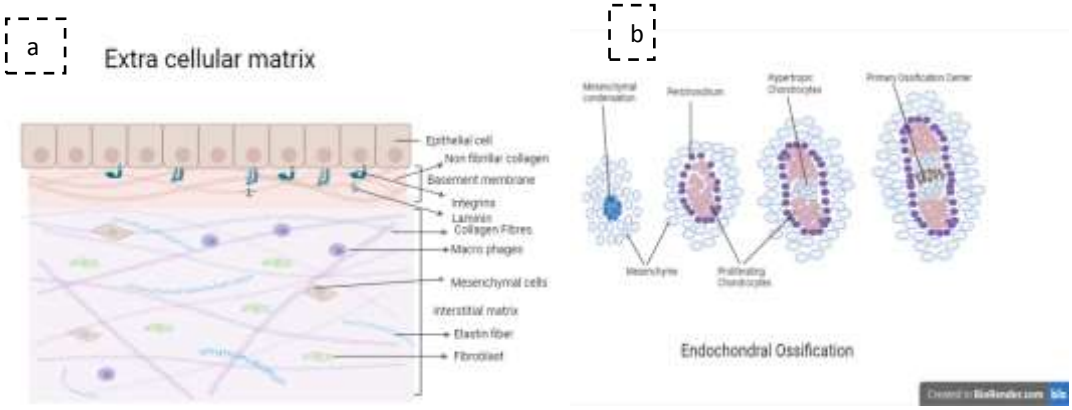


Fig-1(a)Extracellular Matrix &(b) Endochondrial Ossification

Poly ethylene terapthalate is a non degradable plastic it is used in the preparation of plastic bottles. Poly ethylene terapthalate converted to PBAT with different reactants like zinc acetate , butyl stannic acid preparation. [1,2].now a days using the esterification reaction the synthesis of PBAT was done by PET .Using poly ethylene terapthalate to the synthesis of PBAT can be useful to reduce plastic pollution.

PBAT (Poly butylene adipate teraptalate) is a biodegradable polymer now a days it is used to synthesis of biodegradable plastics,packaging material due to the elasticity and their strength.before the synthesis of PBAT was done by di methyl terapthalate.[3–5]

PBAT is a easily bio degradable, hydrophillic, and non toxic to the fibroblat cells.due to this reason the PBAT is selected for bone tissue engineering[6],.Bone tissue engineering contains the bone cells are made up of osteoblast which are belongs to epithelial cells. The epithelial cells are differentiated into cuboidal cells, sqamous epithelial cells,rectangulaar cells etc. in this the bone cells are belongs to cuboidal cells.these cells are a composite of collagen,elastin, and fibronectin.

Before bone scoffolds bone regenaration process was done by an autologus cells(from patient cells) ,Allogenic cells(other human beings), and xenogenic cells (different specimens) are used to form bone regenaration.when different cells are used it forms an immunological diseases[7].Now a days the problems are solved by cells producing on bone scaffolds.

The bone scaffolds are need to satishfy the(1) bio compatabilityforcell adhesion, (2)porous structure is greater than 100 for cells migration.(3)hydrophilicity for cellattachment (4)immobilisation for receptor attraction,(5)Mechanical strength for loading cell weight, (6)no toxicity for cell growth and survival.[8]The bone scaffolds are metals, ceramics, and natural and synthetic polymers are used.

First titanium metal was used in bone regenaration process but it needs to remove after bone regenaration due to their non degradable property and again surgery was needed to remove a titanium metal [9].The polymers are bio degradable and it is easily degradable through the process of bone formation.

