BIOINSPIRED MATERIALS SCIENCE AND ENGINEERING

EDITED BY GUANG YANG,
LIN XIAO, AND LALLEPAK LAMBONI

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Foreword

Centered on materials, this book explores the full scope of products inspired by nature. The process of learning from biological structures and principles for the development of advanced and multifunctional materials as novel resources that revolutionize human life is discussed, presenting fundamental concepts and methods of biofabrication. Examples are offered that showcase currently trending compounds and macromolecules with their properties, their potential, and their contribution to the fabrication of bioinspired materials. Concrete applications are discussed as well with an accent on biomedically engineered materials, that will take the reader into the realm of such seductive biomaterials.

With currently captivating topics such as biotemplating, microfluidics, self-assembly, mussel-inspired surface modification, 3D biofabrication and more, this book represents a source of inspiration for the design of novel materials, and an important tool for updating active researchers. Additionally, its comprehensive approach will be of great interest to the beginner in the field who will discover the concept of bioinspiration from its fundamentals to its applications. Although the book emphasizes biomedical engineering, the multidisciplinary aspect of the subject will make it appeal to many research areas, such as biologists and engineers, while not leaving out chemists, physicists, and technicians.

Although an old concept, by proposing natural materials with superior features and low cost as models, bioinspiration has re-emerged as an essential tool for overcoming various limitations in current materials science and engineering, thereby solving many of mankind's substantial problems, such as the shortage of resources and the environmental concerns. Hence, this book deals with an important topic of the moment, which concerns numerous researchers across the world and should also be of interest to the general public. As illustrated by the authors, many different talents need to come together to make this approach a reality, and this book will inspire, instruct, and involve both current and the next generations in advancing the field.

April 13, 2017

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Preface

Bioinspiration is an old concept which can be described as the process of learning from nature and its biological principles. Taking advantage of the properties and nanostructures of natural compounds, the science of bioinspired materials aims at developing new and formerly non-existent materials, which exhibit novel and multifunctional properties, in the attempt of meeting the current requirements of human well-being. The idea is to take inspiration from natural mechanisms and the problems they are set to solve, in order to design advanced materials which are solutions to problems encountered in human life. Indeed, the focus of materials science is being increasingly shifted towards the development of bioinspired materials, prompted by the shortage of resources, the low cost and superior characteristics of natural materials, and the environmental and climatic concerns. The first step to engineering bioinspired materials is understanding biological materials and the processes involved in their production, and thence, develop biofabrication or bioinspired fabrication approaches. This leads to the highly interdisciplinary character of this field, which brings together natural scientists (biologists, chemists, and physicists), engineers, and technicians. Thus, an active interaction across disciplines is the key to the real development of this old research area, which is now attracting many researchers worldwide. However, as underlined by several recent reviews on the subject, this condition is yet to be fully met, due to the rather limited understanding of the building principles of living entities which are numerous and complex, and because the definition of the scope and novel applications remain to be further clarified. Hence, approaches for conveying information in the field and storing the bioinspired solutions already uncovered are of real importance, and would contribute significantly in propelling this promising research area.

Biofabrication approaches are developed by studying and exploiting unique and basic biological aspects, including evolution, growth, and structure (formation and performance), which are non-existent in engineering materials. Based on the “growth and functional adaptation” concepts, the strategies adopted aim at creating hierarchical structures and self-assemblies (dynamic strategies), while those associated with the “damage repair and healing” principles design self-repair or self-healing materials.

The purpose of this book is to introduce a comprehensive view of the bioinspired materials science and engineering, discussing biofabrication approaches and applications of bioinspired materials as they are fed back to nature in the guise of biomaterials. Some biological compounds will also be brought up, as of what is learned from them, and how they can be useful in the engineering of bioinspired materials. Thus, this book will include 3 main sections: biofabrication, biomacromolecules, and biomaterials. Illustrating the bioinspiration process from materials design and conception to application of bioinspired materials, this book will present the multidisciplinary aspect of the concept, and represent a typical example of how knowledge is acquired from nature, and how in turn this information contributes to biological sciences, with an accent on biomedical applications. We anticipate that this book will be suitable for different classes of the scientific community including undergraduate, graduate, and senior researchers in all areas of bioinspired materials. We hope that it will stimulate new thoughts and research in this field.

