Engineering Materials

Felipe Avalos Belmontes
Francisco J. González
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Green-Based Nanocomposite Materials and Applications

Springer

Engineering Materials

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Preface

The answer to the development of new light, easy processing, and versatile materials for the fabrication of environmentally friendly products is certainly through the development of new green materials. Furthermore, it is desirable to optimize resources and processes with the subsequent decrease in production costs and energy required for its fabrication.

This book exposes a systematic review of the different stages involved with the knowledge and preparation of environmentally friendly nanocomposite materials. That is the combination of biodegradable and biocompatible polymer matrices with nanoparticles, leading to the generation of finished products preferably prepared by sustainable methods.

Even though there exist many references in literature about nanotechnology and green materials, the present book presents an opportunity to go further into the issue of the prospects, development, production, and applications of environmentally friendly materials. Therefore, it is presented as a reference to continue the research of new materials and applications of green-based nanocomposites.

The content of this volume describes the fundamentals, trends, and general applications of green materials. Moreover, the book comprises the reintegration of these materials into the environment, followed by some biomedical, biological, and energy storage applications.

Despite the efforts made to cover different topics of green-based nanocomposites from several points of view, we are aware that due to the growing literature on biocompatible and biodegradable polymers, nanoparticles used and novel applications constantly developed, it is not feasible to cover the entire materials and applications of eco-friendly nanocomposites. However, we consider that the chapters discussed in this book include the updated reviews of the most relevant research results carried out and reported in the literature by experts worldwide on this interesting type of eco-friendly materials. We hope that our readers have the certainty that the people who collaborated on this volume did their best for them, with a fresh and reliable bibliographical source on the different topics discussed.

In summary, we expect that this book will be a tool for both established researchers and students who are starting in this fascinating world of green-based nanocomposites, providing them with the fundaments and the new contributions in this innovative and continuously changing materials science area. Finally, we also expect that readers can contribute with their discoveries to continue with our task of taking care of our planet's environment.

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Introduction to Green Based Nanocomposites: From Polymer Discovery to Eco-friendly Materials

**Felipe Avalos-Belmontes, Francisco J. González,
and Miguel Ángel López-Manchado**

Abstract The origin of synthetic polymers could be established at the dawn of the eighteenth century, replacing products commonly obtained from the slaughter of mammals currently in danger of extinction. Advances in chemistry and macromolecular science made possible to manufacture synthetic polymers at a commercial level. However, natural and synthetic polymers were not able to cover the entire technical requirements demanded in certain applications; therefore, it was necessary to combine polymers with materials of other nature and properties, leading to the appearance of composite materials. Advances in composite materials science, the discovery of the capabilities of nanoparticles, and the manufacture of nanocomposites reinforced the growth in the use of plastics. However, the plastics industry, especially the packaging sector, has alerted the ecologists, as our culture of consumerism has not taken care of the rules for recycling these materials. Fortunately, new eco-friendly nanocomposites, along with the latest environmental protection laws make it possible to have commercially available biodegradable and biocompatible polymeric products. This introductory chapter presents an overview of each stage, from the discovery of synthetic plastics to the development of eco-friendly plastics. Finally, a brief compendium of the chapters presented in this book is given.

Keywords Nanocomposites · Biocompatible · Biodegradable · Ecofriendly

1 History

The history of polymers is directly related to the history of life on earth. Specifically, it refers to the spontaneous reactions that gave rise to gradually more complex chemical compounds, including the so-called amino acids, which at one point were combined

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to form larger structures called proteins, which are the fundamental component of the tissues that form organs, organelles and that eventually form living organisms. Other forms of biomolecules with this same origin are those formed through glycosidic bonds, whose combinations allowed the formation of structural materials such as cellulose, amylose, amylopectin, chitosan, and natural gums among others. Those materials are polymers of natural origin with biocompatibility and biodegradability characteristics that are implicit because of the origin of the raw materials used during their synthesis.

On the other hand, the origin of synthetic polymers could be established at the dawn of the eighteenth century, with the synthesis of Parkesite by Alexander Parkes, whose synthesis process was later improved by Wesley Hyatt, founding the Celluloid Manufacturing Company [1–3]. Later, Leo Baekeland developed Bakelite, replacing products made from ivory, commonly obtained from the slaughter of mammals currently in danger of extinction, such as elephants and rhinoceros [4–6].

With the advancement of the knowledge of chemistry, along with the important contributions to macromolecular science made by extraordinary scientists such as Flory [7], new materials could be developed. The latter fact grew especially since World Wars demanded most of the production of existing materials such as metals, ceramics, and natural polymers. Therefore, it was possible to manufacture synthetic polymers as we know them today at a commercial level. Thus, two of the best-known polymers were born for the world; the nylon developed by polycondensation mechanisms by Wallace Carothers [8], and polyethylene [9], synthesized by addition polymerization, the two most extensive processes used for obtaining polymers [10–12].

2 Composites and Nanocomposites

Despite the remarkable characteristics and versatility of polymer materials, natural and synthetic polymers are not able to cover the entire technical requirements demanded in certain applications; therefore, it was necessary to combine polymers with materials of other nature and properties. As a consequence, new materials were created with the opportunity to compete with their counterparts synthesized from metals and ceramics. This fact gave birth to composite materials, products that combined the characteristics of the starting raw materials with the advantage that they allowed the development of customized parts that could be “designed” to meet the technical requirements for specific applications [13, 14].

Due to the characteristic forward-looking personality of scientists, they began to investigate the combination of polymers with ceramics and metals in addition to their geometric shapes. They found that it was possible to improve the design of composites as a function not only of the particle concentration in the polymer matrix but also tuning their shape and distribution within the manufactured polymeric parts. Moreover, they discovered that the geometries with the highest diameter-length ratios (L /D), were the ones that provided the greatest resistance to composite materials.

Consequently, they discovered that fibrous materials are the geometric shapes that allow reaching a higher elastic modulus and hence, a higher mechanical performance [15–17].

Despite all the progress achieved up to that date, technologists continued to demand more, better, and cheaper materials. Starting from the premise that large L/D ratios in the reinforcements used optimized the resistance of composites, scientists raised the possibility of using structures at the nanometric level, under the argument that this would optimize not only the resistance but in addition, with the use of nanoparticles, substantial savings could be made in the weight of the manufactured parts, due to the low concentrations used during the manufacturing, giving birth to the nanocomposites [18–21].

The above technological advance gave rise to the wide use of polymers in several applications since they were able to cover practically any technical requirement, from common applications such as paints, fibers and threads for clothing design, frames, tubes, and doors for construction, electronics, automotive, aeronautics, and medical applications. However, the versatility of these materials began to generate problems due to their disposition at the end of their half-life of use [22, 23].

3 The Environmental Issue and Laws

Taking as a starting point Rachel Carson's book in 1962. Silent Spring [24], considered a milestone in environmental conservation, due to the criticism of the uncontrolled use of dichlorodiphenyltrichloroethane (DDT) as a pesticide in agriculture and the implications to the health of organisms involved. As a result, environmentalists began to be concerned about using plastics in different applications. Finally, the bomb exploded when one of the most important applications went out of control: packaging with plastic materials.

Unfairly attributed or not, one of the industries that have caused a strong questioning about the use of polymers is the packaging industry [25–28]. As a consequence, plastic prohibition is a current dilemma. However, it seems that our lack of culture is the fact that has altered our environment. This is since we usually give a single-use to most of the polymers used as containers when the truth is that practically all these containers could be reused and recycled. Hence, effective programs agreed between citizens and the authorities are demanded, to reuse plastic materials effectively [29–31].

Another important issue to be highly considered as a risk, especially in the case of the bottle industry, is the potential health hazard of bisphenols (BPA). BPA are organic compounds commonly used in the synthesis of plastics that are used to manufacture bottles and other products. These molecules present two phenol functional groups with the potential ability to react with estrogen receptors, which can result in endoncrine disruptors in our organism [32–36].

After all, it must be recognized that not only the plastic packaging industry has generated this great controversy about polymer pollution, since all parts made with plastics run the risk of ending in soils, lakes, and seas in the form of micro and nano plastics [37–41]. Those environments that are the habitat of microorganisms and bigger organisms that finally end as part of the human food chain, which may become a real risk to human health [42–47].

In addition to all the previously stated, it is necessary to consider the substantial increase in plastic pollution derived from the pandemic caused by COVID-19. This applies to disposable masks and all plastic pieces consumed due to this terrible health situation in the world [48–54].

4 Development of Eco-friendly Polymers, Nanocomposites and Applications

Considering the evolution in the use and final disposal of plastics, in the last two decades, science has turned towards the development of eco-friendly polymeric materials and composites, focusing on the development and production of plastics with biodegradability and biocompatibility. It is desired that the final disposal of the manufactured parts can return as raw materials, following the circular economy model, to preserve the environment free of contamination by this type of materials [55–63].

Focusing on eco-friendly nanocomposites, we introduce to readers a brief review of the chapters contained in this book. They present well-documented and updated reports on polymers and their nanocomposites with biodegradability characteristics. Biodegradability of these materials occurs through conventional methods of composting or burial underground so that through the specific enzymatic processes of the microorganisms existing in these environments, they are able to neutralize or at least minimize the polluting effect that plastics have at the end of their life cycle. In addition, some of the polymer nanocomposites are also biocompatible with human and animal tissues, blood, and organs.

In Chapter "Nanofillers and Nanomaterials for Green Based Nanocomposites", F. J. González et al. present a review of the most used nanoparticles and nanostructures in the formulation of nanocomposites where the polymer matrices are conventionally biodegradable. These nanostructures comprise carbon-based nanoparticles such as nanotubes (CNT), graphene, and fullerenes, as well as the most widely used nanoclays and metal nanoparticles such as gold and silver. Finally, it deals with the issue of biodegradable nanofibers such as crystalline cellulose and chitosan nanoparticles. In summary, in this chapter, together with Chapter "Sustainable Fillers for Elastomeric Compounds" of Utrera-Barrios et al., they present most relevant nanoparticles are discussed in detail according to their nature, paying special attention to their sustainable synthesis and applications in polymer-based and elastomeric compounds.

In Chapter "Overview of Natural Nanocomposites and Applications", Saenz-Galindo et al. present a general overview of the different formulations of natural nanocomposites. The chapter covers their synthesis routes, conventional methodologies, and the obtention alternatives of these nanocomposites. Moreover, aspects such as their chemical and mechanical properties are presented, along with their biodegradation and biocompatibility in diverse applications.

Thermoplastic starch is another of the most widely used eco-friendly polymers in different applications, either as a single material or formulated with plasticizers and nanoparticles. One of the most common applications is in the packaging industry, although in that case, it has flexibility limitations. In Chapter "High Performance Thermoplastic Starch/Vermiculite Bionanocomposites", Rodriguez-Cueto et al., authors of some patents registered on the topic, present a review of this type of material and the experimental results of obtaining thermoplastic starch nanocomposites formulated with vermiculite. They observe that it is possible to obtain deformations of up to 150%, in addition to high elastic modulus, which are promising results to expand the number of applications of this group of biodegradable polymers.