Bone Scaffold Preparation:

Bone scaffolds are fabricated by different techniques that is wet methods.which are fabricated by using solvents.Wet methods are Solvent casting, Thermal induced phase separation,electrospinning techniques are used.Solvent free methods are gas forming

techniques,miceo cellular injection, melt extrusion,batch forming,sterno lithography techniques and blending and particle leaching techniques are used.[10]

Bone Scaffold Mechanism:

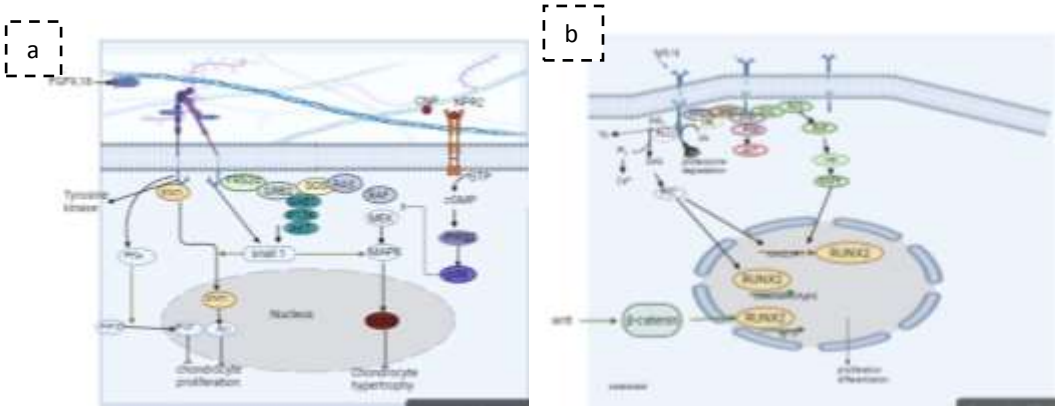


Fig-2:Chondrocyte Hypertropy & Osteoblast Prolifiration

The bone is formed by connective tissue.The connective tissue contains an Extracellular matrix.Extra cellular matrix made up of collagen fiber,elastin fiber, Mast cells, Fibroblast and Macrophages. Bone scaffolds are mainlysupportive environment to the Extra cellular matrix[11].PBAT polymer shows good biocompatability with Extracellular matrix and fibroblast cells . PBAT has good tensile strengh and young modulus.[12]

Bone scaffold seres as a Extracellular matrix due to their bio compatability and bioadhesive when bone scaffold are induce at a fracture bone it allows the Extra cellular matrix due to their pore size[13].Mesencheymal stem cells starts an aggregate intereact with fibronectin receptors in extracellular matrix releases kinase and phosphorylation reaction in Mesenchymal stem cells.

The reaction induces a wnt signaling pathway and bone morphogenetic protins releases transcrptional factors like RUNX2 and SATb.The transcriptipnal factors change mesenchemal stem cells to osteoprogenarators and chondrogenesis.these are type-I and type-II collagen.when chondrocytes are improve it forms pre hypertropic chondrocytes.the pre hypertropic chondrocytes are developed into a chondrocyte hypertropy through a process of IHH path way,para thyroid hormone,collagen.

IHH path way,para thyroid hormone,collagen changes RUNx2,RUNx3 transcriptional changes in chondrocytes.Chondrocyte hypertropy converts into chondrocyte maturation.[14]

Angiogenesis process: (Formation of Blood vessels):

vascular endothelial growth factors are intereact with endothelial cells .after Endothelial cells are migrate and proliferate due to gene expression in Chondrocyte Hypertropy.Migrated Endothelial cells intereact with fibronectin receptor through the integrins in ECM.where the firbronectin receptors are present endothelial cells form a tubular structure .these are blood vessels in bone.[15]When chondrocytes are mature and convert into osteoblast cells.The osteoblast forms spongy bone.

PBAT/CNT was degraded through a hydrolysis reaction.CNTs enhance PBAT strength, stiffness, and impact resistance by reinforcing the matrix, increasing durability and toughness, and enhancing Young's modulus.The characteristics and performance of PBAT-CNT composites are impacted by CNT type, concentration, fabrication method,

and processing conditions. Dispersion and alignment within the PBAT matrix considerably impact composite characteristics, requiring careful processing processes. These PBAT/CNT scaffolds facilitate cell adhesion, proliferation, and differentiation, allowing bone marrow regeneration to occur.

