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A NOVEL HYDROTHERMAL SYNTHESIS OF HYDROXYAPATITE NANOTUBES USING *CEIBA PENTANDRA* (KAPOK) AS TEMPLATE FOR BIOMEDICAL APPLICATIONS

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Biotemplates from natural origin as a new source for designing and fabricating tubular rnanostructures has potential applications in the field of nanomedicine. In this study, a facile, surfactant free and controllable size of hydroxyapatite(HAp) of tubular morphology was produced using hydrothermal method with kapok as natural template. After heat-treatment at 900 °C, powders showed a hollow tube of HAp with an average inner radius of 10 nm and length of several hundred nanometers. This was mediated through nanofibrillated cellulose with diameter of 10 nm and microfibrillated cellulose of diameter 40 nm, a constituent of kapok fibre. The biocompatibility of the prepared nanotubes was tested against HeLa cell lines using MTT assay. The Holoxan drug release profile of synthesized biocompatible nanotubes showed that the nanotubes served as drug carrying vehicles for hard tissue repair in bone cancer treatment and bone regeneration.

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1. Introduction

Bone defects are one of the primary causes of disability in elderly patients, resulting in poor health [1]. According to recent statistics, around 2.2 million bone graft surgeries take place annually all over the world and the number of graft surgeries gradually increases every year [2-4]. In general,forbone repair treatment, the materials should possess excellent compatibility, biodegradability and non-toxic and non-immunogenic response to bone tissue [5-8]. Among the materials, Hydroxyapatite (HAp) acts as a promising candidate for bone tissue repair because of its resemblance to the mineral constituents of human bones and teeth [9, 10]. HAp is highly useful in reconstruction of damaged bone due to its excellent biocompatibility, bioactivity, osteoconductive potential, slow degradation, non-inflammatory, non-cytotoxicity and non-immunogenic properties [11,12].Hydroxyapatite nanoparticles have been synthesized by different methods such as sol-gel [13], mechanochemical [14], combustion [15], precipitation [16], solvothermal [17], sonochemical precipitation [18], hard-template method [19] and hydrothermal method [20].

Synthesis of nanodimensional structures with controlled size and shape,especially hollow structures like nanotubes is considered as a challenging area in nanotechnology especially hollow structures like nanotubes [21]. Yuan et al synthesized HAp nanotubes with a diameter of 100 nm using porous alumina template [22]. Balasaheb et al employed porous anodic alumina template to form calcium phosphate based tubular structures with diameter ranging from 39 to 330 nm and HAp nanotubes with diameter ranging from 140 – 350 nm [23,24]. According to Lester et al, tubular morphology of HAp was produced with an inner diameter of 30-70 nm using a continuous-flow hydrothermal reactor which is more complex and laborious [25].Template mediated synthesis

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of nanomaterials with defined structures is a low cost, easy and flexible method. After the desired material is grown over the templates, the templates area removed completely to form freestanding nanostructures [26].Recently, using kapok based cellulose fibers for synthesis of various nanostructures has become a growing interest due to its advantages such as cost-effectiveness, non-toxicity, biodegradability and light weight properties [27].

In this work, we have used a novel hydrothermal synthesis of HAp nanotubes using kapok fibers as natural templates. The hydrothermal treatment was set up at 180 °C for the duration of 20 hrs at 18 atm pressure. The entire reaction was carried out under nitrogen atmosphere. The morphologies of the samples that were prepared by hydrothermal method were investigated byPowder X-Ray Diffractomter (XRD), Fourier Transform Infrared spectrometer (FTIR), Field Emission- Scanning Electron Microscope (FE-SEM), Transmission Electron Microscope (TEM). From the various analyses, we report the successful formation of HAp nanotubes with average inner radius of 10 nm and length of several hundred nanometers. Moreover, these HAp nanotubes were found to be biocompatible in HeLa cell lines. The synthesized hollow HAp nanotubes can be employed as an efficient nanocarrier for bone tissue engineering applications.

