ABSTRACT

According to the International Osteoporosis Foundation in India, approximately 26 million osteoporosis patients were reported in 2010 and this figure is expected to reach 36 million in future. Autografts, allografts and xenografts bone substitutes have their limitation such as lack of availability, immunogenicity and zoonotic disease transfer, whereas metal implants may fail due to corrosion, post-operative loosening or failure to osteointegration. These intrinsic problems associated with the currently available clinical strategy led to the introduction of bone tissue engineering as a clinical alternative. Bone tissue engineering (BTE) is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve bone tissue function or regeneration of new bone. The main strategies involved in the development of these substitutes are biomaterial selection (Natural/Synthetic polymer, Ceramic), scaffold fabrication, superior biocompatibility, bioactivity, mechanical property, controlled biodegradation, and tissue-specific differentiation potential.

This thesis aims to design and develop chitosan and gelatin-based nanocomposite scaffolds with the integration of other bioceramics like nanobioglass, tricalcium phosphate and nano-hydroxyapatite and nanoparticles like copper and graphene oxide to enhance their mechanical properties and bioactivity using the lyophilization process.

Lyophilized porous nanocomposite scaffolds were developed from Ch/G blends. Among the different blend ratios, 2:7 w/w of Ch/G was the optimal concentration for achieving a set of superior scaffold properties in comparison to pure Chitosan scaffold. These scaffolds were incorporated with different ratios of CN (0.01%, 0.02% and 0.03%) to examine their application for tissue engineering application. The CN was synthesized using a chemical reduction approach and it was characterized before being applied in the study for its morphology (SEM, TEM), and structural and functional characteristics (XRD, FT-IR). The developed scaffolds were characterized by their morphology (SEM), structure (XRD, FT-IR), surface properties (including swelling ratio and biodegradation), porosity and mechanical strength. The average pore diameter was determined to be 71μm. These scaffolds showed enhanced porosity of 80% after the addition of CN, improved swelling index and biodegradation, and enhanced mechanical activity of 3.12 ± 0.21MPa in the case of 0.03% CN scaffold but it did not show enhanced metabolic activity in comparison to the control scaffold (Ch-G). While the addition of 0.02%CN into scaffolds resulted in 2.75 ± 0.02MPa and for 0.01% it was observed to be 2.27 ± 0.045MPa which was comparatively better in comparison to the control(1.7 ± 0.11MPa) scaffold. However, the scaffolds with lesser percentage of CN showed better metabolic activity of cell and they still retained sufficient strength for supporting various types of tissue regeneration. Among all these

scaffolds, Ch-G-0.02CN was chosen for further study due to its better mechanical strength and biocompatibility.

To enhance the osteogenic potential of this scaffold and the mechanical strength, nanohydroxyapatite (nHAP) was further added into this scaffold at 3.5%(w/v) concentration which led to the development of Ch/G/nHAP/CN scaffolds which was studied for its efficacy for BTE applications. The porosity of the developed scaffolds was between 80-85% which was approximately same to the control scaffolds with 87% porosity. Among the different concentrations of CN tested, Ch/G/nHAP with 0.03% CN showed the optimal compressive strength of 3.5 ± 1.23 MPa which was again higher in comparison to the previous study yet the ALP activity of MG-63 cells on Ch/G/nHAP/0.03%CN was significantly ($p < 0.05$) lower than that of 0.02%CN throughout the culture period. It was almost similar for both 0.01%CN and 0.02%CN but it was comparatively lower than the control scaffolds. The cell adherence of MG-63 cells and their growth on the surface of 0.03%CN revealed lesser metabolic activity in comparison to the other CN-based scaffolds. Therefore, these results prove that the Ch/G/nHAP/0.02%CN composite scaffold provides a superior osteogenic platform for MG-63 cells when compared to the previous study.