Materials:

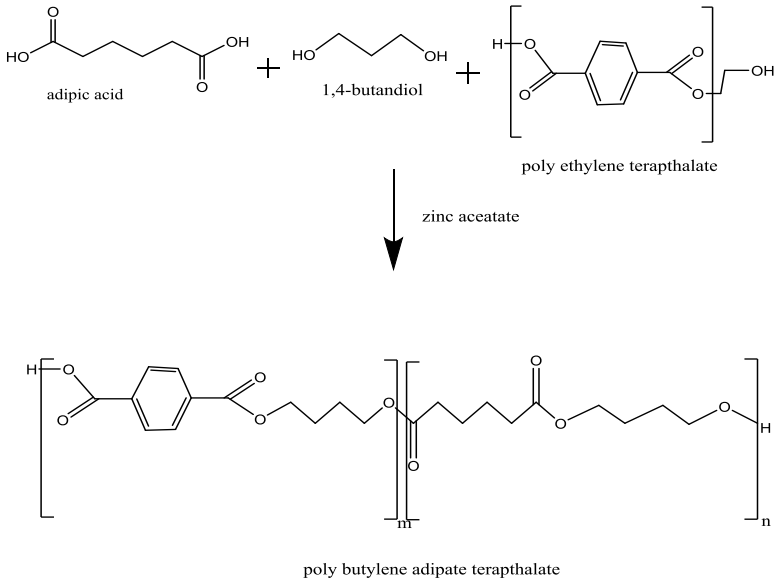
Materials and chemicals:

PET(poly ethylene terapthalate) ;Adipic acid; 1,4-butandiol; Butyl stannic acid. PBAT(polybutylene adepic terapthalate) ; Maleic acid ; Benzoyl peroxidase;Chloroform; Acetone.MWCNT,$NaNo_2$,Ethylene diamine, and H_2So_4 .G-PBAT,amine CNT,Ethyl carbodimaide.

Synthesis:

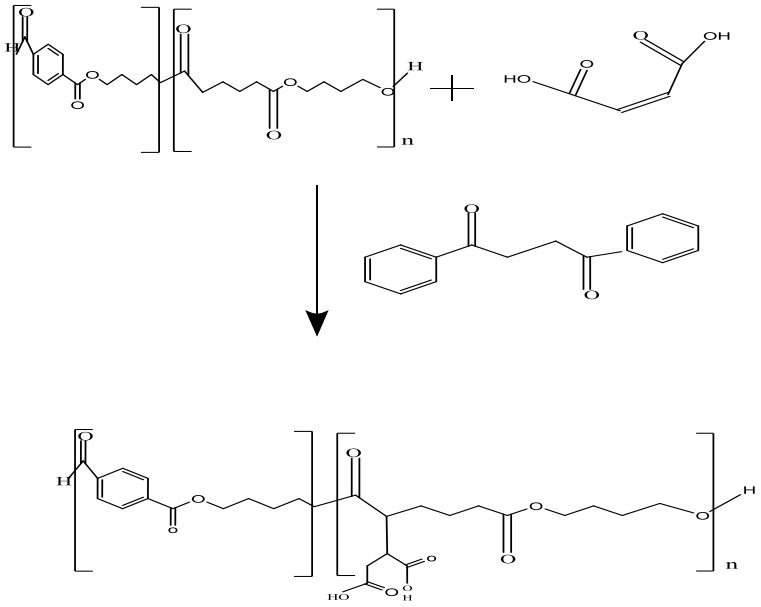
PBAT SYNTHESIS:

Polyethylene terapthalate (50gm), adipic acid(37 gm), 1,4-Butandiol(95gm) and Butyl stannic acid (0.5gm) was taken in a 500 ml round bottom flask Stittred with 250 rpm at 145℃ for 3 hours to produce an esterification reaction.after the reaction was proceed at 250℃ for 250 rpm for 4 houurs to produce polymerisation reaction.the solution was kept in vaccum at 250℃ for 1.5 hour to remove an excess 1,4-Butandiol

