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## Preface

In the last decade, there has been a boom in three-dimensional (3D) cell culture methods. This type of cell culture aims to completely recapitulate the 3D organization of cells and cell-cell and cell-matrix interactions in vitro to closely mimic a typical organ microarchitecture and microenvironmental signaling. Thereby, 3D cell cultures represent more accurate and physiologically more relevant models and thus bridge the gap between more traditional 2D cell cultures in Petri dish and live organism. They provide a malleable platform and a less costly and more ethical alternative to in vivo models. 3D cell cultures have revolutionized biological research: they have enabled studies of fundamental biological questions, improved drug efficacy and toxicity screening, and fuelled tissue engineering for clinical applications.

The intention of this book is to provide an overview of established 3D cell culture assays, presented with detailed methodology and useful tips. To this end, leaders in the field have been invited to share their protocols and know-how. Their contributions cover a wide spectrum of techniques and approaches for 3D cell culture, from organoid cultures through organotypic models to microfluidic approaches and emerging 3D bioprinting techniques, which are used in developmental, stem cell, cancer, and pharmacological studies. The first chapter provides an introduction to the 3D cell culture field and a guide to the methods presented in the book. Next, in the first part of the book, several chapters are devoted to the production of hydrogels and scaffolds for 3D cell culture, including biohybrid hydrogels, decellularized extracellular matrix, stiffness-tunable interpenetrating networks of reconstituted basement membrane matrix and alginate, and calcium phosphate foams. The second part of the book covers protocols for conventional, manually assembled 3D cell cultures. They include 3D mammary, prostate, lung, and intestinal organoid cultures, and organotypic assays, such as invasion assays, confrontational co-cultures, and a procedure for the production of full-thickness skin equivalents. The third part of the book presents techniques for 3D cell culture micropatterning, including photomask, micro-needle, soft lithography, and two-photon polymerization techniques. Microfluidic approaches for 3D cell culture are described in the fourth part of the book. They include several techniques for spheroid production and embedding into ECM as well as protocols for production of organs-on-a-chip. Another state-of-the-art approach to 3D cell culture, 3D bioprinting, is presented in the fifth part of the book. At last, but not least, techniques for imaging and image analysis of 3D cell cultures are provided in the sixth part of the book.

I hope that this volume on 3D cell culture will be useful to a wide readership of scientists, especially in the fields of developmental and cancer biology, pharmacology, medicine, and tissue engineering. I envision that the book will serve not only as a collection of protocols to be strictly followed but also as an instrumental guide for assay adaptation and establishment of new models. I believe that the book will inspire development of novel 3D cell culture techniques according to specific scientific needs and interests towards new generation of physiologically relevant and realistic 3D cell cultures.

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