Proceeding with the study of specific biopolymers, Chapter "Chitin and Chitosan Nanocomposites: From the Synthesis to the Application" of Oyervides-Muñoz et al. presents an exhaustive review of the synthesis and applications of chitin and chitosan. Chitin is a bio-based and biodegradable polymer and the second most abundant natural polysaccharide on the planet after cellulose. Chemically it is composed of *N*-acetyl-D-glucosamine, and it is found on the exoskeleton of different organisms such as crustaceans, insects, annelids, mollusks, plants, and some microorganisms. On the other hand, chitosan is generally obtained by the controlled deacetylation of chitin in the presence of alkali, composed of randomly bound D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit).

These two polysaccharides have different physicochemical and biological properties, which can be modified and/or improved by nanocomposite formulations based on this polymer. In Chapter "Composites Based on Chitosan and Inorganic Materials for Biomedical Applications", S. Silva et al. offer a review on chitosan and inorganic materials in biomedical applications.

Although it is known that acrylic polymers are not normally biodegradable, it is also true that when biobased and/or biodegradable polymers are copolymerized with acrylic monomers, the acrylic section of these grafted copolymers is subject to enzymatic biodegradation. In Chapter "Biodegradable Acrylic Polymers and Nanocomposites" of F. Avalos et al. provide an updated review on biodegradable acrylic polymers and nanocomposites, such as starch, cellulose, PLA, chitosan, alginate, and natural gums. This chapter details the grafting polymerization and the cross-linking methodologies of the materials obtained, as well as the preparation of nanocomposites. Applications of these nanomaterials cover the removal of toxic chemicals in water and fluids, biocompatibility for the controlled drug release, the dosage of fertilizers in agricultural activities, and the obtaining of superabsorbent materials. To summarize, the use of acrylic monomers for graft modification of biobased and/or biodegradable polymers allows wide versatility for these materials, minimizing their environmental impact.

Collagen is a natural polymer with the ability to generate three-dimensional (3D) complex matrices formed by nanometric scale fibrils. These materials have promising biological and biomedical properties. They constitute a natural scaffolding where mammalian cells can perform their vital functions; therefore, they have been widely used in fields such as regenerative medicine, controlled drug release, in vitro cell culture models, cell phenotype modulation, and tissue engineering. On the other hand, acrylic polymers have been widely used for functionalization with collagen, designing nanocomposites with different architectures, mechanical properties, adhesion, swelling, and biofunctionalities. On this topic, in Chapter "Nanocomposites Comprise of Collagen and Acrylate-Derived Polymers for Biomedical Applications" of Cano-Salazar et al., they present the fundamentals of the synthesis processes, the characterization and applications of nanocomposites based on collagen and polymers derived from acrylate in biomedical fields.

One of the most environmentally friendly synthetic polymers is polyvinyl alcohol (PVA). This material has been studied extensively due to its easy processability, hydrophilic characteristics, biocompatibility, and interesting properties when combined with other polymers. In Chapter "PVA Blends and Nanocomposites, Properties and Applications: A Review", Vera Garcia et al. present a review of the studies carried out on mixtures of PVA with different polymers and nanocomposites with nanoparticles, their properties, and applications.

Among the most studied biopolymers, polyhydroxyalkanoates (PHAs) have attracted greater attention due to their thermoplastic, biodegradable, biocompatible, and renewable nature. F. Rivera-Gálvez in Chapter "Diversifying Polyhydroxyalkanoates: Synthesis, Properties, Processing and Applications" describes a general overview of the synthesis, processing, properties, and applications of this polymer family and their nanocomposites. Specifically, polyhydroxybutyrates (PHB) are the most widely used PHAs, as they provide similar properties to several synthetic plastics, including polypropylene. However, PHB have some disadvantages, such as their high cost, slow crystallization rate, and their relatively high crystallinity degree. Fortunately, these problems can be fixed by modifying the polymer formulation to obtain nanocomposites. The development of PHB-based materials promises to expand the use of biodegradable materials, expanding their applications, including properties such as antimicrobial performance with the use of nanotechnology. Estrada-Monje et al., in Chapter "Polyhydroxybutyrate-Base Nanocomposites and Blends. Applications", present a review of PHB-based nanocomposites, including cellulose nanocrystals and silver nanoparticles, and the applications of the materials obtained.

Poly(butylene succinate) (PBS) is one of the most attention-grabbing biodegradable polymers to replace non-biodegradable and polluting petroleum-based polymers. The ease of processing and its adequate balance in thermomechanical properties make this polymer suitable for applications in sectors such as food packaging, agriculture, and medicine. The results of the latest research on biodegradable polymer nanocomposites based on PBS promise to compete with some of the main commercial polymers (polyethylene and polypropylene) in an expected short time since it offers the possibility of processing by conventional processing techniques such as extrusion, injection, thermoforming, foaming, etc. Chapter “Poly(Butylene Succinate). Functional Nanocomposite Materials and Applications” of Medellin-Banda et al. presents a review of current research on the preparation, properties, and applications of PBS and nanocomposites, with reinforced and functional properties such as those required in electronics, robotics, biosensors, etc., which may offer an opportunity to mitigate current and future environmental problems.

Chapter “Sustainable Approach of the Natural Rubber” of Aguilar-Bolados et al. deals with the environmental problems related to natural rubber, a material obtained from plant sources, such as *Hevea brasiliensis* and *Parthenium argentatum* plants that are also capable of synthesizing cis-1,4-polyisoprene through rubber transferase. A special focus is given to understanding the fundamental aspects of the biosynthesis of cis-1,4-polyisoprene, as well as its biological degradation. The chapter addresses the reactions, processes, and additives used in natural rubber according to the applications needed and how these can hinder the degradation of this polymer. Within this same context, in Chapter “Sustainable Fillers for Elastomeric Compounds” presented by Utrera-Barrios et al., alternative solutions are addressed through the formulation with nanoparticles like chitin, chitosan, lignin, and cellulose as sustainable fillers in elastomeric compounds. Finally, they provided brief information on the bacterial and fungal strains recently identified for the degradation of both polymers and fillers.

One of the most environmentally friendly bio-based polymers that have been gaining importance in the market are tree gums. They can be divided into three categories according to their origin: plant exudate gums, microbial gums, and seed gums. Gums are abundant, cheap, harmless, biodegradable, and biocompatible. Gum nanocomposites are used in various industries and applications, such as packaging, tissue engineering, drug delivery, and environmental applications. S. Thakur, in Chapter “Gum Based Green Nanocomposites and Their Applications”, provides an overview of the state of the art of natural gums-based nanocomposites and discusses their application in various fields.

For their part, Cruz-Delgado et al. present in Chapter “Nanocomposites Based on Biodegradable Polymers for Biomedical Applications” the biomedical applications of nanocomposites. They present a review on the new alternatives of regenerative medicine using biocompatible, biodegradable materials with low or no cytotoxicity. The most promising biodegradable polymers for these applications are PLA, PVA, and PHA, which have served as support or matrix. Moreover, different biopolymers such as chitosan, chitin, cellulose, collagen, alginate, and combinations of them have been used as a bridge to increase the effectiveness of the stimuli of the receptor organism, obtaining and improvement on the biological response through synergistic effects.

In the Chapter “Review and Analysis of Biological Tests on Nanomaterials to be Applied in Biological Areas” of Cuellar-Gaona et al., they provide an approach that combines the analysis and review of standards and methodologies tests carried out on nanomaterials or devices that allow us to determine if they can be considered biological nanomaterials. Some examples are interaction with blood (hemolysis), sensitization, implantation, toxicity, immunocitotoxicity, genotoxicity, and carcinogenicity, resulting in reliable and comparable data to determine whether or not a nanomaterial is toxic in a biological setting. In addition, authors supply some examples about how different biological assessments have been applied in recent years.

This book also covers specific applications of eco-friendly nanocomposites. Chapter "Harnessing the Potential of Fibrous Polyester Composites Meant for Bioactive Medical Devices" of G. Morales et al., provides a compilation of several studies on the development of submicron fibers based on two important polyesters for biomedical use, poly(3-hydroxybutyrate) and PLA. Studies are focused on fibers developed using hydrodynamic techniques, like electrospinning, electrospraying, and centrifugal spinning with the incorporation of zinc oxide or hydroxyapatite into polyester fibers, which results in materials that inhibit bacterial growth or induce bone cell proliferation.

Pabon-Mora et al., in Chapter "Nanotechnology Applied to the Management of Fungal Diseases in Tropical Crops", present a review of recent advances in the research and development of nanotechnology in the management of fungal diseases that are applied in tropical areas. In this context, the reports on the main causes of these and the control mechanisms are presented, as well as the nanobiocontrollers, which are systems normally manufactured through nanocomposites for controlled release of the active ingredients capable of slowing down, stopping, or eliminate the fungi and bacteria that cause these diseases.

Finally, Lopez-Cudero et al. in Chapter "The Role of Polymer-Based Materials in Sustainable, Safe, and Efficient Metal Batteries" present a detailed review of energy storage and the use of polymers and biopolymers in electrodes, binders, and electrolytes, components of batteries. A detailed study on synthesis, preparation and processing strategies of new polymer-based battery components is carried out, focused on the progress towards the development of cleaner and more sustainable processes for batteries, including the potential application of new green chemistry and engineering approaches.

5 Conclusions

Despite the efforts made to cover different topics of green-based nanocomposites from several points of view, we are aware that due to the growing literature on biocompatible and biodegradable polymers, nanoparticles used in formulations and novel applications constantly developed, it is not feasible to cover the entire materials and applications of eco-friendly nanocomposites. However, we consider that the chapters discussed in this book include the updated reviews of the most relevant research results carried out and reported in the literature by experts worldwide on this interesting type of eco-friendly materials. We hope that our readers have the certainty that the people who collaborated on this volume did their best effort, with a fresh and reliable bibliographical source on the different topics discussed here.

In summary, we expect that this book will be a tool for both established researchers and students who are starting in this fascinating world of green-based nanocomposites, providing them with the fundaments and the new contributions in this innovative and continuously-changing materials science area. Finally, we also expect that readers can contribute with their discoveries to continue with our task of taking care of our planet's environment.

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Nanofillers and Nanomaterials for Green Based Nanocomposites

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Abstract During the last decades, nanotechnology has allowed important and numerous advances in diverse scientific and technological fields by providing the bases for developing nanoscale materials that exhibit novel characteristics that differ from that of bulk materials. On the other hand, following the circular economy model, biodegradable polymers have emerged as substitutes for non-biodegradable polymers in several technological applications. This chapter discusses the most relevant nanoparticles in detail according to their nature, giving special attention to their synthesis, processing, and applications. Additionally, some of the most relevant nanostructures for biodegradable polymers are presented.

