Chapter 1. Introduction

1.1 Background

A combination of remarkable strength, stability and structure defines the hybrid connective tissue, Bone. This natural biomaterial plays a supportive and structural role in regulating the quality of life [1].

Elderly patients represent the large fraction of the world population facing bone related disease and disability. Due to a large population of patients experience bone related defects or diseases, driving effort in developing novel biomaterial for bone tissue integration. In recent years, a large number of patients have suffered from bone related tauma or injury. It dramatically cater the need of regenerative bone practices to support improvement in the overall health and quality of life [2]. Investigations are in progress in developing new material and processing techniques favoring bone tissue regeneration [3-5].

This supports in reducing the mismatch between current *in vitro* and clinical approaches [6]. In recent clinical practices, related to bone regrowth, grafts have been used as defect substitute [3]. In the last ten years the common bone reconstruction practice was to incur autografts for orthopaedic related difficulties [7]. Autografts represents an excellent substitute, due to inbuilt non-immunogenicity and osteoconductive-osteoinductive nature, but still have limited usage due to shortage in availability and donor site morbidity [8]. Using the reamer-irrigator aspirator (RIA) technique as the conventional approach in

clinical setting, the patient bone tissue was collected from the injured defect-site or from the iliac crest of the patient intact long bone [9]. With success rates touching ~90%, autografts suffer limited availability and donor implant site morbidity [10]. However, allograft poses risk due to immunological illness. For years the prosthetic orthopedic implant integration in the surrounding healthy tissue had raised question due to mismatch with the host bone. It lead the focus on alternate available procedures to reconstruct bone after trauma, tumour resection, and congenital diseases [11]. This led to the development of approaches exploiting the bone tissue regeneration via tissue engineering.

1.2 Significance of Tissue Engineering

Tissue-Engineering was given by Langer and Vacant, 1993 [12] states Tissue Engineering (TE) involves controlled stimulation of target cells via systematic combination of molecular and mechanical signals [13]. TE has been a multidisciplinary research for the therapeutic reconstruction by the creation of novel tissue substitutes.

As a promising alternative, tissue engineering of bone evolved by involving materials that induce bone formation in response to the neighbouring tissue [12]. The materials play template for the seeded bone cells, supporting cell attachment, proliferation, migration and extracellular matrix (ECM) production [14]. Bone tissue engineering revolutionizes the regeneration of bone to prominently improve the life of patients with severe bone defects [8, 12]. In the regeneration of an injured organ, many tissue engineered biodegradable yet bio-mineralised biomaterial based composites were proposed.

In bone tissue engineering, different techniques have been combined along with different system [15]. The easiest approach was to deliver the tissue-inducing signalling molecules to the place of injury or disease. The different techniques rely either directly on the primary cell line (e.g. bone cell) or on the primitive cell substitutes (e.g. MSCs and ESCs). Using these cells in combination with the different materials as matrix substitution was followed

to mimic and preserve the function of the natural intercellular space without inviting the immunological reactions. Cells loaded materials were used as the open or closed system. The cells anchored in the material and integrated into the injured tissue have been defined as the open system. Whereas, in the closed system the cells were encapsulated in the matrix, allowing only nutrient-waste exchange but were isolated from the host immune system.

The materials and methodologies used in the bone regenerative studies were corresponding to the techniques used in bone bioengineering or tissue engineering.

1.3 Scaffolds for Bone tissue Engineering

During the past years, an extensively studied class of biomaterials has evolved in bone tissue engineering from both natural and synthetic in origin [16], and when seeded with donor bone cell for use as bone graft stand a chance in successful bone repair.

It is interesting to develop composite mimicking native ECM because it is inspired by bone architecture [17]. Scaffold raw materials mimicking ECM component properties be an effective strategy in restoring majority of bone related deformities. Hence for the scaffold, combined advantage of every raw material contributes in improving the scaffold behaviour. The ‘inorganic-organic’ ECM based structures defined by the bone ECM where non-stoichiometric hydroxyapatite (Ha) crystals based inorganic phase embedded in-between the collagen I based organic phase [18-19]. Recent work on combining organic, inorganic and ceramic materials by freeze drying has shown significantly improved practical applications (namely osteogenic behaviour, bioactivity, biocompatibility and mechanical properties) [20-24].

1.4 Objectives of this study

The focus of this study was the comparison among the different types of bone cell seeded-scaffolds which were to be implanted clinically as bone substitutes. The scaffold preparation involved the 'gelate freeze-dried' scaffold slurries and subsequent lyophilisation method creating sites for bone cells differentiation and attachment. Bone cell of rabbit and human origin were cultured on these composite scaffolds *in vitro* to determine their applicability for bone tissue engineering. Here, composites were expected to provide information for cell sustainability followed by ECM deposition to meet bone tissue engineering requirements.

The objectives of my research domain are as mentioned below:

1. To design, characterize and evaluate the scaffolds based on various components w.r.t. the natural bone extracellular matrix features.

2. To characterize the optimized gelatin/chitosan/hydroxyaptite based scaffolds *in vitro* using the isolated, grown &/or differentiated osteoblast from both the bone and bone marrow mesenchymal stem cells.

3. To test reproducibility of the osteogenesis achieved in *in vitro* 'bone cell cultured in the scaffold' set-up *in vivo*.

4. To study the behaviour of human osteoblast seeded and cultured in the optimized scaffolds *in vitro*.