We would like to acknowledge the National Natural Science Foundation of China for the financial support on this book. Then we would like to express our appreciation to China-Germany Center for Science, which supported the Sino-German Sympoisum on Bioinspired Materials and Engineering held from May 11-15, 2014 in Wuhan, China, co-chaired by Prof. Guang Yang (HUST) and Prof. Cordt Zollfrank (TUM). This memorable symposium brought together outstanding scientists working in the bioinspired material field from China, Germany, Japan and the rest world. It is this symposium that first inspired the conception of this book. Many of the symposium attendees then accepted our invitation to contribute to this book. We offer special thanks to them. We also would like to express our appreciation to all contributing authors and to staff at John Wiley & Sons, Inc., for their patience and never-failing support of this project. Finally, we would like to express our sincere gratitude to Prof. Lina Zhang at Wuhan University, Prof. Lei Jiang at Technical Institute of Physics and Chemistry CAS, and Prof. Deyue Yan at Shanghai Jiaotong University for their on-going support and guidance.

Guang Yang, Lin Xiao, Lallepak Lamboni
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Introduction to Science and Engineering Principles for the Development of Bioinspired Materials

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I.1 Bioinspiration

Bioinspiration refers to the process of learning from nature and its biological principles. The science of bioinspired materials aims to develop novel functional materials with advanced and multi-functional properties by using the nano-, micro-, meso-, and macro-structures and features of natural materials with the aim to meet the requirements of human well-being. Natural mechanisms and biological materials can be exploited to design advanced materials to solve the problems encountered in human life. Indeed, the focus of materials science is being increasingly shifted toward the development of bioinspired materials, prompted by the shortage of resources, the low cost, and the superior characteristics of natural materials, and the environmental and climatic concerns. To this end, understanding the biological phenomenon, natural biological materials, and the processes involved in their natural production is essential, and hence, developing biofabrication or bioinspired fabrication approaches.

I.2 Bioinspired Materials

Bioinspired materials are synthetic products fabricated to mimic the structure and mechanical features of natural biological materials [1]. Biological materials are inherently multi-functional in nature but may have evolved to optimize a principal mechanical function such as the impact of fracture resistance, for armor and protection, for sharp and cutting components, for a light weight for flight, or special chemical and mechanical extremities for reversible adhesive purposes. These functions are regulated by the nano-, micro-, meso-, and macro-structures of the materials. Further, these structures determine the mechanism of the biological systems to adapt themselves to the external mechanical stimuli. These inherent functions and structural properties are inspiring scientists and engineers to design novel multi-functional synthetic materials with a wide range of structural features and a broad spectrum of potential applications. In the past few decades, several natural biological materials have been examined by researchers for various aspects to explore their potential in different fields. Studies reveal that the inherent multi-scale structures of natural biological materials possess several functions. Nature as a school for scientists and engineers has served as a great source of inspiration to fabricate new materials [2]. At present, biomimetic and bioinspired approaches have been adopted for the fabrication of several biological materials with multi-scale structures for function integration, as summarized in Table I.1. An interdisciplinary collaboration of materials science and engineering, chemistry, biology, physics, and bioinformatics, etc. may lead to the design and fabrication of novel multi-functional bioinspired materials.

To date, several biofabrication approaches have been developed by studying and exploiting unique and basic biological aspects, including evolution, growth, and structure (formation and performance) which are not found in engineering materials. Based on the "growth and functional adaptation" concepts, the strategies adopted mainly aim at creating hierarchical structures and self-assemblies (dynamic strategies) and those associated with the "damage repair and healing" principle designs, and self-repair or self-healing materials. To achieve these objectives, several models have been presented by the researchers to describe the design, fabrication, and optimization of properties of bioinspired materials. Modeling of biological materials helps in rational understanding of

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Table I.1 Typical biological materials with function integration.

Biological materials	Functions	Ref.
Butterfly wing	Superhydrophobicity, directional adhesion, structural color, self-cleaning, chemical sensing capability, fluorescence emission functions	[3–7]
Brittlestar	Mechanical and optical functions	[8]
Cicada wing	Anti-reflection, superhydrophobicity	[9]
Fish scale	Drag reduction, superoleophilicity in air, superoleophobicity in water	[10]
Gecko foot	Reversible adhesive, superhydrophobicity, self-cleaning	[11]
Lotus leaf	Superhydrophobicity, low adhesion, self-cleaning	[12]
Mosquito compound eye	Superhydrophobicity, anti-reflection, anti-fogging	[13]
Nacre	Mechanical property, structural color	[14, 15]
Peacock feather	Structural color, superhydrophobicity	[16]
Polar bear fur	Optical property, thermal insulation	[17]
Rice leaf	Superhydrophobicity, anisotropic wettability	[12]
Rose petal	Superhydrophobicity, structural color, high adhesion	[18–20]
Shark skin	Drag reduction, anti-biofouling	[21]
Spicule	Mechanical and fiber-optical properties	[22–24]
Spider capture silk	Water collection ability, mechanical property, elasticity, stickiness	[25–27]
Spider dragline silk	Mechanical property, supercontraction, torsional shape memory	[28–35]
Water strider leg	Durable and robust superhydrophobicity	[36]