Keywords Biodegradable polymers · Nano-fillers · Nanotechnology · Nanocomposites · Green synthesis

1 Introduction

Polymer and plastic derived materials are type of materials part or our everyday life. These materials usually contain additives and fillers in their formulation. Both additives and fillers are aimed to improve the processability and properties while reducing production cost. Fillers were used at the beginning of the plastics industry with the objective of decreasing the cost of the final products. Nevertheless, current technological applications require the development of efficient polymer-based materials where the fillers are used not only to reduce the production cost but also to improve or to give specific properties to the materials, i.e., mechanical, electrical, thermal, and barrier properties among many others [1].

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Nanomaterials are commonly defined as materials that present a nanometric size in at least one dimension [2]. The development of materials that share the particular feature that at least one of their dimensions is in the nanometric scale has attracted great scientific and industrial attention up to the point that nanotechnology has been established as a discipline in constant evolution. This interest is reflected in the large efforts that have been made in the synthesis, improvement, and refinement of the properties of nanomaterials. One of the main characteristics of nanomaterials is that their size provides remarkable properties, i.e., optical, electrical, and higher surface area in comparison with bulk materials. In that context, nanotechnological applications have been continuously under research in diverse areas like electronics, energy, medicine, catalyst, and materials science. Nanofillers can be classified according to their function, structure, shape, or nature. In this book chapter, nanofillers are classified as a function of their nature, being addressed as carbonaceous materials, inorganic clays, metallic, and biodegradable particles.

Fillers can be addressed according to their dimensionality into three categories as follows: fibers (1D), flakes (2D), and particles (3D). Fillers with different dimensionality are shown in Fig. 1. Along with dimensionality, filler aspect ratio, defined as the ratio of the longest to the shortest dimension of the particle, plays an important role in composite materials because it is associated with the surface energy of the filler. Surface energy has a large effect on the dispersibility and compatibility of the filler with the polymeric matrix [3, 4]; hence, higher-aspect ratios imply a high interaction filler—polymeric matrix, and incorporation of relatively small concentrations of higher-aspect ratio fillers induces notable changes in the properties of polymer-based composites. By considering the relationship between aspect ratio and surface energy, it results convenient to diminish a particulate dimension of the filler from micro- to nanoscale with the aim of increasing several orders of magnitude its aspect ratio, which, in turn, improves the filler—polymeric matrix interaction.

Among other applications, nanotechnology has been applied to overcome the environmental problems caused by plastic pollution. The development of sustainable materials heading to the circular economy model and green chemistry principles has become a necessity. In this respect, a recent scientific and technological interest has grown in the development of composite materials based on biodegradable polymers, using nano-size fillers or nanofillers. In contrast to traditional composites, superior properties have been achieved in nanocomposite materials by incorporating a small amount of the nanofiller. Particularly to biodegradable-based nanocomposites, the latter argument is very relevant because although some nanofillers are not biodegradable, the amount used in these nanocomposites is low, usually below 5 wt%. Therefore, it is ensured the biodegradability of the greatest part of the manufactured final piece. A lot of research has been focused on biodegradable polymers during the last decades. Some of the most studied biodegradable polymers are polylactic acid (PLA), polybutylene succinate (PBS), polyhydroxyalcanoates (PHAs), chitosan (CHT), polyvinyl alcohol (PVA), cellulose, starch, and alginates, some of which can have a synthetic or natural origin.

In this book chapter, we present a general overview of some of the most employed nanofillers, emphasizing their synthesis, classification, processing, properties, and

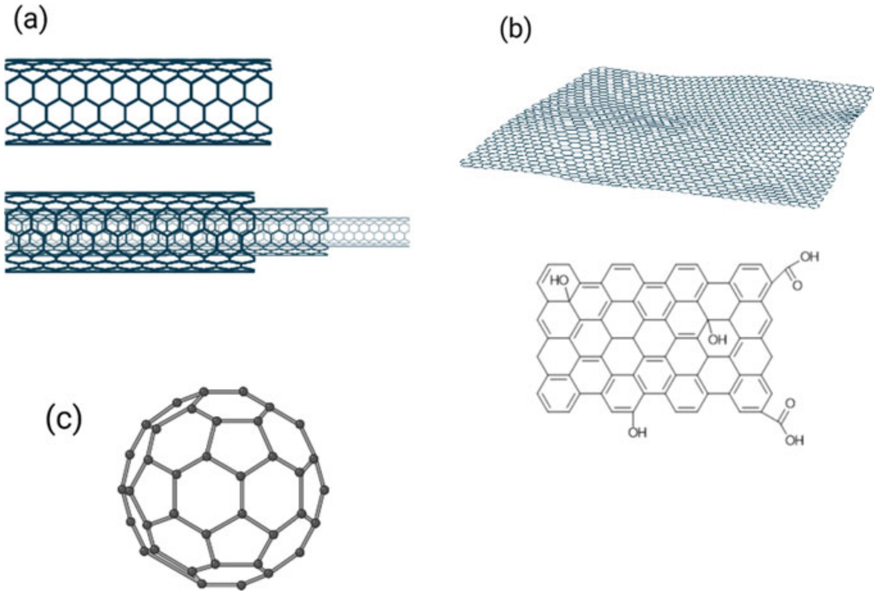

Fig. 1 Carbon-based nanoparticles commonly used as fillers in green polymer-based matrices: **a** 1D materials: single and multiple wall nanotubes; **b** 2D flakes: graphene and reduced graphene oxide; and **c** fullerene as a 3D nanoparticle. Created with BioRender.com

general applications. In later sections of this book, more details about specific biodegradable polymers as well as green-based polymer nanocomposites and novel applications will be presented.

2 Carbonaceous Nanofillers

The incorporation of different types of nanofillers is not only aimed at tuning specific properties but also at inducing new and fascinated properties to polymeric matrices. Respecting green polymer nanocomposites, carbon-based nanofillers, as those presented in Fig. 1, have been regarded as one of the most promising nanomaterials for improving and inducing thermal and electrical conductivity, flame resistance, thermal stability, antimicrobial features, and biodegradability [5–7].

Carbon-based nanofillers can be further modified to extend even more their dispersibility and compatibility, as well as to impart them unique properties [8–12]. Besides, nanofillers of various types can be incorporated into the preferably biodegradable polymer-based matrix to take advantage of their functional synergism [13–16]. The aforesaid features make the application of carbonaceous nanofillers in nanocomposites to be a vast field of study. Consequently, the most common used carbon nanofillers and their characteristics and preparation methodologies are further discussed thereafter.

2.1 Carbon Nanotubes

Carbon nanotubes (CNTs) have been the focus of particular interest due to their outstanding properties. In comparison with traditional carbon fibers, CNTs possess high tensile strength (between 50 and 100 GPa) and modulus (in the order of TPa), and exceptional thermal and electrical properties [17]. There are two main types of CNTs: the so-called single-walled (SWCNTs), which can be conceptualized as a cylinder made up of a single layer of graphite that is one atom thick, with a diameter of a few nanometers, and a length of thousands of times its diameter, as shown in Fig. 1a; and those with multiple walls (MWCNTs) made up of multiple layers of graphite that form concentric cylindrical structures, as depicted in Fig. 1a. Although SWCNTs have better mechanical properties than MWCNTs, the latter are the most widely used because their production cost is considerably cheaper than that of single-walled nanotubes. Methods for CNTs synthesis include arc discharge, laser ablation, and chemical vapor deposition (CVD) [18].

When incorporating into a liquid matrix, regardless of their viscosity, CNTs tend to form agglomerates due to attractive Van der Waals forces. Therefore, it makes difficult to process and disperse CNTs in polymeric matrices. This fact, together with the smooth and inert nature of the nanotube surface, results in less interfacial adhesion and inhibits an efficient transfer of CNT properties to the matrix [19]. On the other hand, the low solubility of nanotubes in solvents and their poor dispersion represent important limitations for their applications. For this reason, it has been necessary to develop a series of mechanical, physical, and chemical methods to improve CNTs dispersion. Mechanical methods include high shear mixing or ultrasonic treatment [19]. Physical methods comprise the oxidation or plasma treatments [8], the use of surfactants on the surface of nanotubes [20], or the technique of wrapping nanotubes with polymers [21] in order to reduce the barrier of interfacial energy between the nanotube and the polymeric matrix. Among the chemical methods, the functionalization of the surface of the nanotubes stands out to introduce specific groups [22, 23] or the functionalization of the polymeric chains itself [24] to improve the compatibility between the load and the matrix through the interfacial interactions.

Biodegradable polymers such as PLA have been prepared by incorporating SWCNTs as a filler or reinforcement [25]. Wu et al. reported the preparation of PLA/MWCNTs composites by using two strategies to improve the compatibility between the polymer and the filler: (1) a graft modification of PLA with acrylic acid (PLA-g-AA) and (2) a functionalization of MWCNTs with hydroxyl groups. Both approaches resulted in the enhancement of thermal and mechanical properties of the PLA/MWCNTs composites [26].

2.2 Graphene and Derivatives

Graphene (GN), a single layer of graphite, consists of an atomically thin layer of carbon atoms arranged in a two-dimensional honeycomb structure. A GN flake structure is presented in Fig. 1b. In the GN layer, the overlap of sp^2 orbitals between neighboring carbon atoms give rise to three σ bonds, meanwhile the rest p_z orbitals form the valance and conduction bands that are associated with the filled π and empty π^* orbitals, respectively [27]. The specific honeycomb structure of carbon atoms with their sp^2-hybridized bonds endows GN with high specific surface area, and remarkable mechanical (stiffness 1 TPa), thermal (conductivity 5000 W m^{-1} K^{-1}), optical (transparency 97.7% transmittance) and electronical properties (charge-carrier mobility 250,000 cm^2 V^{-1} s^{-1} at room temperature) [28–31]. In spite of its outstanding properties, functionalization of graphene is carried out for increasing its dispersion and overcoming limitations in technological applications such as in semiconductors and sensors, as GN is inert to reaction and possesses zero band gap [11, 32].

