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SYNTHESIS AND CHARACTERIZATION OF NANOHYDROXYAPATITE/POLYURETHANE NANO-COMPOSITE SCAFFOLDS

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Abstract:
A novel type of nano hydroxyapatite (nHAp) / polyurethane (PU) nano composite was created for application in tooth and bone tissue engineering in this work. The wet chemical precipitation technique was used to successfully manufacture nano hydroxyapatite. In this study, hydroxyapatite/polyurethane nano composites were produced, and the compound was characterised using Fourier transform infrared (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS).

Keywords: FTIR, XRD, SEM, EDS, Hydroxyapatite (HAp), Polyurethane (PU).

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I INTRODUCTION:

For the replacement or repair of tissues in the human body that have been destroyed by illness or injury, a variety of clinical methods are employed. The current therapies are based on replacing damaged tissue with donor graft tissues, such as autografts, allografts, or xenografts. The lack of donors or donor locations, the volume of donor tissue that may be safely extracted, donor site discomfort and morbidity, the likelihood of adverse immune responses, disease transmission, and transplant rejection are all concerns associated with this technique [1]. Tissue engineering plays a critical part in this process. The goal of tissue engineering is to produce biological replacements that restore, maintain, or increase tissue function rather than replacing injured tissues (with grafts) [1, 2]. Cells in natural tissues are kept together by an Extracellular Matrix (ECM), which aids in tissue growth and regeneration. Tissue engineering aims to create substitutes for the natural ECM in order to aid the development of new functional tissue in vitro or in vivo. Tissue engineering is built on biological triads, such as signalling processes, cells, and the extracellular matrix (ECM), and requires the effective interplay of three components.

The primary idea is to combine engineering concepts with the body's inherent biological reaction to tissue injury. Tissue engineering's most researched field is bone tissue regeneration. By targeting osteogenic development of multicomponent mesenchymal stem cells of the bone marrow, equivalents of bone tissue can be produced, according to bone tissue engineering. It's in the realm of implant surgery, where the goal is to create the ideal tissue-engineered bone construct [4, 5]. The fundamental goal of bone constructs or skeletal tissues is to offer mechanical support for moving the structures enclosed and to create the right mechanical environment for functional repair [6]. Once the treatment is complete, the superfluous item must be removed from a clinical and biomechanical standpoint.

As a result, degradable polymers are becoming more popular in tissue engineering since they may be utilised as an implant and do not require a second surgical procedure to remove. It's also extremely simple to adjust synthetic polymers' mechanical characteristics, degradation periods, and other features to suit the three applications. Polyurethanes are a significant subset of the polymers chosen for tissue engineering. Polyurethane characteristics may be changed by altering the molecular weight of the polyol and the composition of the hard segments for use in tissue engineering, whether for soft tissue repair or bone reproduction.

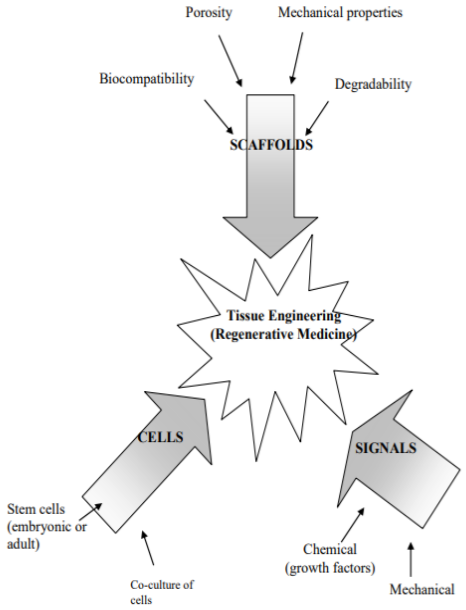

Fig 1 Tissue engineering triad

II RELATED WORK:

The major goal of this research is to create a nano-scale composite material containing cellulose nanocrystals (CNC) and hydroxyapatite (HAp). Composition revised simulated body fluid (r-SBF) is used to precipitate nano-sized HAp on the surface of functionalized CNC. Variations in reaction conditions like as temperature, pH, and the molar ratio of calcium ions per functional group on the CNC surface are attempted to regulate the size and orientation of HAp crystals, which have not been accomplished in earlier research. Furthermore, HAp-covered CNC is used to create oriented films with high-strength composites. Particles or plate-like materials have been utilised for inorganic/CNC films in previous studies. As a result, making coated CNCs that align themselves in the films should be extremely unique. The films are made by depositing a well-dispersed solution on a highly hydrophilic glass substrate. The study's ultimate objective is to create directed structures with composites, which will serve as the first step toward creating structures that resemble bones.

III. METHODOLOGY:

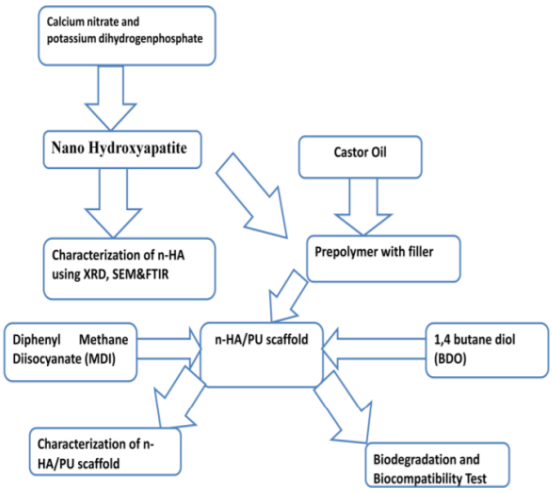

Fig 2: Methodology Flow

STEP 1: Synthesis of Nano Hydroxyapatite using Sol-gel method:
By using the sol-gel method, Nano-HA will be made from calcium nitrate and potassium dihydrogen phosphate as calcium and phosphorus precursors, respectively.

STEP 2: Characterization of nano-hydroxyapatite:
X-ray diffraction will be used to determine the phase composition and crystallinity of the nano hydroxyapatite powder obtained through the sol-gel method. The chemical groups and potential bonding are tested using Fourier transform infrared absorption. Scanning electron microscopy (SEM) and transmission electron microscopy will be used to examine the scaffold's surface morphology, particle size, and pore size (TEM).

STEP 3: Functionalization of nano-HAP particles
Characterization of prepared unmodified and modified hydroxyapatite nanocrystallites for phase composition, morphology (e.g. SEM, TEM), structure (e.g. XRD), and composition (e.g. FTIR) and surface modification of synthesised hydroxyapatite nanoparticles using coupling agents and surface modification of synthesised hydroxyapatite nanoparticles using coupling agents (e.g. contact angle)

STEP 4: Synthesis of n-Hap/PU scaffold:
Create an n-HA/PU scaffold utilising a foaming technique using castor oil as the soft segment and nano hydroxyapatite as the filler at various weight percentages. First, castor oil and n-HA will be combined in a nitrogen-free environment, and then TDI will be added at the desired temperature. BDO will be introduced drop by drop.

STEP 5: Characterization of n-Hap/PU scaffold:
X-ray diffraction (XRD), Fourier transform infrared absorption (FTIR), and scanning electron microscopy (SEM) methods will be used to examine the n-HA/PU scaffold. In-vitro biocompatibility, TEM, and biodegradability using Simulated Body Fluid (SBF)

IV. MATERIALS AND METHODS:

Material
Calcium hydroxide (Ca(OH)2) and ammonium dihydrogen phosphate ((NH4)H2PO4) from Merck (India) and polyurethane from Sigma Aldrich were utilised in this investigation. The solvents were distilled water, ethanol, and N,N-dimethylformamide (DMF).