2. Experimental Section

2.1 Materials

Calcium nitrate and di-ammonium hydrogen phosphate were purchased from Himedia. Ammonia solution was purchased from Merck. All chemicals were analytical grade and used as received without further purification.

2.2 Preparation of raw fibers

Kapok fibers were isolated from the matured fruit and washed with distilled water and organic solvents to remove the dirt and impurities. Then they were air-dried to remove the moisture content.

2.3Preparation of HAp nanotubes

Kapok fibers were immersed in calcium nitrate solution of 1mol/l for 1 hour. Di ammonium hydrogen phosphate solution of 0.6mol/l was then added to the above solution under constant stirring and pH was adjusted to 10 by using ammonia solution. The hydrothermal treatment was set up at 180 °C for 20 hrs duration at 18 atmospheric pressure. The recovered samples formed into three layers and the top most floating layer was isolated for further analyses. The samples were recovered and dried at 80 °C overnight followed by calcination at 900 °C for 2 hrs. The samples were finally washed with ethanol to remove the presence of impurities.

3. Characterization

The XRD patterns were recorded using Panalytical, Netherland & X'pert Powder Diffractometer. Fourier Transform-Infrared spectrometer (Schimadzu, Japan) was used to analyze the spectral assignments of the prepared nanotubes.The JEOL JEM 2100 High Resolution Transmission Electron Microscope (HRTEM),Carl Zeiss Microscopy Ltd, UK and Sigma Field emission Scanning Electron Microscope (FESEM) were used to analyze the morphologies of the prepared samples.The human cervical cancer cell line (HeLa) was purchased from National Centre for Cell Science (NCCS), Pune. It was grown in Eagles Minimum Essential Medium with 10% fetal bovine serum (FBS) and the cells were incubated at 37 °C, 5% CO_2 and 95% air.

4. Results and discussion

4.1 Powder X-Ray Diffractomter (XRD) Analysis

Fig. 1 shows the X-ray diffraction patterns of HAp nanotubes. The XRD patterns showed a strong peak at around 32.2° corresponding to (211) planes of HAp crystalline structure and the other characteristic peaks were at (101), (200), (002), (112), (202), (310), (222), (303). The obtained samples were in good agreement with the XRD pattern of a HAp standard available in JCPDS (09-0432).

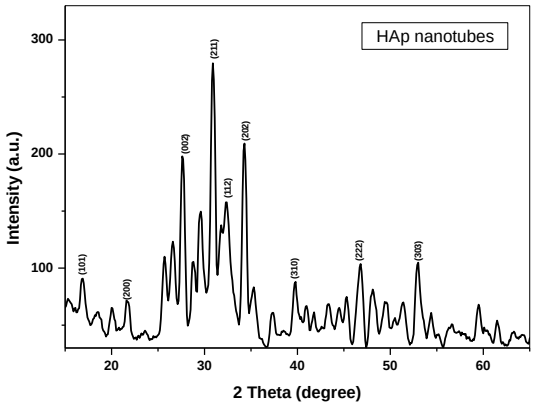

Fig. 1: X-Ray diffraction patterns of HAp nanotubes

4.2 Fourier Transform Infrared (FTIR) Analysis

Fig. 2 represents the FTIR spectral assignments of the prepared HAp nanotubes.The peak at 3568 cm^{-1} was assigned to O-H stretch. The intense broad peak at 1041cm^{-1} corresponds to phosphate group (PO_4^{3-}). The peak at 723 cm^{-1}may be due to the OH group of HAp. The peak at 570 cm^{-1} was due to asymmetric bending vibration of PO_4^{3-} [28].

