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## Preface

The last decade has continued to bring tremendous advances in our understanding of bone biology. The genes responsible for the majority of rare inherited bone disorders have been identified and much progress has been made in the identification of genes in polygenic disorders, such as Paget's disease and osteoporosis. Studies of genetically modified mice have resulted in the identification of other genes with profound effects on bone. These studies have uncovered many new pathways which form the focus of research by bone cell biologists to understand the mechanisms by which these genes and gene products affect bone mass and bone strength.

The second edition of *Bone Research Protocols* contains a catalogue of protocols to assist researchers in the pursuit of mechanistic studies. In the tradition of the *Methods in Molecular Medicine* series, the chapters are practical laboratory protocols that should enable the reader to carry out the techniques from scratch.

We have concentrated on laboratory techniques, rather than clinical methods of assessment and have tried to tailor the methods to the study of bone cells and bone tissue. For example, there are no differences in the analysis of DNA and RNA from bone or other tissues, but special considerations apply to isolation of DNA and RNA from bone and these are described. Equally, histological and histochemical procedures for soft tissues are often easily adapted to bone and are not specifically covered, apart from those included as analysis tools in various chapters. Tissue fixation, embedding and sectioning of bone, however, present unique problems and such methods are described as part of the chapters dealing with electron microscopy and immunostaining.

Much progress has been made in digital image analysis recently and several chapters (dealing with confocal microscopy, bone resorption assays, and histomorphometry) include a detailed description on how to make best use of this powerful technology. New chapters have also been included on the analysis of bone tissue by Fourier transform infrared microscopy and Raman Spectroscopy in view of the increasing interest in these techniques as methods of assessing bone quality. The chapters on bone imaging have been updated and extensively revised with new chapters on quantitative analysis of radiographs and real-time bioluminescent imaging.

Generally, one method is given for each technique, with the exception of in vitro osteoclast formation studies, for which several protocols are described, illustrating that many methods, often only subtly different, are described in the literature for different species and for different applications. Increasingly, high-throughput methods are used and some of the culture techniques described are suitable for such studies. Those interested in this field are encouraged to read all methods first before deciding which one is most appropriate for their particular application.

The section on osteoblast cultures has been updated and new chapters have been included on primary osteocyte cultures, analysis of osteocyte cell lines, and osteogenic differentiation of bone cells from mesenchymal stem cells.

A new section has been added on biochemical and molecular analysis of bone cells to cover topics, such as transfection, analysis of intracellular signaling, promoter reporter assays, gel shift assays, and chromatin immunoprecipitation assays.

The section on mechanical loading techniques has been updated from the previous edition and expanded by inclusion of a new chapter on *in vivo* loading techniques.

We hope that *Bone Research Protocols* will help those entering the bone field to establish new techniques in their laboratories. For those already experienced in bone research, we hope that they will benefit from the detailed description of the methods, in particular the many pointers and pitfalls, which the authors were specifically asked to discuss in the Notes section. We certainly learned a lot!

We express our sincere thanks to all authors for their willingness to share their trade secrets and to Prof. John Walker at Humana Press for giving us the opportunity to publish a second edition of Bone Research Protocols; both he and the authors have been most patient during the edits of this volume.

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