In the synthesis of GN, mechanical exfoliation, epitaxial growth, and thermal reduction of graphene oxide (GO) are the most used methods; nevertheless, the last two have preference because they enable large-scale GN production [10, 11, 31–33]. With respect to GO, it is obtained by incorporating epoxide, carbonyl, carboxyl, and hydroxyl groups in the GN structure. In consequence, GO possesses a 2D layer structure that is formed by amorphous domains related to sp^3 C–O bonds that results of the incorporation of aforesaid oxygenated functional groups, as well as by crystalline domains of sp^2-hybridised carbon atoms arranged in a hexagonal lattice [29]. The structural modifications described above are accompanied by new and interesting physicochemical properties, as the incorporated functional groups serve as anchor sites for further tunability of GO physicochemical properties through chemical functionalization, whilst the polar oxygen functional groups make GO strongly hydrophilic, facilitating its dispersion in diverse solvents [10, 11, 27, 32, 34]. GO results to be convenient for large-scale production, and its functionalization and easy dispersion allow to develop diverse graphene-based structures with controllable electronic, optoelectronic, mechanical, and transport properties [29, 32, 34]. Nonetheless, some applications demand to partially restore the properties of GN. In this respect, the oxygenated functional groups of GO are chemically or thermally removed, resulting in reduced graphene oxide (RGO), as seen in Fig. 1b. Among other strategies, it is worthy mentioning that the in-situ GO reduction via either polymerization or melt process in a polymeric matrix could be advantageous in developing polymer-based nanocomposites reinforced with RGO [35–37]. Very recently, it was reported the use of RGO with a biodegradable starch-chitosan matrix for active food packaging [38].

2.3 Fullerenes

Fullerenes are carbon-based compounds that have a closed mesh network of small rings that constitutes a geodesic spheroid, being formed by *sp*^{2}-hybridized and highly-isomerically-diverse atoms that frequently develop multi-layered structures [39, 40]. A fullerene is depicted in Fig. 1c. Although CNTs can be considered as fullerene-related particles with a quasi-one-dimensional behavior, fullerenes have attracted large attention due to their unique topology [41]. The feasibility of endohedral, substitutional and exohedral doping make fullerenes promising materials in fields such as nanotechnology, electronics, and biotechnology by imparting new and fascinating structural, magnetic, superconducting, electrochemical, and physicochemical properties [41–43]. Additionally, fullerene-containing polymers and biodegradable polymers have been synthesized, resulting in potential materials that combine the properties of the polymers with those of fullerenes [44, 45].

3 Inorganic Nanoclays

Inorganic clays are one of the most conventional fillers used for polymer-based nanocomposites. Inorganic fillers are commonly incorporated for mechanical enhancement and to reduce manufacturing costs. Some of the most employed inorganic fillers are talc, silica, and clays. As previously mentioned, a distinct characteristic of the polymer nanocomposites is the significant improvement of desired properties by using a small concentration of nanoparticles, avoiding undesirable characteristics such as density increment and transparency loss [46]. Some of the main motivations for the use of nanoclays-based nanocomposites is due to their low cost, availability, and easy processing [46–48].

Phyllosilicates or layered silicates are one of the most used materials to prepare nanocomposites based on clay-polymer systems. The structure of these layered silicates consists of layered structures formed by different coordination tetrahedral sheets and octahedral sheets. Depending on the structure of the sheets, different coordination cations can be found, such as Al^{3+}, Mg^{2+}or Fe^{2+} [49]. The most extensively studied clays in polymer nanocomposites comprise montmorillonite, hectorite, halloysite, saponite, and sepiolite [49–51].

Three interactions are described to occur between the polymer matrix and the clays that are (1) agglomerated or phase separated, (2) intercalated, and (3) exfoliated structures [46, 47, 49, 51]. The agglomerated structure is typical of microcomposites where it exists a phase separation between components. The intercalated structure occurs when polymer chains are located between the inorganic layers of the phyllosilicate, forming an ordered clay-polymer structure, and finally the exfoliated structure, where the phyllosilicate layers are uniformly exfoliated and dispersed in the polymer matrix.

The interactions described above can be obtained depending on the processing techniques. The three most common are the following: (1) intercalation in a polymer solution, (2) in-situ polymerization, and (3) melt intercalation [47, 48, 51]. The latter technology becomes very relevant on an industrial scale to avoid the use of additional solvents. Depending on the clay-polymer interactions, some compatibilizer agents are added to improve the interactions, especially in low polarity polymers like polypropylene [52]. One approach is the clay modification with alkyl ammonium or the use of copolymers in the formulation like polypropylene grafted with maleic anhydride [53]. Clay nanocomposites have been incorporated in biodegradable polymer composites like PLA [54–56], starch [57], chitosan [58, 59], and PHAs [60, 61].

4 Metal Nanoparticles

Metallic or metal nanoparticles have acquired great importance due to their unique properties. These nanoparticles are generally prepared from noble metals like silver, gold, platinum, or metal oxides and have been used in diverse fields, especially in optics, catalysis, electronics, and biomedicine [62].

There are two main approaches for the synthesis of metallic nanoparticles: the top-down and the bottom-up approaches [62]. In the top-down approaches, generally, the macroscopic bulk material is subject to a chemical or physical process that turns the macroscopic material into nanoparticles. Examples of these methods are mechanical milling, laser ablation, or sputtering. On the other hand, in the bottom-up approaches, nanoparticles are formed from the joining of particles of smaller sizes. i.e., small molecules, ions, or atoms. Numerous methods have been developed, such as physical or chemical vapor deposition, chemical reduction of metallic salts in liquid or gas phase, or electrochemical methods.

In accordance with the current research trend, green synthetic methods avoiding the use of toxic solvents and reagents for the synthesis of nanoparticles are highly recommended. In this regard, it exists a recent and growing research towards the synthesis of metallic particles using plants and extracts. [63–65]. Briefly, this biogenic method consists of the use of plant extracts as a reducing agent of metal ions to subsequently form nanoparticles [66]. Due to its benign synthesis of these nanoparticles, which avoids the use of other synthetic reagents, this methodology has been widely followed and applied for biomedical applications. It is worthy to note that the plant extract used during the synthesis of metal nanoparticles has a direct effect on the shape, size distribution, and antibacterial properties [67].

4.1 Gold Nanoparticles

Synthesis of gold nanoparticles (Au NPs) is usually carried out in liquid aqueous solutions to obtain a colloidal dispersion of the nanoparticles. In a typical synthesis. $AuCl_3$ salts are normally reduced by a chemical reagent to produce chemical ions. After that, a nucleation process occurs that leads to the formation of the Au NPs. This reaction is normally carried out by adding a surfactant to obtain a stable colloidal solution [68]. Applications of Au NPs include labeling, drug delivery vehicles, hyperthermia treatment, and optical sensors [62, 68].

Because of the electrical properties of noble metal particles, Au NPs have been successfully used in biodegradable polymer-based nanocomposites to improve electrical conductivity, dielectric properties, and ion mobility of the polymer [65]. For instance, eco-friendly nanocomposite materials have been prepared by using different plant extracts and green synthesis of Au NPs [67]. In the aforesaid nanocomposites. Different concentrations of nanoparticles were added to solutions of carboxy methyl cellulose (CMC) to form a film with thickness ranging from 55 to 100 μm. An improvement in electrical conductivity of 3 magnitude orders was observed compared to pure CMC at a temperature of 353 K. Similarly; another report confirms the effectiveness of Au NPs used as active fillers in green nanocomposites based on PVA [69]. More recently, nanocomposites based on a mixture of biodegradable polymers reinforced with Au NPs have been described. Au NPs were synthesized using the leaf extract *Mentha Spicata L.* The synthesized Au NPs were added to a solution that contained a mixture of PVA/CMC in a 70:30 weight ratio. An increase in the direct electrical conductivity of 4 magnitude orders was observed, as well as a decrease in the dielectric permittivity [70].

Another interesting approach toward the green synthesis of Au NPs is using biodegradable polymers like chitosan as a reducing and stabilizing agent [71]. In this respect, a solid film of the nanocomposite material was prepared and used for surface-enhanced Raman Scattering (SERS) for the detection of tryptophan as analyte model molecule. Begines et al. reported the synthesis of Au NPs stabilized by sugar-based polyurethanes to form a nanocomposite [72]. The selected material was then processed and used by an inkjet printing technique for active packaging applications due to its antibacterial properties or in a cellulose/keratin polymer nanocomposite for controlled drug release [73]. Another nanocomposite made of xanthan gum grafted with polyacrylic acid by an in-situ microwave-assisted polymerization with green synthesized Au NPs has been reported [74]. Au NPs were prepared from the plant extract *Nepeta leucophylla*, which, posteriorly, were incorporated in a biodegradable hydrogel for controlled drug release of amoxicillin. In addition to the effect of the nanoparticles to control the diffusion rate, a maximum drug loading efficiency of 85% was obtained. It is worth mentioning that other biodegradable polymers such as alginates, polysaccharides, poly(ε-caprolactone) (PCL) copolymers, and PVA copolymers have been used as stabilizer agents for Au NPs [75].

4.2 Silver Nanoparticles

Silver nanoparticles (Ag NPs) are well known due to their antibacterial, optical, electrical, and catalytic properties [76]. Ag NPs synthesis is usually carried out by chemical reduction methods, probably due to their simplicity. A typical synthesis of Ag NPs involves reducing agents like sodium citrate, sodium borohydride, thioglycerol, hydrazine, and 2-mercaptoethanol [77, 78]. However, the toxicity of the above-mentioned reagents has led to prefer green synthesis methods especially for biomedicine and packaging applications.

Ag NPs were synthesized by using a *M. frondose* leaf extract as reducing agent. A nanocomposite material based on the Ag NPs, chitosan, and gelatin was prepared by solution casting for food packaging as an ecofriendly material that extended the shelf life of carrots [79]. In another green synthesis, Ag NPs were prepared using lemon juice as reducing agent, and, subsequently, incorporated as nanofiller in a starch matrix by using solvent casting method to form a polymer film [80]. These films demonstrated an effective antimicrobial capacity and a decrease in water vapor permeability, which made them a suitable material for food packaging applications. Taking advantage of the antibacterial properties of Ag NPs and the biocompatibility of Au NPs, silver-coated Au NPs were prepared [81]. These core–shell nanoparticles were deposited on cellulose paper to develop an effective antibacterial biodegradable film. An extended review of green nanocomposites based on Au and Ag NPs used for food packaging applications has been reported in the following references [82, 83].

A cellulose-Ag NPs composite using *Ocimum sanctum* as the reducing agent was reported [84]. In this case, dip processing was carried out by dipping the cellulose films previously impregned with the plant extracts in $AgNO_3$ solutions. The material showed good efficiency with the *E-coli* bacteria, having promising applications in medical dressing.

The capacity of chitosan to act as a reducing agent and stabilizer in the synthesis of metal nanoparticles is well known. Popescu et al. prepared a hydrogel composed of Ag NPs, PVA, and chitosan [85]. The material was crosslinked by the action of a freeze-thawing method and an ionic crosslinking made by oxalic acid. The crosslinked gel showed an effective antimicrobial activity against three species of pathogenic bacteria: *S. aureus, P. gingivalis, and K. pneumoniae*. Due to its outstanding antimicrobial properties, this material was proposed as a candidate for periodontitis treatment. On the other hand, a PVA/chitosan/Ag NPs nanocomposite was prepared by electrospinning. Firstly, a chitosan/Ag NPs composite was synthesized. After that, a mixture of PVA with the previously prepared nanocomposite was blended and processed by electrospinning to prepare a fiber mat. The hybrid materials showed that mechanical properties such as elastic modulus or hardness were improved with the addition of Ag NPs in comparison with the control materials, which turned them into promising materials for biomedical applications [86].