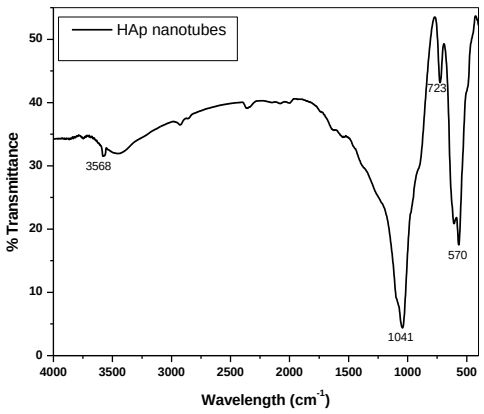

Fig. 2: FTIR spectra of the HAp nanotubes

4.3 Field Emission- Scanning Electron Microscope (FE-SEM) images of Kapok fibers

The kapok fibers were processed under hydrothermal condition at 180 °C for 24 hrs. After the treatment, most of the fibers became completely flattened which was due to the removal of oxygen (fig. 3).The EDAX results showed that the raw kapok fibers consisted of carbon and oxygen.

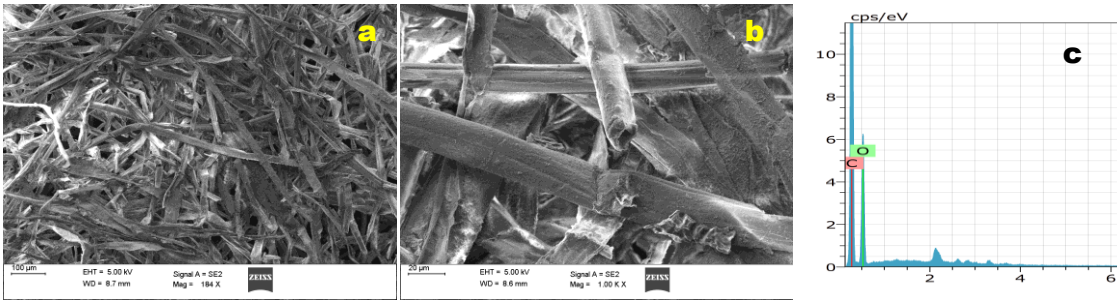

Fig. 3: a, b) FE-SEM images of raw kapok fibers, c) EDAX

4.4 Transmission Electron Microscope (TEM) and FE-SEM images of HAp Nanotubes

The schematic representation for the synthesis of HAp nanotubes is shown in Fig. 4. Nanofibrilated cellulose (NFC) is finer cellulose fibrils that consists of 36 cellulose chains arranged in 1β crystal structure with diameter of 10 nm [29].

