Preface

In this book, the preparation, characterization and applications in tissue regeneration of bovine type 1 collagen are reviewed. Furthermore, collagen is often used in regenerative medicine. In this book, the authors report on the helicity of collagen molecule in fibrils by circular dichroism spectroscopy and the thermograph of fibrils by differential scanning calorimetry (the structural aspects of collagen molecules). In the last chapter, second harmonic generation (SHG) microscopy is reviewed and its potential to visualize collagen fibers in a variety of connective tissues.

Chapter 1 – In the human body, collagen is the most abundant structural protein, forming up to 35% of the entire protein content of the whole body. Type I collagen, as the main collagen in various connective tissues, presents mostly in fibrous tissues such as skin, ligaments, and tendons. Type I collagen is also related to wound healing, and is frequently found in scar tissues as well as tissues formed through reparative processes. Heterogeneous type I collagen, which possesses similar structural characters with human type I collagen, shows outstanding biocompatibility and potential bioactivity. As a natural product, it is considered to have favorable biological properties that artificial materials do not have. Therefore, it was employed as ideal biomaterials in many medical applications. In recent decades, the research on the application of type I collagen in tissue regeneration was blooming and lots of achievements were reported.

In this chapter, the preparation, characterization, and application in tissue regeneration of bovine type I collagen were reviewed with recent literatures and the authors' own studies. The preparation process of bovine type I collagen was divided into three stages: pretreatment stage, extraction stage, and purification stage. The features of these three stages were introduced and

the processes were described accordingly. The problems most frequently encountered during the preparation process were illustrated, mainly based on the authors' research. According to the standards issued by ASTM and ISO, the characterization of bovine type I collagen was introduced. Meanwhile, the arguments on the risk of animal-derived starting materials for tissue engineering medical products were presented.

The application of bovine type I collagen in tissue regeneration ranging from 2D materials, such as surface coatings and thin membranes, to 3D tissue scaffolds such as sponges and hydrogels, were then summarized, focusing on the recent progress in the latest decade. The classification of biomaterials derived from bovine type I collagen was briefly reviewed according to fabrication approaches. Their state of the art clinic application in reparation and regeneration of bone, skin, ligament, etc. were introduced with focusing on the collagen-based hydrogels in cartilage defect reconstruction. Furthermore, the recent improvements on collagen fundamental researches, based on the studies trying to uncover the intriguing roles of collagen in cell-material interactions, were also introduced.

Chapter 2 – Type I collagen molecules spontaneously form insoluble fibrils at physiological buffers and at neutral pH. Insoluble fibrils play an important role in not only structure stabilization in tissue but also maintenance of each cell. Recently, collagen has been used as one of cell scaffolds in regenerative medicine. In cell culture conditions, the structure of molecule in collagen fibrils would be different from that of collagen monomer. Then, the study in ultrastructure of fibril is more important than that of monomer to understand cell behaviors. However, the structural information is not well understood yet. In this Chapter, the authors report on the helicity of collagen molecule in fibrils by circular dichroism spectroscopy and the thermograph of fibrils by differential scanning calorimetry. Furthermore, they observed the appearance of mouse neutrophil on collagen scaffolds showing imperfect fibrils. The authors' data indicated that the ultrastructure of collagen molecule depends on monomer or fibrils. The authors suggest that the formation of collagen fibrils prior to cell seeding is necessary for the interaction between cells and extracellular matrices.

Chapter 3 – *Osteogenesis imperfecta* (OI) composes a group of inherited connective tissue disorders, mainly characterized by bone fragility. It is primarily caused by mutations in the genes coding for pro-α1 and pro-α2 chains of type I collagen. The primary OI clinical manifestation is related to defective bone type I collagen fibers, although the condition is also displayed in other type I collagen-containing tissues, as skin. This means that the disease

signature (collagen fibrils abnormally organized, oddly small, or both) could be readily detected in skin, which is more accessible than bone for ex vivo analysis. The molecular diagnosis of OI is nowadays based on laborious and time-consuming biochemical and/or histological methods, to evaluate the collagen from biopsy-derived dermal fibroblasts. Therefore, there is a clear need to develop a rapid noninvasive method to diagnose OI.

Second Harmonic Generation (SHG) has emerged as a powerful nonlinear optical contrast mechanism with potential to visualize collagen fibers in a variety of connective tissues. SHG signals depend on non-linear interactions of light with a sub-micron noncentrosymmetric environment. Due to the type of fibrillar structure, collagen is one of the major sources of such signals in biological materials. The aim of the present chapter is to show that SHG microscopy, in combination with appropriated quantification methods, applied to human skin provides an OI diagnostic tool to discriminate diseased from normal tissues. Moreover, this method provides a fine tone approach to differentiate clinical sub-types of OI disorders, being highly advantageous over present primarily clinical diagnostic methods, providing fast and clear confirmation.