Synthesis of nano Hap
The sol-gel technique was used to create a combination of FHA nanoparticles containing 50 percent HA and 50 percent FA. As Ca, P, and F precursors, calcium nitrate, TEP, and ammonium fluoride (NH4F) were employed. TEP was first hydrolyzed in ethanol with a little quantity of distilled water to make FHA nanopowders. The OH/P molar ratio was held constant at 1/6. After that, a suitable amount of ammonium fluoride powder was added to the TEP solution and stirred for 24 hours. The necessary quantities of TEP solution were added dropwise to the calcium nitrate solution to obtain a stoichiomet-ric ratio of Ca to P of 1.67. This solution was agitated for 1 hour before being aged for 24 hours at 25°C and then for 72 hours at 40°C. 22 The mixture was dried at 80°C in a vacuum oven. Finally, the powder samples were heated in the air for 1 hour at 550°C.

Synthesis of PU/Hap nano composites
Solid–liquid phase separation and subsequent freeze-drying were used to make pure polyurethane (sample control) and nanocomposite foams containing 5, 10, and 20% wt percent nFHA. The initial step was to make a PU solution in dioxane with a volume ratio of 3% (w/v). Appropriate quantities of nFHA powders were added to the polyurethane solution after homogenous mixing for 24 hours. The mixture was agitated for 30 minutes before being quickly cooled to a temperature of 5°C. For solvent sublimation, the samples were freeze-dried under vacuum (0.1 mbar). Finally, the porous samples were dried at room temperature in a vacuum oven at 40°C until a consistent weight was achieved.

Synthesis of polyurethane
A two-step procedure was used to make the polyurethane. The PCL and HMDI were mixed together in the first stage, and the mixture was

reacted at 80°C in a glass reactor under nitrogen environment for 4 hours to form a viscous prepolymer. The stoichiometry ratio of HMDI to PCL was calculated to be 6:1. In the vacuum oven, the surplus HMDI was distilled under decreased pressure at 80°C. The prepolymer was then reacted with BDO at 80°C for 30 minutes under nitrogen environment in the second stage. In distilled water, the polymer was submerged. To eliminate unreacted monomers, the polymer was precipitated in an 80 percent ethanol solution at 37°C for 48 hours. Finally, the polymer was dried for 24 hours under vacuum at 40°C.

V EXPERIMENTAL PROCEDURE:

The wet chemical technique was used to make hydroxyapatite nanoparticles. 500 mL of 0.4 mol diammonium hydrogen phosphate (pH 4.0) was rapidly agitated at room temperature in a 2 L beaker, then 500 mL of 0.6 mol calcium nitrate tetrahydrate (pH 7.4) was added drop-wise over 4 hours. 0.1 M sodium hydroxide was used to keep the pH of the system at 10.8 during the stirring procedure. Overnight, the mixture was allowed to stay stirred. It resulted in the formation of a white precipitate. The precipitate was vacuum dried and cleaned three or four times with distilled water and ethanol at the same time. The powder that had been produced was used for additional testing. Figure 1 depicts a schematic representation of the method. According to Bouyer et al., Yagai and Aoki were the first to propose this precipitation process for the production of hydroxyapatite nanoparticles (2000).

CONCLUSION:

In comparison to plain polyurethane scaffolds, cytocompatibility and cell survival were considerably enhanced using ED-nHA/PU and TEA-nHA/PU scaffolds, according to the study. These novel nanocomposite scaffolds featured surfaces that improved cell development in vitro cell culture studies, and they may be used to efficiently regenerate tissue (bone) in vivo. The researchers also discovered that ED surface modified nHA based PU scaffolds had greater cell survival and proliferation than raw plastic/plane surfaces, and that TEA-nHA/PU scaffolds offered a better environment for healthy cell development. The research may be expanded to include more advanced advances in the field of tissue engineering, as well as some of the significant research projects that could be performed in the near future.

Future work:

1.The same study may be expanded by using various capping agents.

2. Scaffolds promote cell development, which can be used to regenerate tissue (bone) in vivo.

3. Various manufacturing methods for synthetic scaffolds, such as phase separation, electro spinning, and particle leaching, can be employed.

4. Scaffolds made of PLA, PE, and PMMA can be used to prolong the research.

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