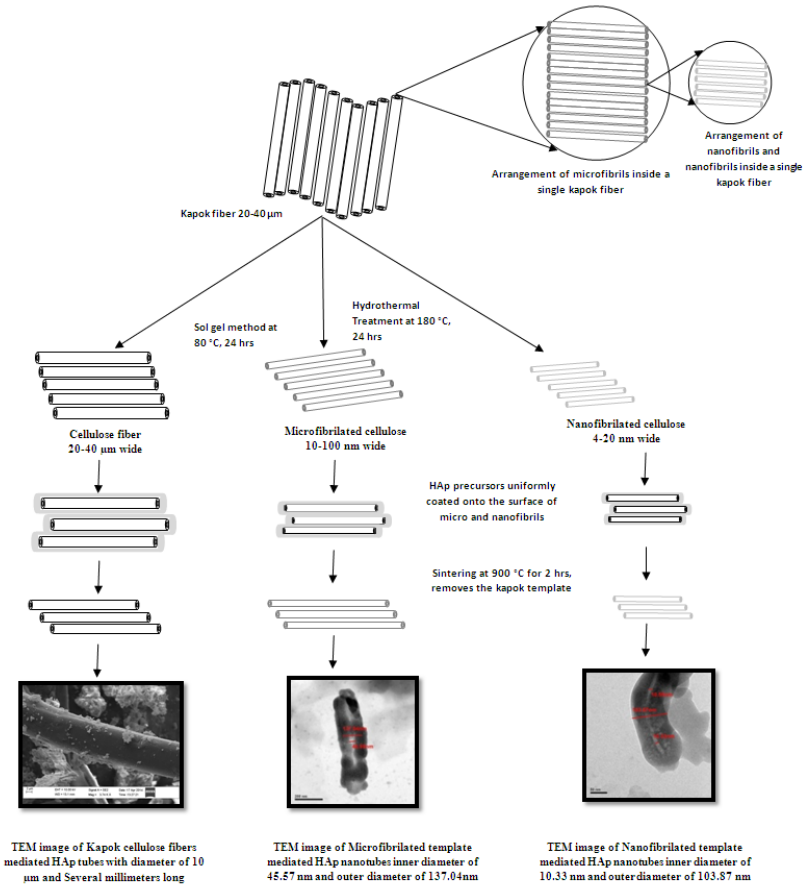

Fig. 4: Schematic presentation of the preparative steps for the creation of HAp nanotubes

The NFC's are obtained from cellulose fiber due to disintegration and found to be negatively charged when dispersed in water between pH ranges of 2-10 [30]. The carboxyl/hydroxyl group of the NFC facilitates the attachment of Ca and P precursors for the formation of hydroxyapatite. Thus the deposition of HAp nanoparticles onto the NFC surface was

achieved by electrostatic interactions.Fig. 5a and 5b shows tubular structure of HAp. Fig. 5c shows energy dispersive X-ray analysis (EDAX) of Calcium (Ca) and Phosphate (P) in the final product. After calcinations at 900 °C, the NFC organic template was removed and hydroxyapatite nanotubeswere obtained; it maintained the size and structural integrity of HAp nanotubes at an inner diameter of 10.33 nm and outer diameter of 103.87 nm which confirms the formation by nanofibrillated cellulose (NFC) templates.

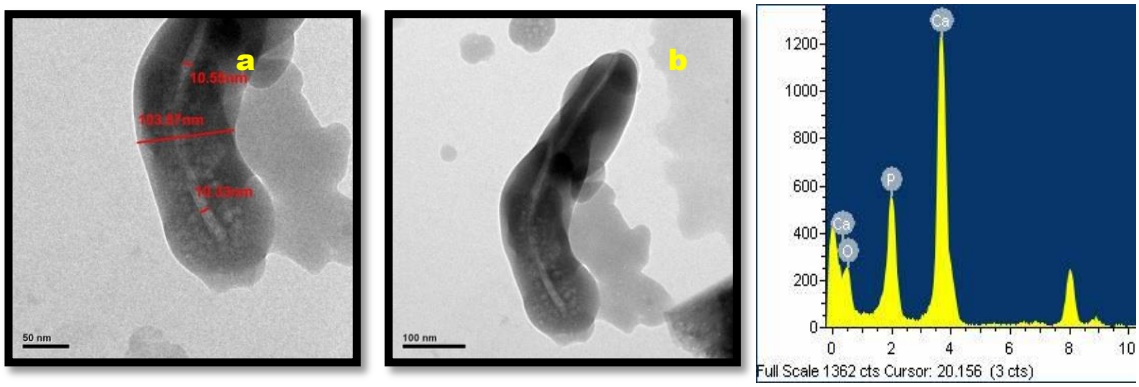

Fig. 5: a, b) Nanofibrilated template mediated HAp nanotubes at different magnifications c) EDAX