Source: Reproduced from [2] with permission from Elsevier.

the design principles which can lead to subsequent designing of bioinspired complements. For example, mechanical modeling of biological materials based on natural materials has attracted immense attention owing to their diverse applications in medicine and engineering. This can be attributed to the structurally hierarchical biomaterials which possess a highly desirable structure-properties relationship and can serve as templates for the fabrication of bioinspired materials. Several approaches, such as single- and multi-scale, micro-structural and phenomenological, and continuum and discrete, etc. have been developed for the mechanical modeling of biological and bioinspired materials [37]. However, further extensive research is required to fabricate bioinspired materials due to their greater flexibility in design variables, such as the selection of material components, the varying degree of constraints among the different available components, the variable boundary conditions, and the novel architectural conformations.

I.3 Biofabrication

Biofabrication is the combination of two words: “bio” means living and “fabrication” means to synthesize or design using templates etc., thus biofabrication refers to the synthesis of living structures using some standard templates or models. Precisely, biofabrication refers to the application principle of engineering and information science to produce an automated robotic assembly of living cells, tissues, and organs, etc. [38]. Further narrowing down the concept, biofabrication refers to the biomedical applications of rapid prototyping or computer-aided additive technologies. It is closely related to tissue engineering and is considered an integral part of it and uses engineering approaches in the assembly of complex tissues and organs. Despite extensive developments in the field of tissue engineering, the transformation of this labor-intensive technology into an automated industry still requires further innovative and creative strategies.

I.3.1 Summary of Part I Biofabrication

In Part I, “Biofabrication,” we discuss various biotemplating principles and recent advances in the one-dimensional and two-dimensional biotemplated formation of inorganic functional materials using natural templates. The chapters in Part I (Chapters 2–6) also discuss microbial-mediated material manufacturing techniques for the fabrication of a variety of functional materials. Recently developed tubular structures are discussed, which serve as templates for *in vitro* recapitulating of highly complex tissues such as blood vessels, etc. and microfluidics-based cell manipulation and development of tubular tissues. This Part also illustrates the fabrication of three-dimensional (3D) tissues with capillary networks by controlling the cell microenvironment with emphasis on 3D-tumor invasion models with blood- and lymph-capillary networks. Furthermore, biofabrication of ordered cellulose scaffolds (nematic ordered) to mediate 3D cell culturing and biomineralization is discussed. As an example of bioinspiration, the preparation and application of biomimetic materials inspired by muscle adhesive proteins are overviewed in detail. Finally, the self-assembly of poly(lactic acid)-based amphiphilic diblock copolymers and their applications in biomedical field are presented.

I.4 Biofabrication Strategies

Biofabrication strategies mainly aim to improve the existing strategies and develop reliable biomaterials-based cell culturing strategies for advances in tissue engineering and regenerative medicines. To achieve such goals, scaffolds have been developed from various biocompatible materials. A scaffold refers to a temporary structure made of biocompatible material and provides support to the growing cells. A scaffold is declared biocompatible when it remains in direct contact with living host tissues without causing any toxic, allergic, or side effects. Scaffolds with well-defined 3D topologies and geometries have been fabricated to introduce various biological molecules with various shapes and sizes. Tissue engineering applications of scaffolds require high porosity, tunable pore sizes, and better mechanical features. For example, scaffolds with large pore sizes allow easy penetration of the impregnating materials, the diffusion of nutrients, the removal of wastes, and the exchange of gases, etc. Further, an ideal scaffold supports adhesion, proliferation, and migration of cells [39]. The following sections describe a few conventional and advanced biofabrication strategies.

I.4.1 Conventional Biofabrication Strategies

To date, a multitude of fabrication strategies have been devised to fabricate 3D scaffolds using various natural and synthetic materials, mainly polymers. These strategies aim to design scaffolds in such a way as to mimic the natural environment of a living cell. To achieve this goal, earlier scaffolds were fabricated followed by the seeding of viable cells. The following overviews some of these strategies.

I.4.1.1 Solvent Casting Strategy

In this strategy, a polymer solution prepared in an appropriate solvent with uniformly distributed salt particles (i.e. porogen) of known size is poured into a mold and the solvent is allowed to evaporate, leaving behind a composite with uniformly distributed salt particles [40]. Thereafter, the composite is immersed in water to allow the leaching out of the salt particles, leaving behind pores according to the size and shape of the salt particles. Thus, a highly porous uniform 3D scaffold is formed on which different types of cells can be seeded. It is worth mentioning here that the size and shape of the pores are directly related to the size and shape of the salt particles, respectively. The size and shape of the pore can be optimized according to the type of cells and specific application. Further, the solvent used should be non-toxic to the seeding cells.