Further efforts were made to enhance the mechanical strength, porosity and bioactivity of the Ch/G scaffolds by incorporating NBG and β-TCP in three different ratios (0:1,1:0,1:1) where the percentage of the additives was kept at 6% as constant(w/w). Among the various compositions tested, it was observed that the Ch-G-NBG-TCP scaffold exhibited the best osteogenic properties compared to the control scaffold (Ch/G). However, the mechanical strength of the scaffolds were not as good as the previous study. The compressive strength for Ch-G, Ch-G-NBG, Ch-G-TCP and Ch-G-NBG-TCP scaffolds were 2.1 ± 0.010, 2.25 ± 0.013, 2.5 ± 0.016 and 2.7 ± 0.045MPa respectively. It is evident that both TCP and NBG incorporation led to improved mechanical strength but when we compare this to the previous study CN incorporated scaffolds exhibited higher compressive strength. Although, the porosity achieved here was comparatively similar to the previous study also yet it can be inferred that addition of NBG and TCP improved the bioactivity and porous network but reduced the mechanical strength which was the aim of this research. Therefore, further efforts were made using NBG with a Ch/G blend to enhance the mechanical strength of the scaffolds using GO nanoparticles. Go nanoparticles were characterized and they were added into the Ch-G-NBG composite in different percentages (30%,60% and 90%) to enhance the mechanical properties of the scaffold (Ch-G, Ch-G-NBG-30%GO, Ch-G-NBG-60%GO, Ch-G-NBG-90%GO) without hindering the osteogenic properties of the scaffold. The morphological characterization and structural and functional characterization of the scaffolds were also performed to assess their physicochemical properties. Also,

their swelling ratio, biodegradation, mechanical property and porosity were examined which demonstrated that Ch-G-NBG-60%GO exhibited optimal swelling and degradation in comparison to Ch-G-NBG-90%GO scaffold. The swelling ratio reduced from 4.9 to 4 in the case of Ch-G-NBG-90%GO and the degradation reduced to almost 20% from the control scaffold. However, the porosity was similar for all the GO infused scaffolds between 75-77% but the mechanical strength improved to 8.5MPa which was the best among all the other developed scaffolds including the previous studies also. Furthermore, the cell viability of MG-63 was tested on these scaffolds which suggested some toxicity effect on the scaffold's surface and therefore to confirm it we further performed ALP activity test which again showed similar kind of results. This proved that Ch-G-NBG-GO scaffolds with 60% GO concentration and Ch-G-nHAP-0.02%CN are two promising artificial extracellular matrix with enhanced mechanical strength, bioactivity and osteogenic properties, making them strong candidates for future bone tissue regeneration applications.

PREFACE

Tissue engineering has emerged as a solid approach to bone tissue regeneration. It has helped in developing artificial bone substitutes that can overcome the limitations of existing bone disease therapies. Bone tissue-engineered constructs can completely reproduce the characteristics of a graft, hence ending the patient's discomfort. Tissue engineering of biological bone focuses on regenerating new functional tissue that successfully merges with the host without generating any adverse effects. In recent years, research in tissue regeneration has focused on developing biomaterials for replacing, regrowing, or restoring damaged cells or tissues. These biomaterials serve optimum support and act as a framework for cellular attachment, replacement and ingrowth of tissues. The literature review suggests that several materials are utilized for bone tissue restoration or regeneration, like natural and synthetic materials. Biomaterials with proteins have found primary utilization in various industries and areas like biosensors, food and agricultural industry, drug delivery mechanisms, pharmaceutical drugs, contact lenses, bone plates, etc. These biomaterials include natural materials like chitosan, gelatin, collagen, alginate, chondroitin sulfate, etc. which exhibit good biocompatibility and biodegradation properties. Similarly, synthetic materials are also there like polylactic acid, polyvinyl chloride, polyglycolic acid, etc. The synthetic materials possess good mechanical strength but there exist some limitations like poor cell cytocompatibility, poor degradation rate, etc. Therefore, both these biomaterials are used in composite forms to attain the desired properties in achieving good cell proliferation and physiochemical properties.

Chapter- 1 focuses on the introduction of tissue engineering and its significance, key challenges in bone tissue engineering and different strategies for bone tissue regeneration. It discusses porous scaffold fabrication and its need in current bone repair technology.

Chapter- 2 consists of a literature survey of bone, its types and the cells involved in its formation. It discussed the various scaffold fabrication techniques and the scaffold's properties. It also covers the requirement of bone tissue engineering in the current scenario.

Chapter- 3 represents the detailed experimental methods and materials involved in this research study. It also gives a detailed description of the instruments involved in this research.

Chapter- 4 discusses the development and evaluation of different scaffolds developed for Bone tissue regeneration. Their efficacy and properties are elaborated via various examinations and tests performed. It includes the chitosan and gelatin-based scaffold with the incorporation of different bioactive nanoparticles. Among all the developed scaffolds, it was observed that the best-optimized scaffold was graphene oxide and nanobioglass-based scaffold (Ch-G-NBG-60%GO) with the highest mechanical strength and enhanced biocompatibility among all the other scaffolds and also CN based Ch/G/nHAP/0.02%CN scaffold with good mechanical strength as well as cytocompatibility.

Chapter- 5 gives the conclusion and future perspective for the conducted research work in the area of Bone tissue engineering