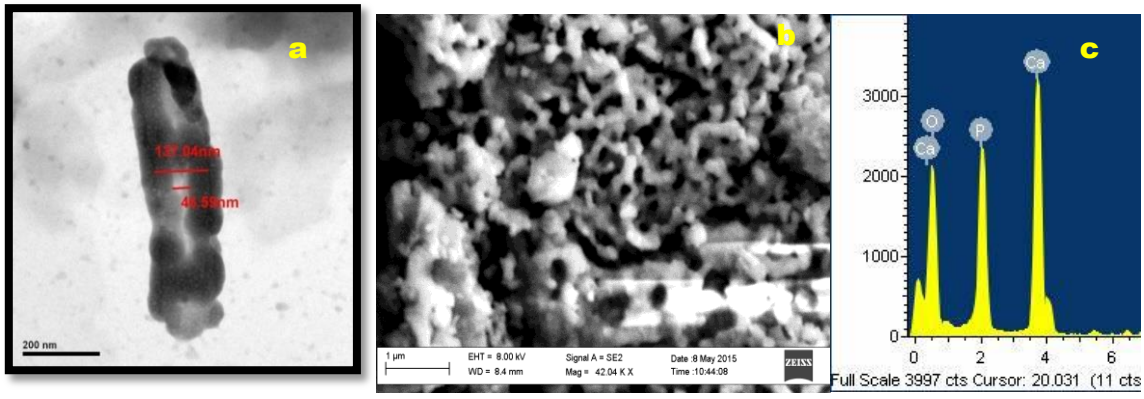

Fig. 6: a) TEM images b) FE SEM images of microfibrilated template mediated HAp nanotubes c) EDAX

Microfibrillated Cellulose (MFC) consists of disintegrated microfibril aggregates with 36 cellulose chains arranged in 1β crystal structure which are 50 nm in diameter. MFC's have high-aspect-ratio fibrils and form strong networks due to fibrillar entanglements. Fig. 6a and 6b shows the formation of HAp nanotubes templated by microfibrillated cellulose (MFC) and fig.6c shows the EDAX spectra of HAp. [29,31,32].The obtained HAp nanotubes have an inner diameter of 45.57 nm and outer diameter of 137.04 nm which confirms the formation by MFC.

4.5 Invitro cytotoxicity study

The MTT assay was performed for HAp nanotubes to evaluate cytotoxicity against HeLa cell lines [33]. The morphology of the cells after exposure with samples for 48 hrs is shown in fig. 7. From the figure, it is clear that the morphology has not been affected and MTT assay reveals atmost identical cell viability with respect to control, which indicates that the compounds does not show any cytotoxic effect (fig. 8).