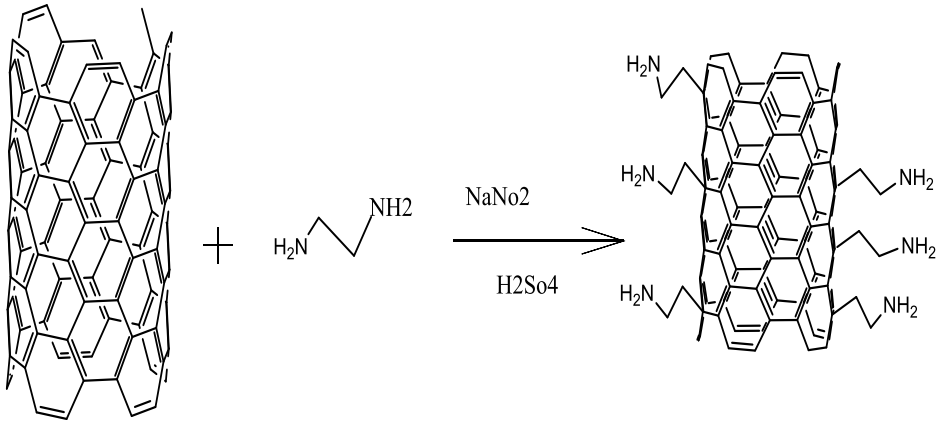


G-PBAT synthesis:

First we took PBAT(polybutylene adepic terapthalate)(10gm), Maleic acid (50mg), Benzoyl peroxidase(10mg). After PBAT was dissolved in 90 ml of chloroform in one beaker and in another beaker, maleic acid was dissolved in 50 ml of acetone. After that, both solutions were added tothe round bottom flask.These solutions were mechanically stirred at 60℃for 3 hours.Then the benzoyl peroxide was dissolved in 90 ml of chloroform and this solution was added to PBAT and maleic acid solution[16]. Finally, both solutions were heated at 60℃ for 24 hours to permit the grafting reaction occur.



Amine CNT synthesis:
First we took MWCNT(0.9gm),$NaNo_2$(12gm),Ethylene diamine(1.1gm), and H_2So_4(0.8ml).After Sodium nitrite , CNT, Ethylene diamine are added into a 100ml of round bottom flask and H_2So_4 was slowly introduced in those mixtures at 60℃ for 1 hour. When the reaction was completed then cool the mixture and50ml of dimethyl formamide was added to the product rinse the solution therefore centrifugethe solution to remove an extra ethylene diamine[17–19].Finally it washed with water and dry the product.



G-PBAT/amine CNT synthesis:Pbat/CNT was synthesised in 5 composites.that is 5% of PBAT/CNT,3.5% of PBAT/CNT,2.5% of PBAT/CNT,1.5% of PBAT/CNT,0.5% of PBAT/CNT.

PBAT/CNT 5%: First we took G-PBAT(10gm),amine CNT (500mg),Ethyl carbodimaide(10mg).Next, these are separatelydissolved in DCM(dichloro methane) and the solution is mixed in a round bottom flask.then these solutionswere mechanically

stirred at room temp for 3 days.After g-PBAT/amine CNT are obtained [20],[21]these were washed and dried in a vacuum.