The synergistic effect of mixing metallic nanoparticles with other nanofillers has been explored. Ag NPs and SWCNTs have been used in combination using PCL as the host polymer matrix [87]. It was reported that SWCNTs and Ag NPs provide a synergic effect that is reflected in an enhancement in electrical conductivity and mechanical properties. This effect was explained due to the nucleation effect of both nanoparticles and that Ag NPs work as a conductive pathway between the SWCNTs. A similar synergic effect was observed with Ag NPs and graphene oxide [88].

5 Biodegradable Polymer Nanoparticles

5.1 Cellulose Nanoparticles

The necessity of fully biodegradable material has motivated the development and current research of biodegradable fillers. To achieve this, natural polymers such as cellulose have been used in preparing biodegradable fillers. The main advantage of cellulose nanostructures is the relatively high abundance of this biopolymer on our planet. In addition to this and heading to circular economy principles, cellulose nanostructures can be obtained from waste derived from agriculture residues [89]. According to its origin and structure, three different cellulose nanostructures are found: (1) bacterial nanocellulose (BNC), (2) cellulose nanofibrils or microfibrils (CNF or CMF), and (3) cellulose nanocrystals (CNC) [90]. Bacterial nanocellulose is obtained by the action of bacteria, mainly *Acetobacters* [91]. CNF or CMF are fibrillar structures with a diameter in the range of 10–100 nm, and micrometers of length [92]. The difference between CNF and CMF lies in the diameter size, with micron-size diameter in the case of CMF and nano-size diameter in the case of CNF [93]. However, both terms are frequently confused in the literature. These materials were firstly obtained by mechanical delamination of fibers under high pressures. Nonetheless, the high energy consumption of the process has led to the development of other alternatives, for instance, the use of homogenizers and microfluidizers, in addition to pretreatments prior to the delamination, such as oxidizing or enzymatic treatments [90]. CNCs, also denoted in literature as nanowhiskers, are highly crystalline cellulose structures with a rod, needle, or whisker shape with a size in one dimension lower than 100 nm [93]. The most applied method to obtain CNC is acid hydrolysis, where the cellulose starting material is treated by harsh acid conditions. After that, the material is usually separated by centrifugation and filtration techniques to obtain the final nano product [93].

Research on cellulose nanostructures incorporated in biodegradable polymers like PLA has been carried out for more than two decades, being the work of Iwatake et al., a pioneer in this field, and which consisted in using CNF as a reinforcement of PLA [94]. These nanocomposites were prepared by gradually adding PLA to a dispersion of the CNF in a water/acetone mixture. Here, the CNF suspension played an important role in the correct dispersion of nanofiller in the polymeric matrix. The solvents were evaporated from the mixture, and the remaining material was further processed using a twin rotary roller mixer. An improvement in the Young's Modulus and tensile strength was notably increased when adding the dispersed nanoparticles.

The use of CNF has been applied as reinforcement for PBS [95]. In addition to the mechanical reinforcement provided by CNC, CNC showed to improve the miscibility between two biodegradable polymer blends like PLA and PBS [96].

Some alternatives can be done to improve the interaction between the polymer and the filler. One is the use of silanes in the case of CNF and PLA [97]. Another method to improve the dispersion of the fibers and the PLA can be achieved by surface modification [98]. In this regard, an esterification reaction of the CNF was done previously to the mixture; after that, the modified CNF was added to a dissolution of PLA using an Ultra Turrax homogenizer.

5.2 Chitosan Nanoparticles

Chitosan nanoparticles (CHT NPs) are another interesting group of biodegradable nanofillers. The two most common methods for the synthesis of these nanoparticles are ionotropic gelation and polyelectrolyte complex. The ionotropic gelation method consists of the nanoparticle formation due to the interactions of the amine group in an acidic solution of chitosan and a polyanion that is added to the mixtures. On the other hand, the polyelectrolyte complex method consists of the formation of CHT NPs due to the addition of a DNA in an acetic acid solution of chitosan due to the charge neutralization between the macromolecules [99]. Chitosan nanostructures can also be found in the form of fibers, where electrospinning is the most common technique for fiber formation [100]. CHT NPs have been widely used for tissue engineering, drug delivery, antioxidizing agent, water treatment, antimicrobial agent, and food packaging, among others [99, 101]. Among those applications, nanocomposites based on CHT NPs have promising applications for food packaging. Likewise other cases, it has been demonstrated that the combination of CHT NPs with other carbonaceous, metal, and inorganic nanofillers can improve the drawbacks of CHT NPs in addition to the antibacterial properties in the final nanocomposite [101].

As seen in previous sections, the combination of nanofillers provides combined properties to the nanocomposites. Therefore, it seems an appealing strategy heading to this new generation of smart multifunctional materials. Figure 2 summarizes the strategies and nanofillers reviewed towards sustainable methods for obtaining multifunctional nanocomposite materials.

6 Conclusions

The high materials demand for satisfying technological applications and the environmental impact caused by the non-biodegradable polymers industry has resulted in the mandatory development of efficient nanocomposite materials based on biodegradable polymers. This review covered some of the most relevant nanofillers and nanoparticles of different nature and characteristics and which are used for preparing novel

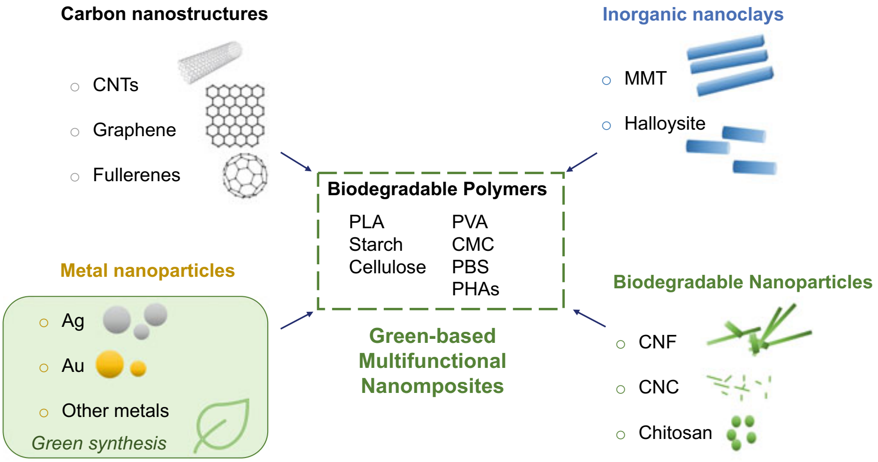

Fig. 2 Strategies using nanofillers of different nature heading towards the development of multifunctional nanocomposites

nanocomposites based on biodegradable polymers. Despite the fact that fillers were initially used as an alternative for reducing manufacturing cost and mechanical reinforcement, nowadays, nanofillers and nanoparticles are used for improving mechanical, electrical, barrier, optical, and antibacterial properties in diverse applications like electronics, biomedical, drug delivery, food packaging among many others.

In addition to the multifunctional properties that nanocomposite materials provide, the circular economy model demands the use of biodegradable polymers in processes and materials. In the case of synthesis of nanoparticles, the use of non-toxic solvents, reagents, and preferably green biosynthesis of these materials is highly preferred. In the case of the greensynthesis of noble metal nanoparticles, there have been numerous advances in recent years. The advantage of nanocomposite materials in comparison with conventional composites is that the theoretical particle concentration in nanocomposites is minimal in comparison with conventional composites to achieve similar properties. However, an excellent dispersion of nanofillers or nanoparticles is usually needed; to achieve this, different physical or chemical modifications of nanoparticles can be done to improve the interactions between the nanoparticle and the polymer. Finally, one interesting approach heading multifunctional nanocomposites is to perform a combination of nanoparticles of different nature. This can be achieved through physical mixtures, core–shell synthesis, or chemical modification. This last approach seems very attractive as many combinations and novel synthetic routes can be developed to obtain materials with dual properties. i.e., electrical and antibacterial properties, and possible synergic effects in the final material.

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Sustainable Fillers for Elastomeric Compounds

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Abstract The development of sustainable rubber compounds is gaining traction due to the environmental issues caused by the extensive use of polymers and the need to move towards a circular economy. However, the conventional fillers used in elastomer compounds to improve their mechanical performance hinder their degradation. Therefore, materials such as chitin, chitosan, lignin, and cellulose, are being considered as sustainable fillers in elastomeric compounds. This chapter presents a review that addresses the promising biodegradable fillers from vegetable sources, their biosynthesis, chemical properties, applications, reinforcing effect, and the improvements in the performance of composite materials based on natural and synthetic rubber matrices. This review also provides brief information on recently identified bacterial and fungal strains for the degradation of both polymer and fillers.

Keywords Elastomers · Rubbers · Sustainable fillers · Sustainable materials · Circular economy

1 Introduction

Polymers outstand over other materials, because of their thermal and mechanical properties, lightweight, wide range of applications, and simple chemical composition mostly made up of carbon and hydrogen. Polymers are mainly obtained from fossil sources. They are also cheap, and have excellent processability. However, conventional polymers are difficult to degrade, and current waste management policies have led to an exponential increase of pollution. This fact has become a big concern, which extends to the elastomers and rubbers, a decisive group of elastic

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Table 1 Environmental impacts of ingredients in a rubber recipe

Material	Source	Potential impacts
Natural rubber (NR)	*Hevea brasiliensis* tree	Loss of habitat in tropical forests, impacts from transportation to markets and from processing including odor
Synthetic rubber	Petrochemicals	Resource depletion of petroleum. Energy consumption and waste during manufacture
Carbon black (CB)	Generally sourced from petroleum stock	During production, transport, manufacture, and disposal of waste products
Zinc oxide	Synthetic	
Sulphur (including compounds)		During production and combustion for energy recovery
Other additives and solvents: age resistors, processing aids, accelerators, vulcanizing agents, softeners, and fillers	Very diverse	Impact in manufacture and transportation. Emissions from tires in use

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polymers, that cannot be reprocessed. Furthermore, almost all additives that are currently used in their recipes have a considerable environmental impact. Recently, an important review of this impact was presented, summarized in Table 1.

The study of the degradation of all these ingredients has become an important issue, especially for the elastomer matrices and the filler systems. The degradation of conventional polymers leads to thermolysis or photolysis, which provides enough energy to promote the scission of hydrocarbon-based macromolecules. The hydrolysis or enzymatic degradation has a poor effect on the degradation of these types of compounds. Several groups are working on the development of sustainable polymers; nevertheless, the properties of these materials are poor, and it makes it impossible to completely replace conventional polymers. However, the understanding of biodegradation sheds light on addressing the development of more sustainable elastomeric compounds.