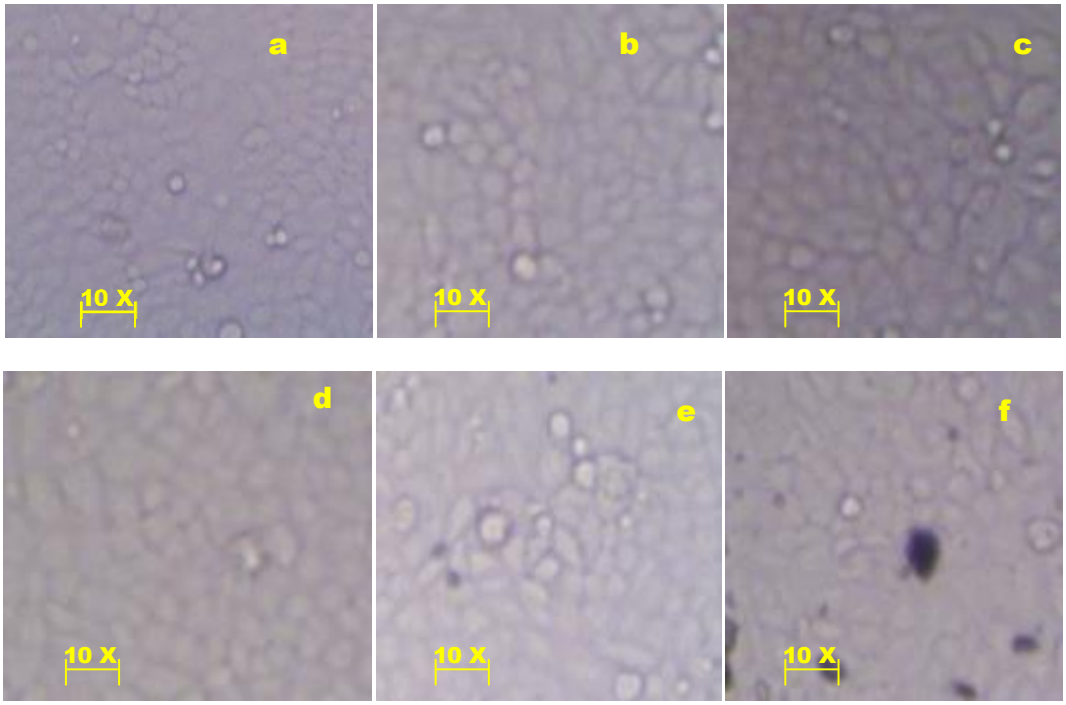

Fig. 7: Photomicrograph (10 X) of HeLa cells after exposed with HAp nanotubes at different concentrations for 48 hrs ((a) Control b) 0.25 µg c) 2.5 µg d) 25 µg e) 50 µg f) 100 µg)

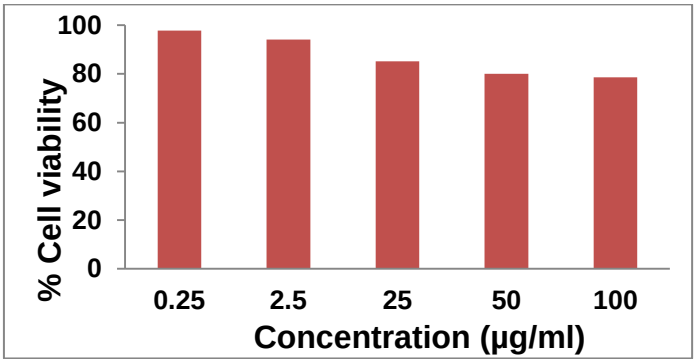

Fig. 8: cell viability by MTT assay

4.6 Holoxan combined with Uromitexan loaded HAp nanotubes

In brief, 0.1 g of HAp nanotubes and 625 µl of (Holoxan combined with Uromitexan) drug were dispersed in 30 ml phosphate buffer solution (PBS). The solution was stirred continously at 4 °C for 3 days. The drug loaded nanotubes were separated from the solution and used for invitro drug release experiment.

4.7 Invitro Drug Release Experiment

In order to study the drug release, the drug loaded nanotubes was sealed in a 30 ml PBS buffer solution and the experiments was performed at room temperature and pH level of 7.4. The release medium (about 3 ml) was withdrawn at the time interval of (0,1,2,3,4,5,6,7,8,21,24,27,30,46,49,52,70,73,76,95,100hrs). The samples were analyzed using an ultraviolet-visible spctrophotometer to determine the amount of (Holoxan combined with Uromitexan) drug released from the HAp nanotubes at (λ 270 nm). The results showed that about 60 % of the drug was released in a sustained manner within the duration of 100 hrs (fig. 9).

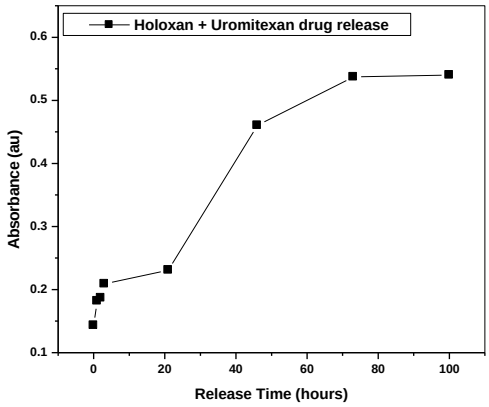

Figure 9: Drug-release profile of Holoxan combined with Uromitexan in PBS solution at room temperature

5. Conclusion

In summary, we have synthesized HAp nanotubes by hydrothermal method assisted by kapok fibrils templates. The prepared nanostructures are of high homogeneity and high purity without any crystalline defects. Hydrothermal method of synthesis produced Hap nanotubes in the range of 10 nm in diameter and controllable size.The synthesized nanotubes were biocompatible with HeLa cells which make them ideal for biomedical applications.

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