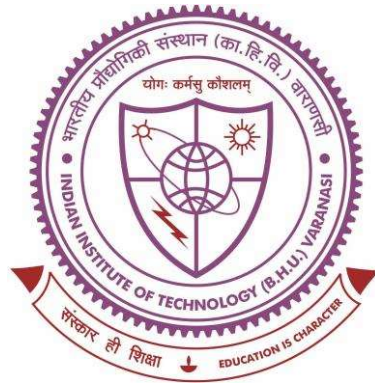


1- and 2-Dimensional (D) Hydroxyapatite Nanocrystals: Synthesis, Growth Mechanism, and Their Effects on Osteogenesis, Antibacterial Activity, and Drug delivery



Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

by

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CERTIFICATE

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Preface

The present thesis has been divided into seven chapters. Chapter 1 introduces the potential of biomimetic materials, particularly hydroxyapatite (HA) nanocrystals, as an alternative to traditional implant biomaterials. Moreover, this chapter provides critical and comprehensive reviews of various synthesis routes for the development of 1- and 2-dimensional (D) HA nanocrystals with a focus to understand the process science. In addition, a systematic discussion has also been made on bone tissue engineering and drug delivery applications of HA nanocrystals, with special emphasis on several essential physical and experimental parameters to achieve desired performance of HA nanocrystals. Toward this end, the motivation behind selecting the 2-D HA nanoplates as the primary research focus, compared to other HA nanocrystal morphologies, is also discussed. Chapter 2 presents the methodology for the development 1- and 2-D HA nanocrystals along with various characterization techniques, such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, Field emission electron microscopy (FESEM), energy dispersive X-ray spectroscopy (EDX), Transmission electron microscopy (TEM) to confirm the phase, morphology, atomic composition, and porosity of the samples. This chapter also provides the drug loading and release experiments, performed on HA nanocrystals, details of various protocols, adopted for the assessment of cellular and antibacterial response of different morphological HA nanocrystals before and after drug loading. Chapter 3 discusses the phase and morphological analyses of HA nanocrystals along with the profile fitting of XRD peaks using FullProf software to reveal the variation in lattice parameters as per the morphology of HA nanocrystals. The closer analyses using TEM have also been carried out to observe the porous nature and crystallographic crystal growth direction of HA nanocrystals. Additionally, this chapter also explores the possible molecular mechanism, controlling the growth of different morphological HA nanocrystals. Chapter 4 focuses on cytocompatibility and

osteogenic activity of 1- and 2-D HA nanocrystals to evaluate the significant variation in their biological response. Moreover, the influence of the morphology of HA nanocrystals on their surface oxygen vacancy, surface charge, and wettability is analyzed using X-ray photoelectron spectroscopy (XPS), zeta potential, and contact angle measurement, respectively. Additionally, the variation in ion release behavior of HA nanocrystals has been explained based on their crystallinity. Further, the role of surface charge, hydrophilicity, ion release, and the size of different morphological HA nanocrystals on hemocompatibility, cell adhesion, proliferation, and differentiation has also been thoroughly discussed. Chapter 5 investigates the antibacterial activity of 1- and 2-D HA nanocrystals using *E. coli* and *S. aureus* bacteria. The antibacterial activity of various concentrations and morphologies of HA nanocrystals is evaluated using a number of quantitative and qualitative techniques. The bactericidal effect of HA nanocrystals is assessed through the quantification of reactive oxygen species (ROS) generation ($O_2^{\cdot-}$ and H_2O_2) within bacterial cells using superoxide dismutase (SOD) and catalase assays. Also, the ROS-induced damage to cellular components like proteins and lipid membranes is evaluated utilizing Lowry protein and lipid peroxidation (LPO) assays. In addition, the detailed mechanism behind the morphology-dependent antibacterial activity of HA nanocrystals has also been explored by considering various factors, such as concentration, size, ion release, particle agglomeration, crystallinity, and surface charge. Chapter 6 explores the antibacterial potential of antibiotic-loaded 1- and 2-D HA nanocrystals with a detailed study of their drug loading and release behavior. The successful loading of the drug onto HA nanocrystals is validated through XRD, FTIR, and electron microscopies. Moreover, the nature of the interaction between DOX and differently-shaped HA nanocrystals are revealed using XPS. The *in vitro* release dynamics of drug from HA nanocrystals are also evaluated as per varying morphology of HA nanocrystals. Further, *in vitro* cell culture and hemolysis assays have been conducted to assess the biocompatibility, including cell viability and hemocompatibility of

drug-loaded HA nanocrystals. Finally, the impact of the sustained release of drug from HA nanocrystals on antibacterial efficacy have also been thoroughly examined, compared to pure drug. Chapter 7 provides the conclusions and future perspectives of this study.