"This book provides an encyclopedic coverage of biomaterials science which, at the same time, has enough to interest the biomedical scientists and engineers. Overall, the book emphasizes the enormous need for the supply of regenerated organs and tissues as the spontaneous capacity for regeneration is limited in the human body."
— **Marthanda Varma Sankaran Valiathan**, National Research Professor, Manipal University, India

"This book has a seminal collection of chapters. I especially liked the chapter on biocompatibility assessment. The case studies described are a good way for any learner to see how basic science can be translated."
— **Abhay Pandit**, Scientific Director, Centre for Research in Medical Devices, National University of Ireland Galway

"Professor Basu has successfully provided an excellent guide in the interdisciplinary frontier field, for students, biomedical engineers and scientists. The fundamentals of materials and biomedical sciences are comprehensively and scientifically detailed for holistic understanding. A rich collection of objective and subjective problems of different formats will greatly benefit the academic community around the world."
— **Kimihiro Yamashita**, Professor, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Japan

"Professor Basu has created a comprehensive textbook for Biomaterials Science and Tissue Engineering that provides both important fundamentals and application areas. The scientific community will benefit greatly from this new resource which highlights key principles in this rapidly growing multidisciplinary field."
— **Tejal A. Desai**, Professor of Bioengineering, University of California San Francisco

"New discoveries in Biomaterials Science and Tissue Engineering increasingly dominate the current scientific literature. The consistent progress in the field demands the training of the younger surgeons and scientists. The pedagogical contribution of this book towards this important mission would certainly help in developing a clear understanding of Materials and Biological Sciences for this societally relevant scientific and clinical field."
— **Guy Daculsi**, INSERM Research Director DRE, Université de Nantes, France

Bikramjit Basu is Professor at Materials Research Center and Associate Faculty at Center for BioSystems Science and Engineering, Indian Institute of Science, Bangalore. He is the recipient of the Shanti Swarup Bhatnagar Prize for Science and Technology by the Government of India and an elected Fellow of the American Institute for Medical and Biological Engineering, Indian National Academy of Engineering and National Academy of Sciences, India.

Cover image: Confocal image of a cell on a biomaterial (top left), 3D printing process description (top middle), volume rendered image of microporous ceramic scaffold obtained using micro-CT (top right), histology image of an implant / host-tissue interface (bottom left), AFM image of micro-patterned biomaterial substrate (bottom second from left), Cell-material interaction (bottom second from right), micro-CT image of a cylindrical implant (red) in animal defect model (bottom right)
Source: Author

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Basu

Biomaterials Science and Tissue Engineering

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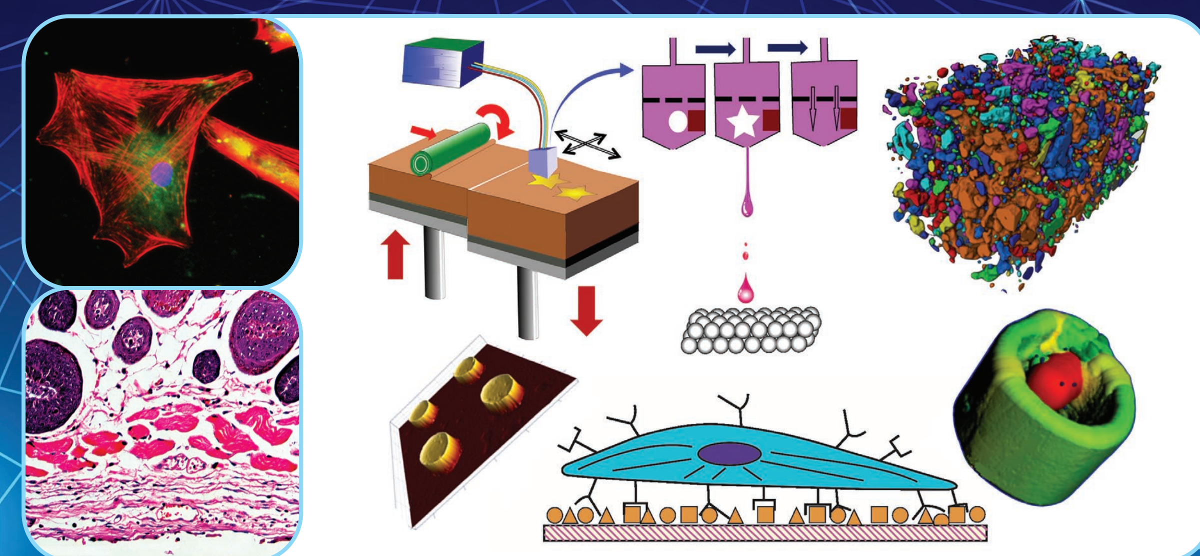
Biomaterials Science and Tissue Engineering