Several conditions influence the biological degradation of polymeric compounds. Figure 1 presents and classifies different factors involved in biodegradation. These factors can be subdivided into the physicochemical variables that are determined by the environment, the properties of the polymer to be degraded, and the type of microorganisms present. If all the conditions listed in Fig. 1 are considered, namely measured, and controlled, it is more possible to yield an effective degradation of the polymeric material [2].

There are two predominant ways by which biodegradable polymer bonds can be broken down: passively by hydrolysis or actively by enzymatic reaction [3]. The latter is more viable to occur in the degradation of natural polymers, such as polysaccharides, proteins (gelatine and collagen), and poly(hydroxy acids). Chemical and

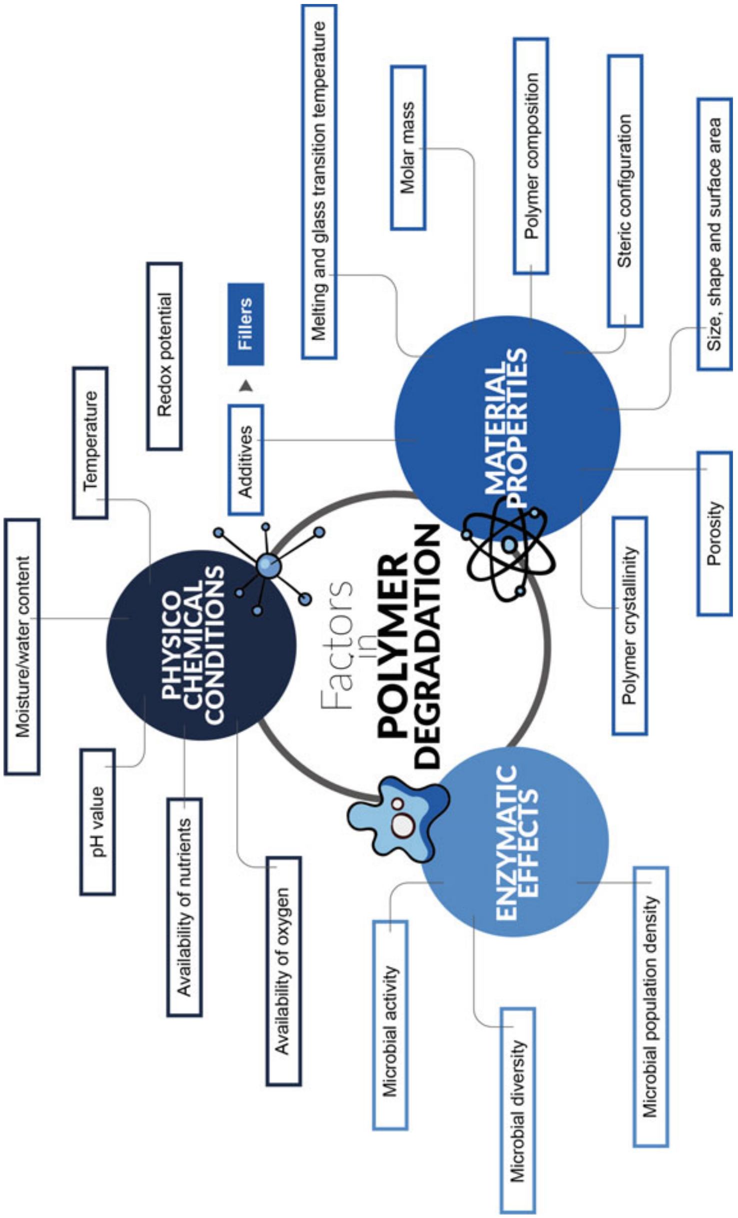

Fig. 1 Factors involved in polymer degradation

physical changes are concomitant with the degradation of biodegradable polymers, like the crystallization of oligomers [4] and monomers [5]. Some of these factors can have a substantial influence on the degradation rate. The most important parameter for evaluating degradation is the molecular weight. Furthermore, reduction of molecular weight has been suggested as a measurement for degradation (such as the loss of mechanical strength and monomer release). These are related but do not necessarily comply with the same kinetics. For instance, complete degradation of poly(L-lactic acid) is known to take more time than the loss of tensile strength [6]. On the other hand, the lack of heterochains in most rubbers and elastomers hinders their biodegradation. In addition, rubbers can host a high number of different types of non-degradable fillers. Consequently, researchers have studied the use of fillers that can be biologically degraded, especially, those based on vegetal sources, such as cellulose, lignin, and zein.

Biodegradation corresponds to the catalysis of organic matter based on carbon, hydrogen, nitrogen, oxygen, etc., promoted by biological metabolic process. This organic matter is decomposed into small molecules, to yield carbon dioxide (CO_2), methane (CH_4), water (H_2O), minerals, and new biomass. Biodegradation works following four consecutive phases: (a) biodeterioration, (b) biofragmentation, (c) assimilation, and (d) mineralization (Fig. 2). The chemical and physical properties of the compound are altered in biodeterioration, while biofragmentation consists of the material breakdown into simpler structures via enzymatic cleavage. The assimilation is the up-taking of molecules by microorganisms and mineralization, and is the final phase, characterized by the release of CO_2 and H_2O, under aerobic conditions, and CO_2, CH_4, and H_2O, under anaerobic conditions [7].

Biodegradation is accomplished using the enzymatic activity of certain microorganisms, such as bacteria and fungi. Microorganisms use their chemical machine, colonizing the surface of the natural polymer and secreting a biofilm of specific enzymes. The excreted enzymes separate the long polymer chains into shorter chain fragments, they are transported using certain tunnel proteins in the cell wall into the internal site of the microorganism where they can be metabolized [2].

The dominant taxa of microorganisms and the degradation pathways associated with polymer degradation are often determined by environmental conditions. When O_2 is available, aerobic microorganisms are predominantly responsible for the decomposition of complex materials, with microbial biomass, CO_2, and H_2O as end products. In anoxic conditions, the anaerobic microorganisms lead to the degradation of polymeric materials. The primary products will be microbial biomass, CO_2, CH_4, and H_2O under methanogenic (anaerobic) conditions or H_2S, CO_2, and H_2O under sulfidogenic conditions [8].

Bacteria and some fungi can form biofilms on the polymeric materials to degrade. These biofilms are multicellular communities held together by an extracellular protein-based matrix, extracellular DNA, lipids, and mono- and polysaccharides [9]. Biofilms impart the optimal conditions for the growth and the protection of microorganisms against adverse environmental conditions and biotic damage from other organisms [10]. Despite all this knowledge, the degradation of conventional polymeric materials, under environmentally available mechanisms and with truly

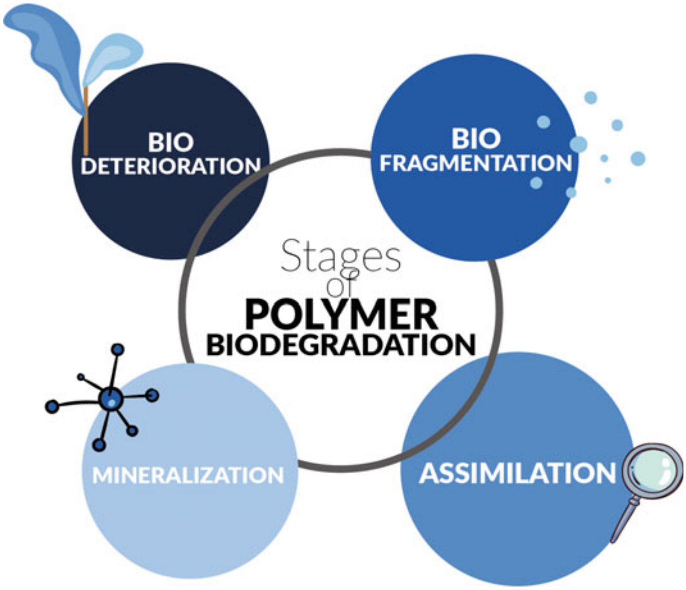

Fig. 2 Stages of polymer biodegradation

sustainable rates is still a pending and difficult task. Thus, actual research is devoted to the study of the additives (e.g., fillers) used in the polymeric formulation, more than to the study of the polymer itself.

2 Biodegradable Fillers from Vegetal Sources

The scientific community and industries' interest in replacing non-sustainable fillers with renewable alternatives, without compromising the performance of rubber compounds, has grown. Likewise, vegetal fibers have been used as fillers to reinforce rubbers. Table 2 shows the composition of some natural fibers used to reinforce rubbers being cellulose and lignin the significant component of several natural fibers. In this chapter, the authors provide a detailed description of cellulose and lignin as the main constituents in most of the natural-occurring reinforcing fillers for elastomers. New families of fillers that are beginning to make their way into the scientific research of these materials, such as starch, and alginate, among others, are also considered.

Table 2 Composition of cellulose, lignin, and pectin of different vegetal fiber resources

Natural fiber types	Cellulose (%)	Lignin (%)	Pectin (%)
Flax	65–85	1–4	5–12
Jute	45–63	12–25	4–10
Kenaf	45–57	8–13	3–5
Sisal	50–67	–	3
Hardwood	40–50	20–30	0–1
Softwood	40–45	36–34	0–1

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2.1 Cellulose

Cellulose is a biopolymer composed solely of β-glucose molecules (Fig. 3); therefore, it is considered a homo-polysaccharide [12]. Cellulose biosynthesis is carried out by the action of cellulose-synthase (CesA), and in this process, the cellulose chains are originated from glucose monomers [13]. The mechanism of cellulose synthase is characterized for being a reaction that uses the sugar uridine diphosphate glucose (UDP-glucose) as a substrate, then the CesA protein unites the UDP-glucose molecules into large cellulose chains. The union of 6 units of CesA forms a sub-unit so-called “rosette”. A rosette is made up of 36 CesA or 6 CesA subunits [14]. The function of the rosettes is to synthesize and join these cellulose chains into a cellulose microfibril, in other words, they are the fundamental structure for the setting up of cell walls and other structures in plants [15].