I.4.1.2 Freeze-drying or the Lyophilization Method

In this strategy, the temperature of a polymer solution is lowered well below its freezing point which results in the solidification of the solvent molecules and leads to the aggregation of the polymer within the interstitial spaces of the scaffold matrix. Thereafter, the solvent molecules are allowed to evaporate via sublimation, leaving behind a highly porous polymeric structure containing well-distributed interconnected pores on the surface and within the matrix of scaffold [41]. Different types of cells can be seeded with the formed interconnected pores. It is worth mentioning here that the pore size of the scaffold depends upon the freezing regime, the concentration of the polymeric material, the size of the ice crystals formed, and the pH of the solution [42].

I.4.1.3 Gas Foaming

Gas foaming is another biofabrication strategy where a polymeric scaffold is first completely saturated using a foaming agent at high pressure, followed by the release of pressure, which results in the solubility of the gas in the polymer. The gas bubbles are formed which grow in the polymer due to the thermodynamic instability [43]. Different types of foaming agents such as CO_2 [44], N_2 [45], or H_2O [46] are used for such purposes, which results in highly porous structures with varying pore size in the range of 100–500 μm [47].

I.4.2 Advanced Biofabrication Strategies

Advanced biofabrication strategies are classified into bioprinting and photolithographic techniques.

I.4.2.1 Bioprinting

Bioprinting is one of the most advanced and innovative technology of this century which has received growing interest worldwide and revolutionized the medical technology and pharmaceutical industries [48]. It refers to the use of 3D printing technology to print various biomaterials with incorporated viable cells to engineer tissue construct applications in tissue engineering and regenerative medicines. Currently, this technology has received immense attention and is widely used for broad spectrum applications, such as regenerative medicines, tissue engineering and transplantation, screening of drugs, and cancer research, etc. It offers several advantages, such as the precise and controlled deposition of cells, hormones, drugs, and growth factors, etc., thus directing improved tissue formation. Further, it provides a base for the development of tissue constructs, organs and organoids, and organ-on-a-chip mimicking the natural ones [49]. Bioprinting is carried out using a 3D printer, which has the ability to print 3D structures such as tissues and organs, etc. using various bioink solutions. A bioink solution refers to a mixture of biomaterial and live cells. A general bioprinting process is shown in Figure I.1. A typical 3D bioprinter has the ability to simultaneously dispense various biomaterials to fabricate structures with high resolution and accuracy and maintain high degree of freedom motion and ensure sufficient motion speed. These 3D printers are user-friendly, fully automatic, easily sterilized, affordable, durable, versatile, and compact instruments [50]. Bioprinting technology is advancing rapidly, however, the technological modalities are based on three fundamental strategies including the inkjet or droplet, extrusion, and laser-based bioprinters, which are described as follows.

I.4.2.1.1 Droplet-based Bioprinting

The droplet-based bioprinting strategy is based on the thermal, piezo, or acoustic-driven mechanisms and uses heat energy, electrical energy, and sound energy, respectively for the generation of droplets of cell suspension in a high-throughput fashion. These bioprinters have received immense attention owing to their simplicity, versatility, agility, and high-throughput potential to dispense a variety of biologics, such as viable cells, growth factors, genes, and pharmaceutics, etc. [51]. These types of printers have a high speed of fabrication of scaffolds, however, this high speed make the strategy difficult to apply to most of the polymer systems as it requires the gelation time to be in accordance with the drop deposition time.

I.4.2.1.2 Extrusion-based Bioprinting

The extrusion-based bioprinting system is a hybrid of a fluid-dispensing system and an automated robotic system for extrusion and bioprinting, respectively. These bioprinting systems use mechanical or pneumatic-driven systems and deposit the viable cells in the form of a filament [52]. In this system, the bioink is dispensed using a