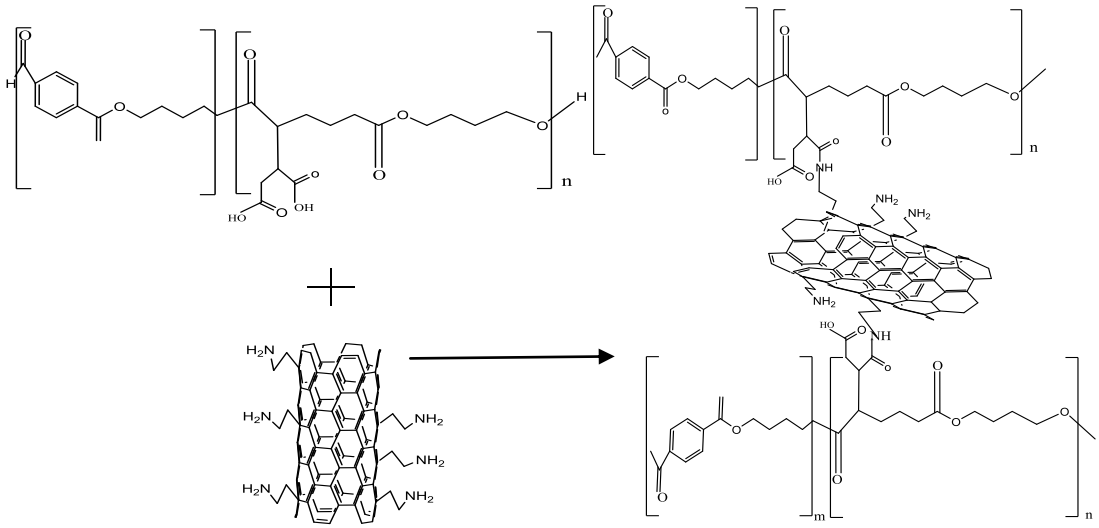
PBAT/CNT 3.5%: First we took G-PBAT(10gm),amine CNT (350mg),Ethyl carbodimaide(7mg).Next, these are separatelydissolved in DCM(dichloro methane) and the solution is mixed in a round bottom flask.then these solutionswere mechanically stirred at room temp for 3 days.After g-PBAT/amine CNT are obtained these were washed and dried in a vacuum.

PBAT/CNT 2.5%: First we took G-PBAT(10gm),amine CNT (250mg),Ethyl carbodimaide(5mg).Next, these are separatelydissolved in DCM(dichloro methane) and the solution is mixed in a round bottom flask.then these solutionswere mechanically stirred at room temp for 3 days.After g-PBAT/amine CNT are obtained these were washed and dried in a vacuum.

PBAT/CNT 0.5%: First we took G-PBAT(10gm),amine CNT (50mg),Ethyl carbodimaide(1mg).Next, these are separatelydissolved in DCM(dichloro methane) and the solution is mixed in a round bottom flask.then these solutionswere mechanically stirred at room temp for 3 days.After g-PBAT/amine CNT are obtained these were washed and dried in a vacuum.

Salt Leaching:

Different compositions of PBAT/CNT liquid material was pour on a glass slide. After that sodium chloride salt was added. PBAT/CNT material was kept for drying at room temp. PBAT/CNT material dipping into a water the salt particles are leached out. Finally the material got a pore sizes.



FTIR (FOURIER TRANSFORM INFRA RED SPECTROSCOPY):

The material infra-red frequencies were measured using perkin-elmer spotlight 400 FTIR. The particle frequency was measured at middleinfra-red region.it is between 500-4000 cm^{-1}.

NMR (Nuclear Magnetic Resonance Spectroscopy):
Proton signals were measured using Bruker Avance 600MHZ NMR Spectrophotometer. The instrument measures Number of signals were measured between0-8.5 ppm. Position of signals were measured for a functional group identification; Peak area of signal were measured for calculation of number of protons and splitting of signals were measured information about neighboring protons.

Tensile Test: Each composite was prepared in 4 dumbbell shape using Hydraulic press at 150℃ /20Pas. The dumbbell shape material had 65mm length,6mm width,0.963mm thickness. Each Composites were tested using Universal Tensile Machine. at a constant rate of 1N min^{-1}.

DSC (Differential scanning calorimetry):
Each sample was weighted from 1.3-1.6 mg. Two aluminum pans were taken to heat the samples. The reference sample was an empty aluminum pans and the samples were sealed in Aluminum pans, the both aluminum pans were heated using DSC (404 F3) at 10℃/min from 30-450℃ under nitrogen atmosphere.

FE-SEM (Field Emission Scanning Electron Microscopy):
The pore size was measured in 100-600 micrometers range.The pore size was measured using Image –J Software.