Principles and Methods



Bikramjit Basu

CAMBRIDGE

IISc Press

Biomaterials Science and Tissue Engineering

In the last couple of decades, the field of biomaterials science and tissue engineering has arrived at the frontier of research and innovation, considering the number of scientific discoveries and their potential impact in treating human diseases. This topical area is the focus of this texbook. The textbook has been divided into four sections. Section I provides an overview of the subject area with a focus on the application in human healthcare. Many important terms related to the field of Biomaterials Science and Tissue Engineering are also defined. Section II primarily focuses on discussing fundamental topics of Materials Science, including manufacturing of biomaterials, probing material structures at multiple length scales as well as mechanical properties. Section III comprehensively covers topics such as structure and properties of cell and proteins, cell-material interaction and biocompatibility, probing cell response, *in vitro*; bacterial growth and biofilm formation and probing tissue response, *in vivo*. The contents of Section III certainly make the book a unique pedagogical asset to readers without any formal knowledge in biological sciences. Section IV discusses several case studies, including corrosion/wear of Ti-based alloys and calcium phosphate-based multifunctional composites for bone replacement applications. This section also closes with author's perspectives on future growth of the field. The book offers extensive pedagogical features including multiple choice questions, fill in the blanks, review questions, numerical problems and solutions to selected problems.

Bikramjit Basu is Professor at Materials Research Center and Associate faculty at Center for BioSystems Science and Engineering, Indian Institute of Science, Bangalore. His current research integrates biomaterials and bioengineering approaches to develop new generation biomaterials and to address clinically relevant research problems for human diseases. Professor Basu is the recipient of the prestigious Shanti Swarup Bhatnagar Prize (2013) by the Government of India and Robert L. Coble Award for Young Scholars (2008) by the American Ceramic Society. He is an elected Fellow of the American Institute for Medical and Biological Engineering (2017), Indian National Academy of Engineering (2015) and National Academy of Sciences, India (2013).

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Biomaterials Science and Tissue Engineering

Principles and Methods

Bikramjit Basu

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Dedicated to
all my family members and students

Contents

Note:

- A number of statements appear within boxes in various chapters. The statements within shaded box refer to definition-like statements, while the statements within unshaded boxes reflect the author's perspective / important concepts.
- Coloured figures are placed at the end of the book and cross-referred in the main text at their respective appearances.

Foreword I

Over the last few decades, biomaterials science and biomedical engineering have been perceived as being among the fastest growing areas of research and innovation within the engineering science community when considering the number of scientific discoveries and their societal impact. To substantiate the relevance for human healthcare, degenerative and inflammatory problems of bone and joints affect millions of people worldwide. Due to the growing geriatric population, these problems account for half of the chronic diseases in people over 50. Osteoporosis, for example, is the most prevalent bone degenerative disease, particularly among the middle-aged population and post-menopausal women worldwide. The upsurge in the clinical demand for reconstructive joint replacements requires new implants with better biocompatibility properties, outweighing existing biomaterial solutions. In spite of remarkable advances in pharmacological, interventional, and surgical therapies, neurodegenerative and stroke disorders remain among the leading causes of mortality and lifelong impairments in humans.

In order to address biomedically relevant challenges in orthopedics as well as neural and cardiovascular diseases, researchers must blend the fundamental concepts of engineering sciences (materials science and electrical engineering), basic sciences (chemistry and physics), and biological sciences (cell and molecular biology) to engineer synthetic tissue replacements and develop novel healing strategies. Such an interdisciplinary research approach requires understanding across the boundary of remotely linked scientific disciplines. Researchers can develop innovative ideas, as well as understand the language of this important research area of societal relevance.

It has been noted globally that many accomplished researchers, as well as young researchers, pursuing the field of biomaterials and biomedical engineering are not formally trained in biology and medical sciences. Nevertheless, they are attempting to think laterally, blending sufficient knowledge of biological systems with engineering sciences to develop biomedical materials, ultimately impacting the field of Biomedical Engineering. Lately, unprecedented growth in the fields of biomaterials and biomedical engineering has revolutionized personalized healthcare.

This book emphasizes the fundamentals of both Materials and Biological Sciences. On the Materials science front, it contains chapters which provide non-specialists with a fundamental understanding on the conventional and advanced manufacturing techniques as well as mechanical properties. Clearly, the strength of this textbook lies in the clear description of the *in vitro* and *in* *vivo* biocompatibility assessment protocols, an asset for non-biologists. The conclusion presents a number of chapters describing case studies, primarily from the author's own research. The number of problem sets and assignments are also important attributes.

I find this much-needed textbook timely and valuable for the biomaterials community.