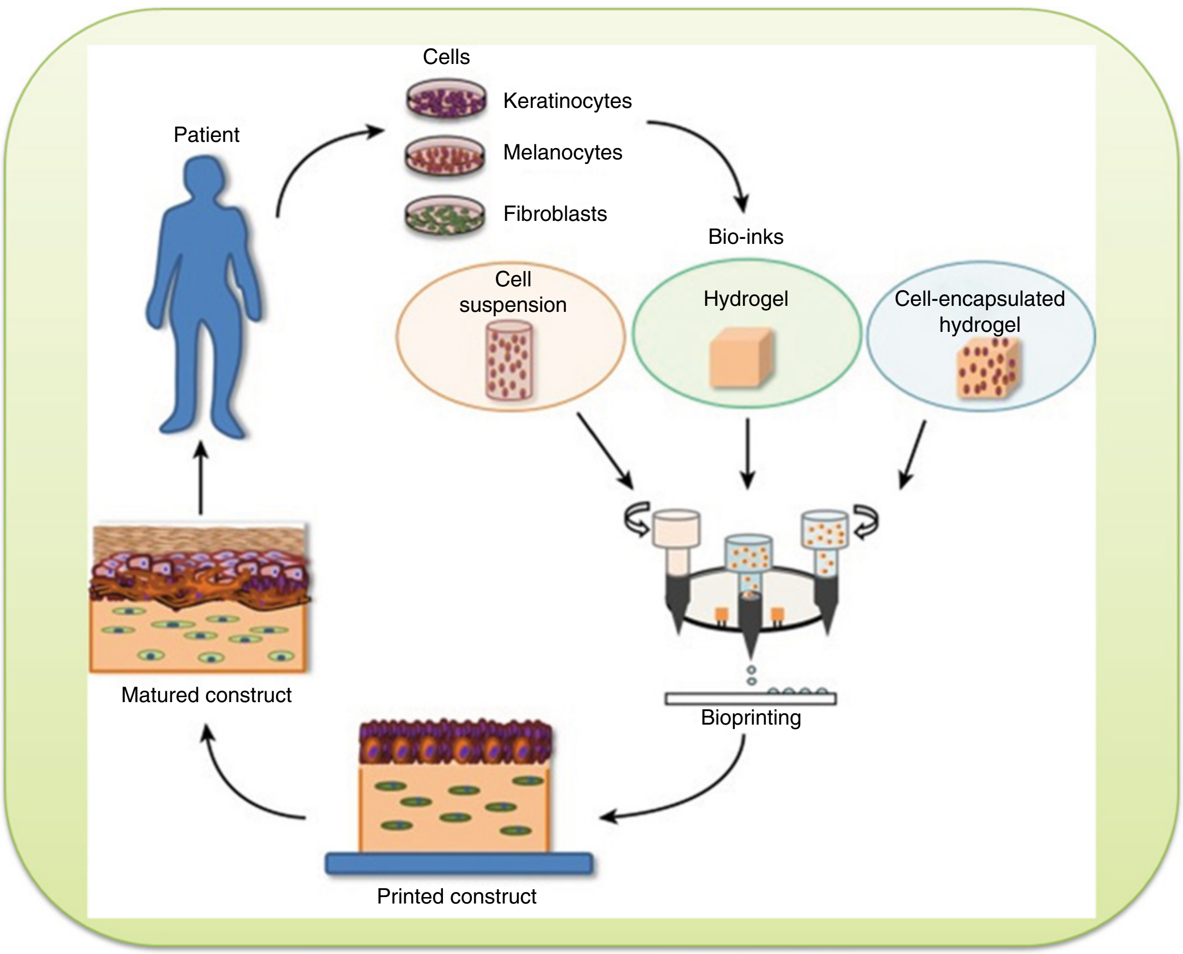

Figure I.1 Illustration of isolation of viable cells, bioprinting of tissue constructs, and implantation into the patient. *Source:* Reproduced from [148] with permission from "Cell-press."

deposition in a computer-aided design (CAD) system which ensures the precise dispensing of viable cells encapsulated in a cylindrical filament. During the printing process, a stage or a surface is moved in a directed pattern controlled by CAD, which ensures the spatial deposition of bioink from a nozzle to fabricate materials of specific structural conformations [53].

I.4.2.1.3 Laser-based Bioprinting

Compared to extrusion-based bioprinting, the viable cells from a donor-slide to a receiver-slide are dispensed without the assistance of a nozzle, using laser energy in a laser-based bioprinting system. This modality offers several advantages in dispensing a variety of biologics such as live cells, biomaterials, growth factors, genes and vectors, and drugs, etc. [54].

I.4.2.2 Photolithographic Strategies of Biofabrication

The photolithography bioprinters have been modified from laser-assisted printers. Similar to a laser-assisted bioprinter, the stereolithography modality uses light or photons for the selective solidification of bioink in a layer-by-layer pattern during the fabrication of a scaffold. Usually, these bioprinters use a digital projector that ensures the same printing time even for the complex planes in a structure and thus this system is more advantageous than conventional bioprinters. Further, such printers are simpler in operation, offer high resolution (100 μm) printing in a short time, and maintain high cell viability [55]. These strategies are used for the fabrication of 2D scaffolds for the growth of cells [56] or the encapsulation of cells in a 3D network of polymers [57]. Photolithographic strategies are further classified into mask-based photolithography, stereolithography, and multiphoton lithography. The mask-based photolithographic strategies of biofabrication use a patterned mask to illuminate selected regions of a polymer. For this purpose, the prepolymer solution is exposed to UV light, which results in the polymerization of the exposed regions of the polymer and thus prevents the formation of a network of 3D porous scaffolds. The unnecessary unpolymerized solution is washed out by immersing in a buffer [58]. Similarly, the stereolithography is a maskless photopatterning CAD strategy used for the fabrication of prototypes. In this strategy, the design of the desired scaffold is first developed using a 3D computer drawing software, which is then processed by software and sliced into a number of layers (25–100 μm thick). The information is then passed to the SL apparatus which prints one layer at a time using a UV laser. Similarly, the multiphoton lithography is also a maskless lithographic strategy which uses a focused laser or a confocal microscope [59–61]. This lithographic strategy offers high lateral (x–y) resolution but little to no control over the axial (z) direction. To solve this issue, several photochemistries have been expanded to multiphoton-based approaches with the potential to confine photochemical reactions in 3D orientation. A comparative analysis of various bioprinters in use is shown in Table I.2.