RESULTS:
FTIR: Ftir spectrum: PBAT grafted to maleic acid peak was observed at 1714. This frequency range shows a highly single carboxylic acidit shows the highest peak for PBAT grafted to maleic acid.
3021 frequency range shows a multi-carboxylic acid group present in small amounts.
below 1000 frequency range shows CH bending and between 1000 to 1500 range shows an ester and alcoholic groups present in it. These peaks are commonly observed in both graphs.
2959 frequency range shows the alkene group present in maleic acid and that peak did'tappeared in the PBAT graph.
FTIR spectrum for PBAT : Here the single carboxylic acid peak shows a smallest peak in PBATthan maleic acid grafted PBAT.here we did not get the alkene group present for PBAT.3360 peak indicates the aliphatic primary amine stretching.3553 peak indicates OH group with intermolecular bond.

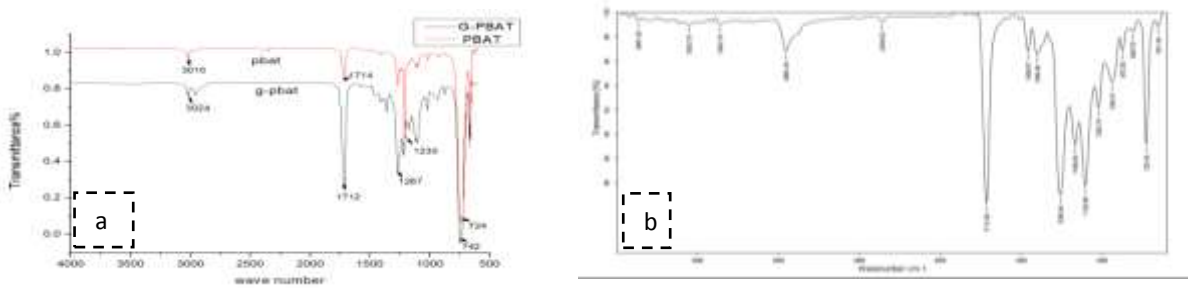


Fig-3:(a)pbat,g-pbat, (b)pbat/cnt.

NMR:

This graph shows the g-PBAT structure Here A,B,C are methyl attached to the oxygen group and D,E,and F shows the methyl groups attached to an estergroup. Here we got a G different ester at 2.33 pap.H and I at the peak of ester groups at 4-4.5 ppmare come.J and k are between 6.5 to 8.10 ppm which shows the aromatic presence. The carboxylic acid group did not defiantly identify due to in COOH the H protonCouldconvert into a D proton but these are shown in C NMR.

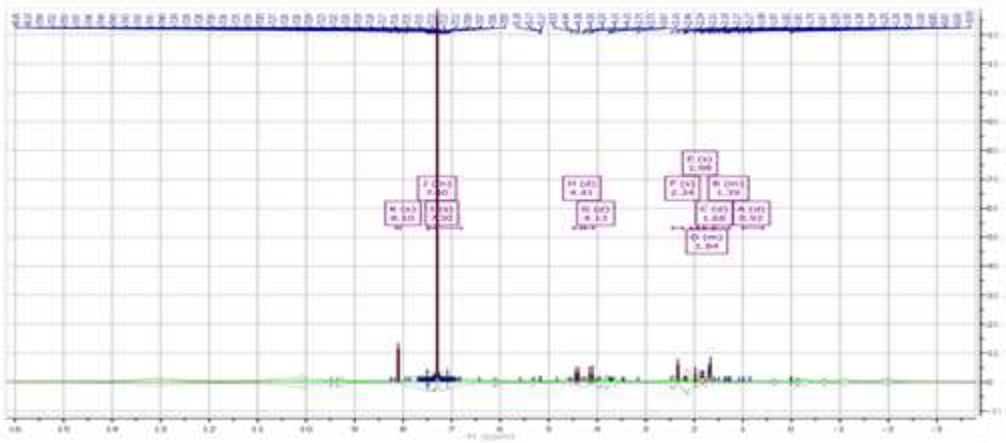


Fig-4:NMR Spectroscopy

Tensile Strength :

PBAT/CNT 0.5% got 7MPa of tensile strength .PBAT/CNT 5% got 6.2MPa of tensile strength .PBAT/CNT 2.5 got 7.3844 MPa of tensile strength. PBAT/CNT 3.5 got 14.3853 MPa of Tensile Strengh.here the strain percentage distance was changed due to increasing load per area. PBAT/CNT 0.5% got high tensile strength than PBAT/CNT 5% due to an agglomeration .it perfectly shows high amount of CNT causes to decrease the tensile strength and young modulus.