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Albert and Wilda Van Dusen Distinguished Professor of Orthopedic Surgery,
Professor of Chemical and Biomolecular Engineering,
Professor of Materials Science and Engineering,
Professor of Biomedical Engineering,
Director, The Institute for Regenerative Engineering,
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September, 2016

Foreword II

Professor Bikramjit Basu's new book "Biomaterials Science and Tissue Engineering" is ideal for biologists who wish to understand, in more depth, the biology-materials connection. On the other hand, materials scientists and materials engineers will find a wealth of information on biological concepts needed to fully exploit applications of materials in biology. The book is extremely well structured and every chapter is critical for anyone planning to design medical devices or implants. For example, the inclusion of a chapter on biofilms is a wonderful addition, which you will not normally find in a biomaterials text. Many implants suffer from infection-related failures.

The basic materials science chapters deal with a variety of biomaterials, principles governing the properties of materials, including materials processing and manufacturing. Then the book develops the principles of biocompatibility, materials-tissue and materials-cell interactions. It impressively integrates materials and biology concepts needed for selecting materials for human application as well as engineering them to optimize their properties, biocompatibility and bio-functionality.

Another attractive aspect of the book, especially for experimentalists and students, is the inclusion of testing and characterization techniques, both materials and biological test methodologies, and the designing and planning of animal experiments, and associated ethical issues to be considered.

The book could be an excellent textbook for a course in biomaterials and a great reference for those working in the field. For the last thirty years I have been teaching graduate level materials science both in the US and India. This would be a book I would use as a mandatory course reference, because I want to include more biological considerations into the standard materials science course.

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Director, Center for Nanosciences,
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April 2017

Preface

Biomaterials, recognized as a new class of materials in the Materials Science community, are being widely developed in last few decades. This specific class of materials has received significant attention because of their potential applications to repair and regenerate tissues in human musculoskeletal system, and thereby augment disease treatment modalities. The field of biomaterials science and tissue engineering has therefore large relevance for human healthcare. The design and development of biomaterials requires the integration of the concepts and expertise from two disconnected disciplines, i.e. Materials Science and Biological Science. While such integration is not an easy task by any means, researchers have put in extensive efforts in this direction. The importance of the field of biomaterials is increasingly being noticed in the Materials community; a compulsory course on this subject is being taught at undergraduate and graduate levels in most top universities around the world. It has been widely perceived that the education and training of next generation researchers can be accomplished more effectively with the availability of a textbook on the subject, which should cater to the requirements of the readers from both materials and biological sciences disciplines.

In the above backdrop, this book "Biomaterials Science and Tissue Engineering: Principles and Methods" begins with an overview of biomaterials and tissue engineering scaffolds (section I). This is followed by three well-structured sections, with section II discussing the fundamentals of Materials Science, as relevant for Biomaterials Science. Considering the significant breadth of the field of biological sciences, Section III of this book describes only the most necessary concepts and techniques of cell and molecular biology with a focus on the application of such knowledge in evaluating the biocompatibility property in a broad sense. The last section essentially illustrates various aspects of biomaterials development, primarily from author's own research. This book is meant for readers, who will be introduced to the broad area of biomaterials. Sections II and III set the floor for the readers to understand necessary fundamentals related to Materials and Biological sciences, as applied to Biomaterials science. Therefore, this textbook will be extremely useful to those readers, who want to pursue research in the field of Biomaterials Science without a formal background either in Materials or Biological science. While conceiving this textbook, the author wanted to motivate young researchers as well as to provide experts in the area with a healthy balance of topics for teaching/academic purposes. It is expected that the book will benefit senior undergraduate as well as graduate students.

In particular, this textbook has the following distinguishing features:

(a) Integration of the concepts of Materials Science and Biological Science, facilitating the use of this book as a textbook for teaching as well as for research purposes.
(b) Coverage of the necessary fundamentals of cell / molecular biology, which is often difficult to extract to an appropriate extent from various available textbooks of biological sciences in qualitative and quantitative manner [structure and properties of cells, tissues, bones, collagen, proteins, cell fate process (migration, differentiation, apoptosis, division) as well as cellular signaling processes].
(c) Detailed discussion on the processing, structure and properties of materials for biomedical applications (metals, ceramics, polymers and their composites), together with techniques and guidelines.
(d) Coverage of *in vitro* and *in vivo* biocompatibility property evaluation of materials for bone, neural as well as cardiovascular tissue engineering applications, together with protocols.