I.5 Part II Biomacromolecules

Part II of the book deals with biomacromolecules. The term "biomacromolecules" refers to the biological molecules with high relative molecular masses whose structure is essentially comprised of multiple repeated units derived from low molecular mass molecules. Generally, a biomacromolecule is synthesized through the polymerization of smaller subunits generally referred to as monomers. Compared to monomers, the macromolecules have exceptionally different physical properties. Similarly, these biomacromolecules are relatively insoluble in water and other common solvents compared to their smaller units and instead form colloids. In general, there are three classes of biomacromolecules discussed in this book: carbohydrates, proteins, and nucleic acids.

I.5.1 Summary of Part II Biomacromolecules

In Part II, "Biomacromolecules," details of the synthesis approaches and applications of electroactive bioartificials are provided. Further, chemical modification of starch and the conformational properties of various linear and cyclic polysaccharide derivatives are discussed. Thereafter, structure, basic properties, and fabrication strategies of silk-based materials with a special emphasis on biomimetic structures are described. Finally, recent developments in polypeptides synthesis by ring-opening polymerization, micro- and nano-structures through the self-assembly of polypeptides, and their applications are presented.

I.5.2 Carbohydrates

Carbohydrates are biological molecules consisting of three main components: carbon, hydrogen, and oxygen. These are represented by an empirical formula $C_m(H_2O)_n$, where m and n can have the same or different values. Chemically, carbohydrates are polyhydroxy aldehydes, ketones, alcohols, acids, their simple derivatives, or their polymers with linkages of the acetal-type. These are categorized according to their degree of polymerization into three main classes: sugars, oligosaccharides, and polysaccharides. Sugars include monosaccharides (e.g. glucose, fructose, galactose, and xylose, etc.), disaccharides

Table I.2 Comparison of four types of bioprinting techniques.

Parameters	Inkjet	Laser-assisted	Extrusion	Stereolithography	Reference
Cost	Low	High	Moderate	Low	[62–65]
Cell viability	>85%	>95%	40%–80%	>85%	[66, 67]
Print speed	Fast	Medium	Slow	Fast	[68–70]
Supported viscosities	3.5–12 mPa/s	1–300 mPa/s	30 mPa/s to above 6×10^7 mPa/s	No limitation	[70–72]
Resolution	High	High	Moderate	High	[63]
Quality of vertical structure	Poor	Fair	Good	Good	[73]
Cell density	Low <10^6 cells/mL	Medium < 10^8 cells/mL	High (cell spheroids)	Medium <10^8 cells/mL	[70]
Representative materials for bioinks	Alginate, PEGDMA, Collagen	Collagen, Matrigel	Alginate, GelMA, Collagen	GelMA, GelMA-PEGDA hybrid hydrogel	[65, 73–77]
Reported applications	Tissue engineering (blood vessel, bone, cartilage, and neuron)	Tissue engineering (blood vessel, bone, skin, and adipose)	Tissue engineering (blood vessel, bone, cartilage, neuron, muscle, tumor) Controlled release of biomacromolecules Organ-on-a-chip	Tissue engineering (blood vessel and cartilage) Organ-on-a-chip	[78–81]

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(e.g. sucrose, lactose, maltose, and trehalose, etc.), and polyols (e.g. sorbitol and mannitol, etc.). Similarly, oligosaccharides include malto-oligosaccharides (e.g. maltodextrins) and others (e.g. raffinose, stachyose, and fructo-oligosaccharides, etc.). Similarly, the polysaccharides include starches (e.g. amylose, amylopectin, and modified starches, etc.) and non-starches (e.g. cellulose, hemicellulose, pectins, and hydrocolloids, etc.). Common examples of carbohydrates used in biomedical research are briefly described here.