Biodegradation of cellulose involves 3 enzymes (cellulases) corresponding to the cellulose-synthase complex: (a) endoglucanase, (b) exoglucanase-cellobiohydrolase, and (c) β-glucosidase. Endoglucanase binds randomly to the surface of the cellulose microfibril, causing the cleavage of a β-1,4 glycosidic bond within the chain [16]. When endoglucanase leaves the surface of the microfibril after cleavage, the

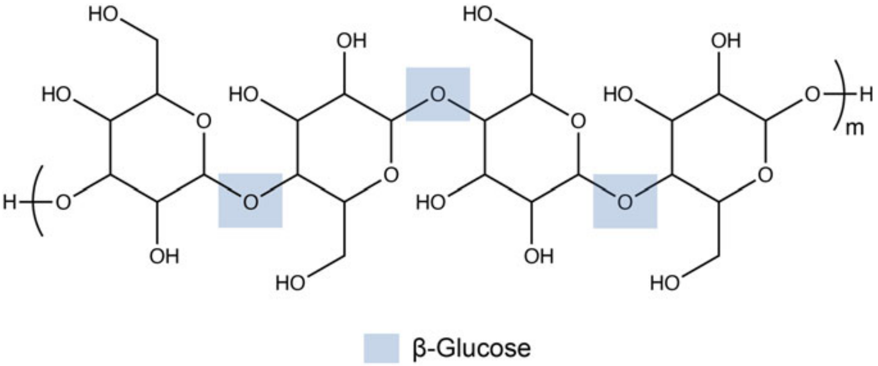

Fig. 3 Chemical structure of cellulose

glycosidic chain is broken, yielding reducing and non-reducing ends. Exoglucanase-cellobiohydrolase reacts only on the non-reducing ends of the chain, splitting a cellobiose unit. On the dimer in the solution, β-glucosidase acts, producing two glucose molecules [17]. Exoglucanase-cellobiohydrolase moves to the newly created free non-reducing ends and continues to release cellobioses from the chain [18]. Endoglucanase continues to release sites on which exoglucanase-cellobiohydrolase can act, resulting in the synergistic degradation of cellulose. Most of the ascomycetes and brown rot fungi produce these enzymes [19, 20]. Many bacteria are also capable of degrading cellulose, half of all described bacteria possess at least one gene for cellulases [21].

2.2 Lignin

Complex racemic aromatic heteropolymers named "*lignins*" (Fig. 4) are mainly derived from three hydroxycinnamic alcohol (monolignols) monomers differing in their degree of methoxylation, coniferilIM1G, sinapyIM1S, and p-coumarylIM1H alcohols [22]. The monolignol biosynthesis begins with the deamination of phenylalanine and means successive hydroxylation reactions of the aromatic ring, follow-up actions by phenolic O-methylation, and conversion of the side-chain carboxyl to an alcohol group [23]. Subsequently, two main processes are identified: (a) dimerization and (b) lignification [24]. The dimerization of monolignols is a simple process compared to lignification, lignification differs substantially from the simple dimerization of monolignols [25]. Only three dimers are produced in the dimerization of coniferyl alcohol, of which at least one of the coniferyl alcohols is coupled at its β position [26]. Conversely, lignification is the cycle by which units are linked together via radical coupling reactions [27]. The arylglycerol-β-aryl ether bond (β-O-4) is the most prevalent [28]. The other bonds are β-5, β-β, 5–5, 4-O-5, and β-1, which are more resistant to chemical degradation [29].

The biodegradation of lignin takes part in the carbon cycle of the biosphere [30]. In this respect, it is known, from studies carried out in the late 1970s on the physiology of ligninolytic fungi, mainly *Phanerochaete chrysosporium*, that the ligninolytic peroxidases "*Iigninases*" play a fundamental role in lignin biodegradation [31]. Regarding the fungal degradation, there are three types of lignin rotting caused by fungal microorganisms: (a) white rot fungi (basidiomycetes and some ascomycetes), (b) brown rot fungi (basidiomycetes), and (c) soft rot fungi (ascomycetes and imperfect fungi).

Lignin is degraded firstly by the action of basidiomycetes and then gradually by some ascomycetes and imperfect fungi [32]. Lignin biodegradation involves a few main enzymes, divided into peroxidases and laccases. Fungal peroxidases (FP) are extracellular fungal enzymes, all members of class II peroxidases, belonging to the superfamily of heme peroxidases. Fungal peroxidases are compounds of (a) Lignin peroxidase (LiP) [33], (b) manganese peroxidase (MnP) [34], and (c) versatile peroxidase (VP) [35]. Moreover, laccase is a very interesting group of enzymes. There

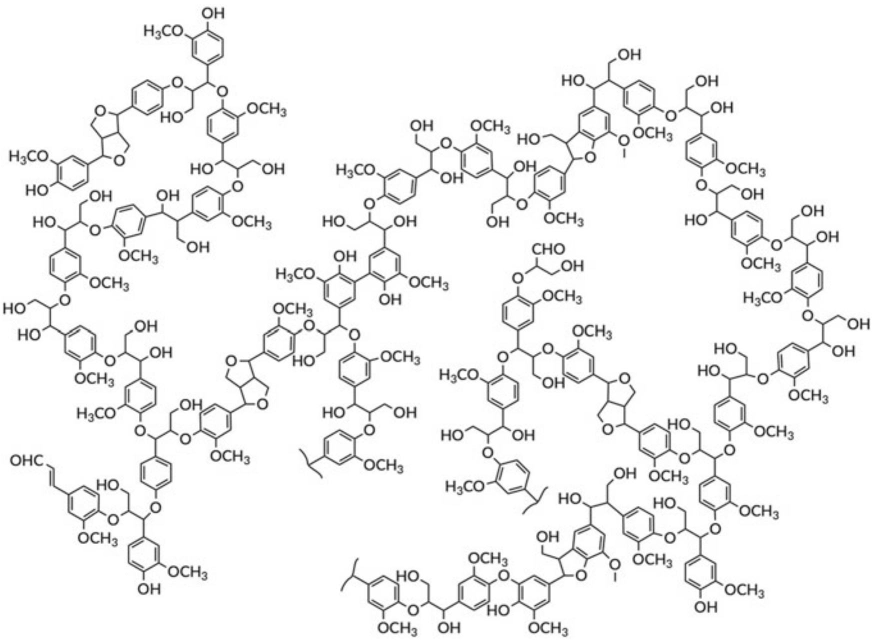

Fig. 4 Chemical structure of lignins

is bacterial and fungal laccase. Fungal laccase is the most relevant and is produced as intracellular and extracellular isoenzymes [36]. These types of multicopper oxidases play a role in de degradation of phenolic compounds such as lignin [37].

2.3 Starch

Starches are natural polymers extracted from tubers (such as potato), cereal seeds (such as rice, wheat, or corn), as well as roots (cassava or tapioca) [38]. All these starches are chemically similar, but their particles are heterogeneous in terms of size, shape, and content of molecular components [39, 40]. Although the structure of starch is still under debate, due to its complexity [41], one model seems to predominate at present. It would be a multi-scale structure, as shown in Fig. 5, consisting of the (a) granule in which we find (b) growth rings composed of (d) blocks made of (c) amorphous and crystalline sheets containing (g) amylopectin and (h) amylose [42].

In general, all starches are made up of those two glucose-derived polysaccharides: amylose and amylopectin [43], whose chemical structures are shown in Fig. 6. Both polysaccharides differ from cellulose in the type of bonds involved in their structure but have a common molecular formula. While cellulose is characterized

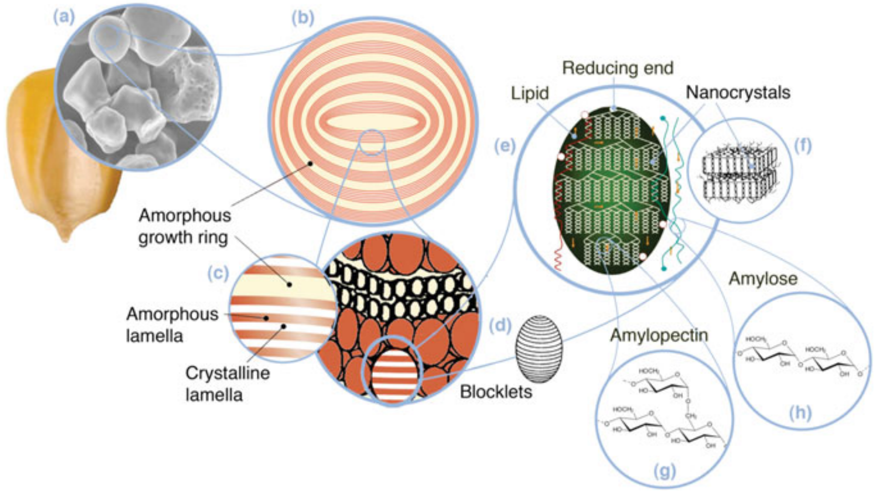

Fig. 5 Starch multiscale structure. Reproduced with permission from [42]. 2010, American Chemical Society

by β-glycosidic bonds (as was shown in Fig. 3), amylose is characterized by α-1,4-glycosidic bonds, whereas amylopectin has a branched structure consisting of short main amylose chains branched with α-1,6-glycosidic bonds [41, 43].

The properties of starch are strongly influenced by the ratio of the polysaccharide's amylose and amylopectin. The molecular characteristics of these polymers affect their viscosity in solution, essential for processing, and their crystallization behavior, critical for the mechanical performance of the material. In addition to amylose and amylopectin, natural starches contain minor non-starch components: particles, extractable surface components, such as proteins, and internal components, mainly lipids [39, 41, 43].

Starch (like cellulose) is not plastic in its original form, but is converted through various processing approaches, including extrusion cooking, functionalization, and plasticization. In addition, it is one of the cheapest biodegradable materials currently on the market [38]. All these potentialities have enabled its use as a sustainable substitute for general-use thermoplastics [44], in thermoplastic elastomers (TPEs) [45], and as a reinforcing filler in rubber compounds [46, 47], with immense potential for use in non-food industries.

2.4 Alginate

Alginic acid or alginate is another widely used natural-occurring polysaccharide. It is obtained from brown seaweeds (algae) such as *Kelp*, *Gulfweed*, *Ascophyllum*, and *Macroalgae* [48], which are naturally available in coastal areas of many countries.

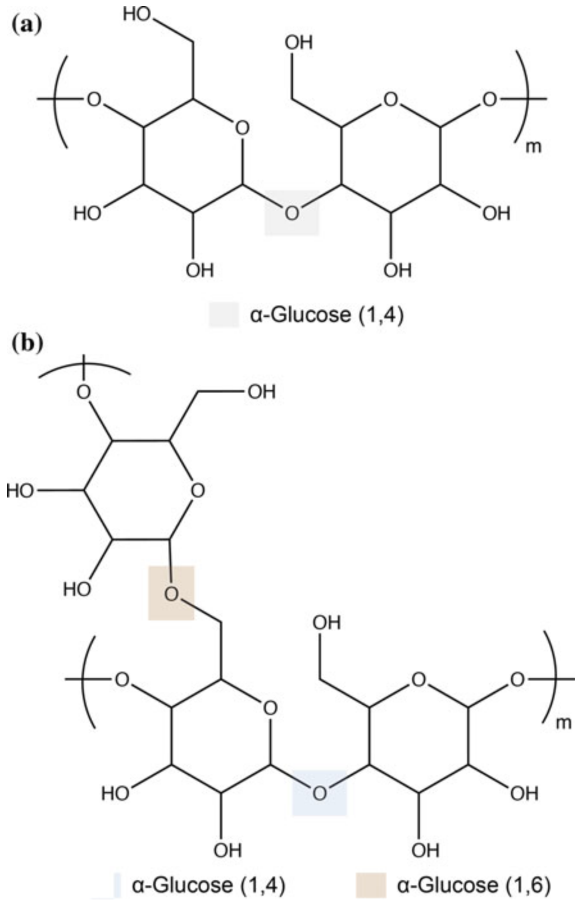

Fig. 6 Chemical structures of **a** amylose and **b** amylopectin

Brown algae have cell walls with a high content of alginate compared to red and green macroalgae [49]. Besides, it can also be produced by microbial fermentation using specialized bacteria [50] (Fig. 7).