Altogether the book contains 18 chapters, with eleven chapters in the fundamentals section and the rest of the chapters being illustrative examples of biomaterials development. In chapter 1, the field of biomaterials is introduced with a special emphasis to distinguish biomaterials as a special class of functional materials and portray how they are different from other material classes. This introductory chapter also outlines the motivation for the development of new biomaterials to mimic the natural tissue properties. Various important terms are defined in this chapter. In chapter 2, the use of different primary material classes (metals, ceramics and polymers) for biomedical applications is discussed, which is followed by the classification of biomaterials on the basis of their biocompatibility. A detailed explanation on the various processing aspects of the tissue engineering scaffolds, with a special emphasis on the surface modification to enhance the biocompatibility properties, has been discussed in chapter 3. The processing of scaffolds and implants are markedly different as the latter involves the conventional processing approaches. To this end, chapter 4 introduces a number of fabrication techniques to prepare metals, ceramics and polymeric biomaterials with an emphasis on the processing science aspects. Chapter 4 also discusses the bulk deformation processes as well as machining and joining techniques, as applicable to metallic implants. After discussing the conventional processing methods, the additive manufacturing techniques are discussed with emphasis on powder based 3D printing technique.

Many researchers, without any formal background in material science, utilize a number of materials characterization techniques to characterise the structure or to measure the physical properties. The fundamental aspects of many of these techniques is discussed in chapter 5 with necessary theoretical background. For bone-tissue engineering applications, the implantable biomaterials need to have a set of desired mechanical properties. While the mechanical reliability of metallic implants is well-established, such reliability for ceramic implants is a matter of major concern for many clinicians. To this end, the fundamental aspects of deformation and fracture of ceramics and polymers are largely discussed in chapter 6. Apart from providing theoretical foundation, the experimental techniques to measure various mechanical properties are also mentioned. An important aspect of this section is the science-based discussion on the origin of brittle fracture and strength variability of ceramics. The concept of fracture toughness, measurement of various mechanical properties as well as brief discussion on toughening mechanisms are also presented in chapter 6. Five chapters in the fundamental section discuss the necessary biological foundation of this book. In particular, chapter

7 discusses the structure and properties of cells, proteins and bacteria. Various cellular adaptation processes as well as cell fate processes are also discussed. In chapter 8, the biocompatibility concept is introduced and the implication of biocompatibility in the context of cell-material interaction is critically discussed. The mechanistic description of cell-material interaction is also discussed. In chapter 9, various *in vitro* biochemical assays for cytocompatibility of biomaterials are extensively discussed. A large number of complimentary assays are mentioned to quantify the cell viability and proliferation. More importantly, many advanced cell biological techniques, like flow cytometry are also critically discussed. The ethical issues related to stem cell study are also mentioned in chapter 9. Bacterial growth and biofilm formation is addressed in chapter 10, which mainly includes bacterial classification, bacteria-material interaction and experiment assessment. Chapter 11 includes tissue compatibility assessment with a particular focus on pre-clinical testing in different animal models as well as ethical issues related to such studies. A few examples on the protocols to be followed in conducting pre-clinical studies.

In section IV, six chapters contain illustrative examples of biomaterials development. In the first of such chapters (chapter 12), the corrosion properties of some selected new titanium based alloy are presented. In chapter 13, the processing of calcium phosphate-mullite composites and their cytocompatibility, genotoxicity and *in vivo* biocompatibility are discussed. The next chapter i.e. Chapter 14 presents the case study on HDPE based hybrid composites using compression molding route and their biocompatibility properties are also summarized. One of the major issues in the development of HA based materials is the bactericidal property without compromising cytocompatibility properties. These aspects have been illustrated while discussing the development of HA-Ag composites in chapter 15. Next, chapter 16 discusses the processing related challenges as well as good toughness and biocompatibility properties together with desired functional properties in HA-based electroconductive composites with $CaTiO_3$ second phase. The next chapter discusses the compatibility of neuronal and cardiac cells on patterned carbon substrates. The proliferation of cardiac tissue-specific cells on PLGA-carbon nanofiber substrates is majorly discussed in chapter 17. At the end, chapter 18 closes with author's perspective on the subject. For the benefit of the students and college teachers, this book also contains an Appendix section with an array of questions of various formats for the self-assessment of the readers and for the examination purpose. The solution of some of the selected problems is also provided.

This book is an outcome of the several years of teaching undergraduate and postgraduate level courses on Biomaterials, being offered to students of Indian Institute of Technology Kanpur, India and Indian Institute of Science, Bangalore. Several chapters also reflect the extensive research by the author's research group, both at IIT Kanpur and IISc, Bangalore during the last two decades, which has been supported by the Council of Scientific and Industrial Research (CSIR), Department of Biotechnology (DBT), Department of Science & Technology (DST), Indo-US Science and Technology Forum (IUSSTF), and the UK-India Education Research Initiative (UKIERI). The author would also like to mention the recent multi-institutional research program 'Translational Centre on Biomaterials for Orthopedic and Dental Applications', supported by Department of Biotechnology, Government of India. Similarly, the funding from Science and Engineering Research Board of DST under the umbrella of 'National Network for Mathematical and Computational Biology' supported recent research in the author's group. The author also acknowledges the ongoing collaboration and interaction under the umbrella of this centre with several colleagues, including

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