I.5.2.1 Starch

Starch, also known as amylum, is a polymeric carbohydrate comprised of repeated glucose units linked via glycosidic linkages. Generally, it is produced by green plants where it serves as an energy storage material. Chemically, it consists of two types of molecules: the linear and the helical-shaped amylose that accounts for 20–25% of the total starch content, and the branched amylopectin accounting for 75–80% of the total starch content. Amylose is a comparatively long linear chain of α-glucans containing about 99% $(1 \rightarrow 4)$-α-linkages and around 1% $(1 \rightarrow 6)$-α-linkages. Similarly, amylopectin contains about 95% $(1 \rightarrow 4)$-α-linkages and 5% $(1 \rightarrow 6)$-α-linkages [82]. Its insolubility in cooled water limits its various applications. This issue has been resolved to great extent by chemical modification, by introducing different functional groups onto its back bone [83]. It possesses several properties which are significant from a biomedical perspective, such as high biocompatibility, biodegradability, and non-toxicity, etc. It has been extensively used for various applications, such as tissue engineering, drug delivery, and enzyme immobilization, etc. [84]. Starch and its derivatives serve as promising materials for various tissue engineering applications, such as artificial skin, scaffolds, bone and cartilage, vascular regeneration, and teeth, etc. owing to its biocompatible, biodegradable, non-toxic, non-immunogenic, and porous structure. Starch has the potential to form nanofibers, highly porous scaffolds, and injectable hydrogels, etc.

I.5.2.2 Cellulose

Cellulose is the most abundant polysaccharide available on Earth and consists of chain of glucose monomers. The molecular formula of cellulose is $(C_6H_{10}O_5)_n$ and is an unbranched homopolysaccharide composed of α-D-glucopyranose units linked by β-$(1 \rightarrow 4)$ glycosidic bonds (Figure I.2). The linear glucan chain forms highly stable regular intra- and inter-molecular hydrogen bonds which stabilize its reticulate structure [85]. It is produced by various sources such as plants, microbial cells (*Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina*, etc.) [86], and enzymes (the cell-free system) [87]. Compared to microbial cellulose, also known as bacterial cellulose (BC), and bio-cellulose produced by cell-free enzymes system [85], which represent the purest forms, the plant cellulose contains several impurities in the form of lignin and hemicellulose, which necessitate its further treatment by various chemical methods. However, BC and bio-cellulose are directly used in various applications without further pretreatment. Further, these possess unique structural, physico-chemical, mechanical, and biological features, such as high water-holding capacity (WHC) (100–200 times its dry weight), slow water release rate (WRR), higher crystallinity (60–90%), high tensile features (with elastic modulus 1 to 15 MPa and elongation at break 10–30%), an ultrafine fiber network, and moldability into 3D structures. which bestow BC with high potential value [85, 88–90] and broaden its applications in different fields, such as biomedicine, opto-electronics, food technology, and separation processes, etc. [91]. BC is rarely soluble in common solvents such as water and organic and inorganic solvents, owing to its highly extended hydrogen bonding [92, 93]. However, several solvents systems have been developed such as LiCl/dimethylacetamide (DMAc) [94], N-methylmorpholine-N-oxide (NMMO) [40], ionic liquids (ILs) [95], and alkali/urea (or thiourea) aqueous, which can dissolve BC [96]. BC has mainly found applications in the biomedical field where it is used as wound dressing material, for burns, artificial skin, vascular grafts, scaffolds for tissue engineering, tissue regeneration, and artificial blood vessels, etc. [89, 97, 98]. Also, it has been used for the preparation of several commercial products such as tires, headphone membranes, high performance speaker diaphragms, high-grade paper, makeup pads, diet food, and textiles, etc. [98]. Furthermore, it is used as carrier in drug delivery systems, enzyme immobilization, and ion exchange membrane, and as biodegradable and biocompatible sensors and actuators [99, 100].

I.5.2.3 Chitosan

Chitosan is an abundant polysaccharide present in nature. Chemically, it is composed of randomly arranged β-$(1 \rightarrow 4)$-linked D-glucosamine and N-acetyl-D-glucosamine, representing deacetylated and acetylated units, respectively. Its chemical structure is similar to that of cellulose except for the replacement of the $-NH_2$ group instead of –OH moieties in the glucose units of cellulose backbone. It is synthesized through deacetylation of chitin shells of crustaceans, such as shrimps, using an alkaline solvent such as sodium hydroxide (NaOH) in excess as a reagent and water as a solvent. The chemical reactions are completed in two steps under first-order kinetic control where activation energy (48.76 kJ/mol) of the first step is higher than the second and yields about 98% chitosan as the final product.