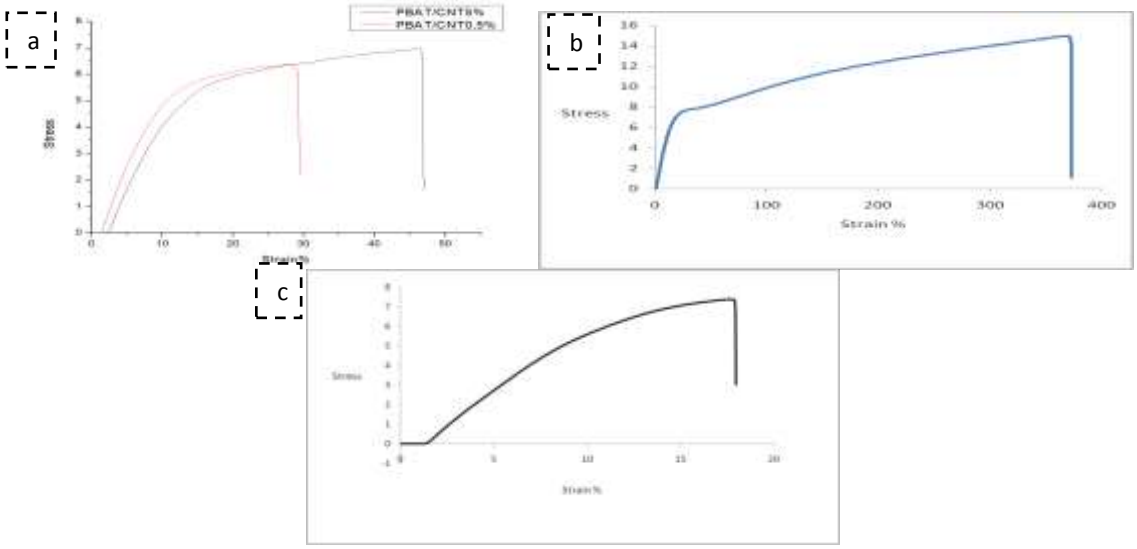


Fig-5:(a)PBAT/CNT 0.5% & 5%, (b) PBAT/CNT 3.5% (c) PBAT/CNT 2.5%.

DSC (Differential Scanning Calorimetry):

In Differential scanning calorimetry the peak temp was observed at 400.99℃ for 5% of PBAT/CNT and their enthalphy is -426.80J/g.The heat of melting(ΔHf) is 2556J/mg for PBAT/CNT 5%.The highest peak temp was observed at 402℃ for 0.5% of PBAT/CNT and their enthalphy was measurd at 712.59J/g.The heat of melting(ΔHf) is 4272.00J/mg.The 0.5% had a highest melting point than PBAT/CNT 5%.

