CHAPTER 1

Introduction

The above figure (Fig.1.1) is not a creation of any science fiction. This is the famous picture of a tissue-engineered cartilage grown in the shape of a human ear on the back of a nude mouse. Joseph Vacanti and his colleagues did this breakthrough work, using chondrocytes seeded onto a synthetic biodegradable polymer fashioned in the shape of a 3-year-old child's auricle (Cao et al., 1997). This figure kindled the hope of millions that, the age-old dream of laboratory engineered tissue or organs for therapeutic applications will be revolutionizing the healthcare research in near future. Langer and
Vacanti (1993) defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue function or a whole organ".

Large bone defects, resulting from resection of malignant bone neoplasms and trauma; bone fracture in patients with osteoporosis, metastases; and metabolic disorders like diabetes, requires therapeutic interventions to assist and accelerate bone regeneration or replacement. Inadequate response to the fracture injury could sometimes lead to mal-union, delayed union, or non-union, which also require clinical attention. Currently, autologous bone graft is regarded as the gold standard for bone regeneration. The bone tissue for the autologous graft is taken from another part of the patient’s own body and the bone graft provides the lattice, the osteogenic cells and the essential osteoinductive factors required for bone healing and regeneration. Limited source and patient morbidity associated with autologous bone graft demand other alternative therapies for healing bone fractures. In India, it is estimated that approximately 5million fractures occur every year. The current fracture healing therapies are accessible to only a limited fraction of the population. The success stories of mesenchymal stem cells (MSCs) in regenerative medicine and the availability of various biocompatible biomaterials led to the emergence of cell based bone tissue engineering as an alternative method of bone regenerative therapy.

Bone tissue is a dynamic tissue with a moderate regenerative capacity. *In vivo* bone healing or regeneration involves osteogenic cells originating from bone marrow mesenchymal stem cells, regeneration template and regulatory growth factors. Bone tissue engineering applies these principles of natural bone regeneration to create functional bone tissue constructs *in vitro* by using osteogenic cells, biomaterial scaffolds, bioactive growth factors, and specialized culture environments (bioreactors). The
environment supporting \textit{in vitro} bone formation is created using a combination of biochemical and biophysical signals presented to the cells in a three-dimensional culture system that allows cell-cell and cell to extracellular matrix (ECM) interactions. The complexity of signalling pathways regulating bone morphogenesis holds significant challenges to engineering a fully viable and functional bone.

The identification of the multi-potential MSCs in various adult human tissues has provided exciting prospects for the cell-based tissue engineering and regeneration. Since MSCs have the potential to differentiate into osteoblasts, they are widely explored for the development of bioengineered bone constructs using various biomaterial scaffolds. MSCs isolated from human adult bone marrow (hMSCs) are considered as the ideal source of osteogenic cells owing to the ease of isolation, high expansion potential, and the ability to undergo osteoblast differentiation and mineralization.

Scaffold requirements for bone tissue engineering include biocompatibility; osteoconductivity and osteoinductivity; controlled biodegradability; and high porosity with interconnected pores to enable mass transport, infiltration of cells and interstitial flow of fluids. Apart from promoting osteoblast differentiation, the scaffolds should also promote osteointegration. Generally, scaffolds are considered as the \textit{in vitro} replacement of the \textit{in vivo} microenvironment surrounding the cells. Therefore, ideally the scaffold should mimic the \textit{in vivo} scenario and thus should support cell adhesion, provide cell-cell and cell-ECM interactions and supply the signals required for cell proliferation and differentiation.

ECM is mainly made up of proteins and polysaccharides that have long chains of glycosaminoglycans (GAGs). ECM along with various growth factors plays a significant role in regulating the properties and functions of cells \textit{in vivo}. The biomimetic approach
of bone tissue engineering is based on mimicking certain advantageous features of the natural ECM, to facilitate cell recruiting, adhesion, proliferation, differentiation and new bone tissue genesis. Therefore, it is very important to understand the role of various ECM components in hMSCs adhesion, proliferation and osteoblast differentiation. This knowledge will help us in selecting the appropriate ECM components for designing and fabricating ideal biomimetic scaffolds for bone regeneration.

Although, many previous studies report the effect of major ECM proteins such as collagen type I, fibronectin, vitronectin and laminin on hMSC adhesion, proliferation and osteoblast differentiation, most of these experiments vary in the experimental protocols with non-conclusive and contradictory results. In addition, the role of various ECM-GAGs in hMSCs adhesion and osteoblast differentiation is a poorly explored area of research.

Since the *in vivo* environment and conditions that influences the cell behaviour is too complex and less understood, it is not easy to simulate such an environment for an *in vitro* culture system. In this research project, we are trying to understand the role of various ECM components in mesenchymal stem cell behaviour and establish a three dimensional culture system that can closely mimic the *in vivo* environment for targeted hMSC differentiation into osteoblasts. In order to achieve this, we have selected four major ECM proteins (collagen type I, fibronectin, vitronectin and laminin) and five major ECM-GAGs (hyaluronic acid, heparin, chondroitin-4-sulphate, chondroitin-6-sulphate and dermatan sulphate) to study their role in regulating hMSCs culture and osteoblast differentiation and mineralization *in vitro*. We have coated the normal tissue culture plates with various ECM components and evaluated their effect on hMSCs adhesion, proliferation and osteoblast differentiation by various established techniques. We used phase contrast microscopy, trypan blue exclusion test and doubling time assay
respectively for assessing hMSCs adhesion, viability and proliferation. hMSCs were directed to osteogenic lineage by growing them in osteogenic induction media and the differentiation was evaluated by phase contrast microscopy, ALP staining, ALP assay, Alizarin red S staining for calcium, von Kossa staining for calcium, calcium assay and real time PCR for gene expression assay.

Chitosan is a naturally occurring cationic polymer having structural similarity to hyaluronic acid and chondroitin sulphates of ECM. It has anti-bacterial, anti-fungal and anti-viral properties. It is known to accelerate wound healing, influence tissue regeneration and osteogenesis. It possesses excellent film forming property and it can form scaffold, gel, nanoparticle and microsphere as well. The free amino (-NH₂) group and hydroxyl group (-OH) of chitosan make it an ideal polymer for protein, peptide and GAGs immobilization. Therefore, with these excellent properties, chitosan serve as an ideal platform for fabricating an ECM based biomimetic scaffolds for bone tissue engineering and regeneration.

ECM mediated regulation of osteoblast differentiation is very complex and is not regulated by only a single ECM component. It is mediated through the integrations and interplay of various components of ECM. In this study, we have used chitosan and selected ECM components with superior osteogenic potential to develop a biomimetic scaffold for hMSCs based bone tissue engineering. This study identifies the ECM components enhancing osteogenesis and applies this understanding for developing a functional biomimetic scaffolds for bone regeneration.