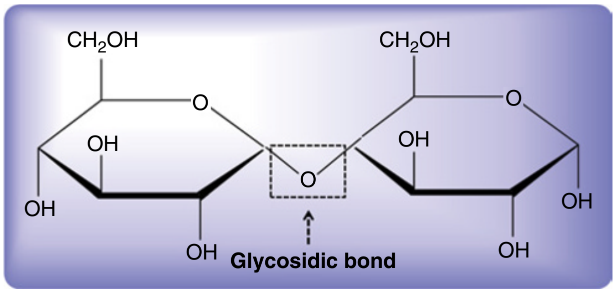

Figure I.2 Chemistry of glycosidic bond formation in carbohydrates.

The degree of deacetylation (% DD) is determined via nuclear magnetic resonance (NMR) spectroscopy, where it ranges from 60–100% for commercial chitosan. The molecular weight of commercial chitosan ranges between 3.8–20 KDa.

Chitosan is highly soluble in dilute acids, highly biocompatible, non-toxic, biodegradable, does not provoke the immune system, is anti-cancerous, and environmentally friendly [101]. It biodegrades slowly to harmless products of oligomers and is absorbed slowly in the body. The biodegraded chitosan accelerates wound healing [102]. Owing to these features, it has found immense use in biomedical applications. It is used for drug delivery and wound care, both alone and in the form of composites with other materials such as BC, owing to its antibacterial activity [103]. Also, it is heavily used in agriculture as a seed treatment and biopesticide against fungal invasion. In the wine industry, it is used as a fining agent and for the prevention of spoilage. Further, it is used as self-healing polyurethane paint coating.

I.5.2.4 Alginate and Other Seaweed-Derived Polysaccharides

Alginate is an anionic polysaccharide isolated from seaweed. Chemically, it is composed of mannuronic acid and guluronic acid units where mannuronic acid units form β (1→4) linkages while guluronic acid units are linked via α-(1→4) linkages. Structurally, mannuronic acid units are distributed linearly and exhibit a flexible conformation while guluronic acid units display a steric hindrance in the vicinity of carboxyl groups. Besides alginate, several other seaweed-derived polysaccharides, such as carrageenan, fucoidan, laminaran, and ulvan have also been used for various biomedical applications owing to their high biocompatibility, easy availability, and simple isolation strategies [104]. Such polysaccharides can form hydrogels through ionic interaction between carboxylic groups (–COOH) present on their surface with a cationic cross-linking agent [105]. Besides, divalent cations such as Ca^{2+}, Zn^{2+}, Ba^{2+}, or trivalent cations (e.g. Al^{3+}) may also exist in these hydrogels. Of the various seaweeds, polysaccharides-based hydrogels, alginate-based hydrogels are extensively used as biomaterials for various biomedical applications including as scaffolds in tissue engineering, as carriers in drug delivery, and as model ECMs for biological studies [106]. Similarly, the carrageenan-based hydrogels are extensively used for the encapsulation of cells, the transformation of growth factors, and the formation of bone and cartilage tissues [107, 108]. The fucoidan and ulvan-based hydrogels are used in the culturing of cells and improving their activity [109, 110].

I.5.2.5 Hyaluronic Acid

Hyaluronic acid (HA), a non-sulfated glycosamino-glycan is constituted of repeated units of D-glucuronic acid and D-N-acetylglucosamine linked together via alternating β-1,4 and β-1,3-glycosidic linkages [111]. In nature, it is present in the form of a long straight chain of anioinc polysaccharide. It is extensively found in the human body where it is dispersed in different tissues. Generally, it has the ability to cross-link by simple freeze-thawing in the absence of any cross-linker or organic solvent [112]. It can be potentially modified into different forms due to the presence of several functional groups in its chemical structure, such as –COOH, –OH, and N-acetyl functional group. It can form hydrogels through various cross-linking methods, such as chemical, enzymatic, or photo-cross-linking which find different applications in the biomedical and electronics fields. It has been extensively used for biomedical applications, such as cell motility, wound care and healing, cell signaling analysis, fabrication of different matrices, and angiogenesis, since it accounts for a major portion of ECM of skin, cartilage, and vitreous humors [91].

I.5.3 Proteins

Proteins are biomacromolecules comprised of one or more long chains of amino acids. Each amino acid is comprised of an α-carbon, amino group ($-NH_2$), carboxyl group (–COOH), and a variable side chain designated as R-group (–R). The amino acid residues are linked together through a peptide bond between the –COOH and $-NH_2$ groups of two consecutive amino acids (Figure I.3). The peptide bond has two resonance forms which contribute some double-bond character and inhibit rotation around its own axis which lead to the coplanar conformation of α-carbon. The protein synthesis is carried out inside the living cells in a two-stage process: transcription and translation, which take place in the nucleus and the cytoplasm of the cell, respectively. During transcription, the information from the segment of DNA known as the gene encodes the information for the formation of messenger RNA (mRNA). The mRNA