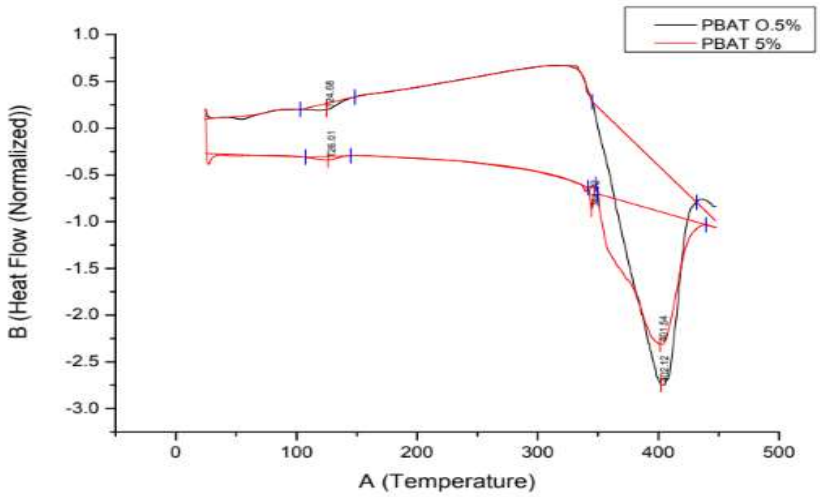


Fig-6:DSC(Differential Scanning Calorimetry)

FE-SEM

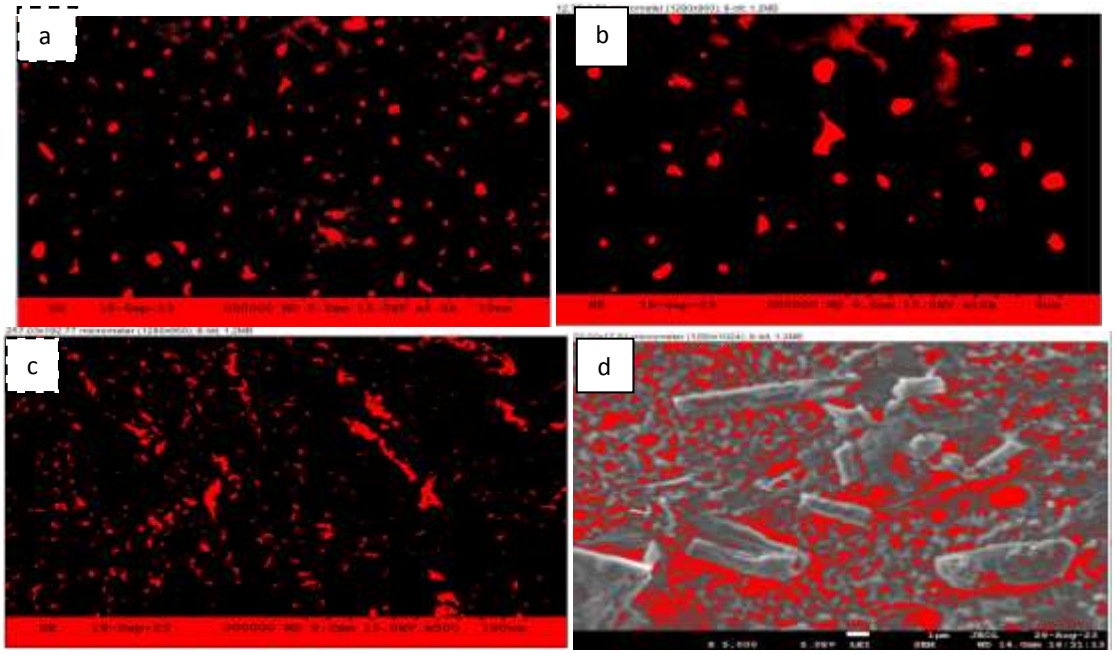


Fig-7: (a) PBAT/CNT 0.5% (b) PBAT/CNT 2.5% (c) PBAT/CNT 3.5% (d) PBAT/CNT 5%

PBAT/CNT 5% got 84 micrometers pore size.PBAT/CNT 0.5% got 124.6 micrometerspore size..PBAT/CNT 2.5% got 202.1% micro pore size.PBAT/CNT 3.5% got 167.8 micrometers pore size .the 84 micrometers is not enough for cell availabilty

because it has less than 100.and other micrometer pore size perfectly enough for cell avilability.

Conclusion:

PBAT/CNT material degraded through a process of hydrolysis at ester bonds.Carbon nanotubes grafting increasing the mechanical strength of PBAT. PBAT 0.5% , 2.5% ,3.5% are perfectly fit as a bioscaffold because it satisfy pore size greater than 100. PBAT/CNT 5% got low mechanical strength and less pore sizes due to carbon nanotubes are aggolomorate.

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