Alginate has important rheological properties, such as gelation, viscosifying, and dispersion stabilization. Furthermore, alginate is non-toxic, biocompatible, biodegradable, biostable and a hydrophilic biopolymer [49], facilitating its use in advanced clinical and biomedical applications [51]. However, all these remarkable characteristics are strongly influenced by their chemical structure.

The chemical structure of alginate presents two isomeric conformations: β-(1–4)-D-mannuronic acid (M blocks) and α-L-guluronic acid (G blocks) [49, 52]. Thanks to this and to the presence of a free carboxylic group, it can easily bond with a variety of metal ions through ionic, covalent, redox, and/or coordination interactions. Some of the most used cations are of divalent (Ca^{2+}, Mg^{2+}) and monovalent (Na^{+}) nature. This characteristic has been used to crosslink alginate, as a common carboxylated rubber [53], for a wide variety of applications, primarily biomedical applications

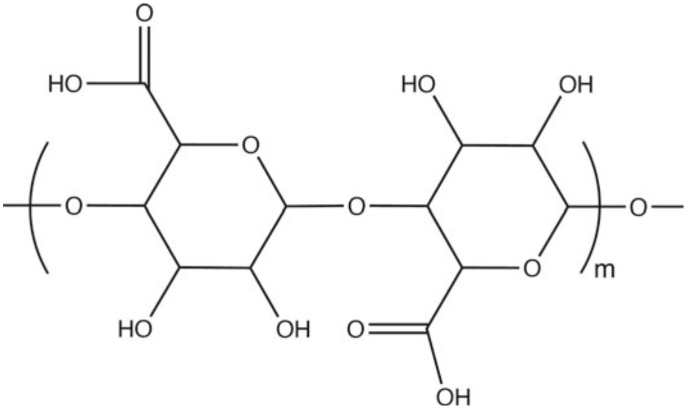

Fig. 7 Chemical structure of alginic acid

[54]. Currently, the authors are working on the use of a new divalent cation (Zn^{2+}), for reinforcement in rubber compounds and advanced properties such as self-healing [55].

As with starch, the most widespread use of alginate is as a polymeric matrix itself [48]; however, its use as dispersing agent [56] and more recently as a reinforcing filler and crosslinking agent [57] is a relevant current issue in Rubber Science and Technology.

3 Rubber Biocomposites

3.1 Rubber Matrices

NR is an elastomer whose chemical structure corresponds to *cis*-1,4-poly(isoprene). It is the rubber with the highest production and consumption worldwide (Fig. 8). It occupies almost 50% of the world's production, while the remaining 50% is distributed among all the existing synthetic variants. The widely used commercial NR originates from the *Hevea brasiliensis* tree, a medium-sized tree native to the Amazon River basin. It is characterized by its excellent mechanical properties, even in the raw state (green strength) thanks to a capacity known as strain-induced crystallization (SIC).

However, not all are advantages in the history of the NR. In recent years, production (essentially concentrated in Southeast Asia) has not been able to meet the growing global demand [58]. This, together with characteristic diseases and pests, and the scarse security conditions in the plantations, has prompted the search for more sustainable alternatives. The two main alternative crop sources are the well-known Kazakhstan dandelion (*Taraxacum kok-saghyz*) [59–61] and the North American Guayule (*Parthenium argentatum*) [60, 62–65]. Dandelion-NR is a fast-growing

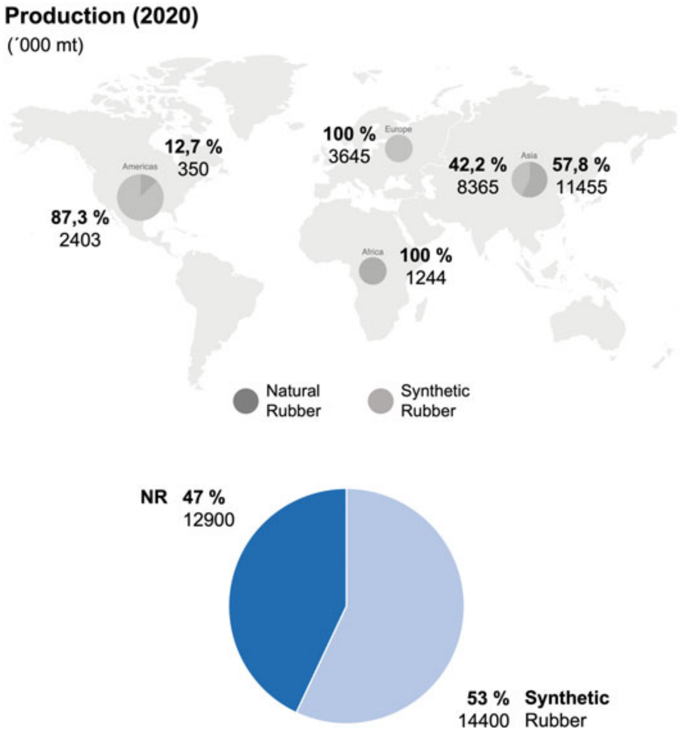

Fig. 8 Global production of NR and synthetic rubbers (prepared by the authors. *Data source* 2020, International Rubber Study Group)

resource and produces large amounts of high-quality polymer, resulting in excellent chemical and physical properties [59]. However, it has a potential drawback: it contains more associated proteins that can cause allergic reactions, making it impossible to use in medical applications. On the other hand, Guayule-NR has molecular weight and physical–mechanical properties analogous to those of Hevea-NR; however, its tensile strength is slightly lower [58, 66].

The biological nature of NR, its dominance in the market, and its renewable origin would explain the reasons why it is the most used material when it comes to applying circular economy strategies. According to the available literature, and the authors' best knowledge, many sustainable fillers are studied in this matrix [46].

The vast majority of synthetic rubbers is destined for the automotive industry. Synthetic rubbers include styrene-butadiene rubber (SBR), butadiene rubber (BR), butyl rubber (IIR), ethylene-propylene rubber (EPM) or ethylene-propylene-diene rubber (EPDM), nitrile rubber (NBR), chloroprene rubber (CR) and polyisoprene (IR), as well as more special-purpose rubbers such as acrylic rubbers (ACM), silicone rubbers (Q) and fluorinated rubbers (FKM and FFKM). This enormous variety of combinations and functionalization that synthetic rubbers allow has broadened the horizon for elastomers; however, its non-renewable fossil fuel origin, hinders its use as a renewable material but opens doors for the application of other sustainability strategies in the circular economy context.

3.2 Reinforcement Effect of Sustainable Fillers in Rubber Compounds

Due to its renewable origin, NR stands out as one of the most used matrices in the development of elastomeric compounds reinforced with sustainable fillers. To a lesser extent, synthetic elastomers are making their way into this space, intending to give them a more environmentally friendly character. Table 3 shows a summary of the articles of the last 5 years (2017–2022) related to the development of rubbers reinforced with cellulose, lignin, lignocellulose, starch, and alginate.

Achieving a strong interfacial adhesion between the filler and the rubber is a key factor for obtaining composite materials with excellent mechanical performance. Thus, it is not surprising that many studies are based on different functionalization strategies for these natural fillers, typically incompatible with most rubbers.

Starting with cellulose, this fiber has gained substantial attention due to its low cost, availability, and performance attributes. In terms of properties, cellulose (cellulosic fibers) possesses a relatively high tensile modulus (20–70 GPa) a tensile strength (~1 GPa) counting on the fibril orientation, and better flexibility than glass fibers [122]. The compatibility between cellulose and NR could be enhanced by modifying the natural fiber and/or NR [46]. It has been reported that the effect of the reinforcing agent on rubber depends on the filler's properties such as particle size, shape, and size distribution, among others [123]. There are different types of cellulose, the most interesting forms of cellulose to be used as filler in composites preparation are cellulose nanocrystals (CNC) and cellulose nanofibers (CNF) (Fig. 9), since their various interactions with rubber.

Nano-sized celluloses can be generated from cellulose fibers by controlled enzymatic hydrolysis, which generates a cross-section of 3–50 nm and a submicron length [123]. For instance, CNC improves rubber properties, accelerates the speed of curing, induces a reduction of the Payne effect, and improves the tear strength toughness [125]. CNC also improves mechanical properties and thermal stability [87]. It has been reported that the CNC also increases the rate of rubber degradation in the soil [126]. Moreover, NFC also increases the degree of biodegradation when these compounds are subjected to the composting process [88].

Chem et al. have made relevant contributions in the use of this material as reinforcing filler in rubbers, specifically in NR. Recently, they presented one approach for the preparation of composites with an excellent degree of rubber-filler interaction [76]. The approach consisted of mixing NR with CNCs and then epoxidizing the entire mixture. With this novel process, the formation of oxygenated groups was achieved not only on the rubber but also on the surface of the CNCs, which resulted in

Table 3 Recent articles published on vegetable-based fillers in rubber compounds

Vegetable-based filler	Type	Rubber matrix	Reference
Cellulose	Cellulose nanocrystals (CNC)	NR	[67–74]
		ENR	[75–77]
		XSBR	[78]
		CR	[79]
		Q	[80]
		NR/NBR/SBR	[81]
		SBR/BR	[82]
	Cellulose nanofibers (CNF)	NR	[83–89]
		SBR	[90]
	Others[a]	SBR	[91, 92]
Lignin		NR	[93, 94]
		BR	[95, 96]
		NBR	[97]
Lignocellulosic[b]	Straw	NR	[98–104]
	Wood	NR	[105]
	Brewers’ spent grain	NR	[106]
	Jute fibers	NR	[107–111]
	Hemp fibers	NR	[112, 113]
	Pineapple leaf fibers	NR	[114]
	Sugarcane fibers	NR	[115]
	Moringa oleifera fibers	NR	[116]
	Flax fibers	EPDM	[117]
Starch	Porous starch	NR	[118, 119]
	Corn starch	NR	[120, 121]
Alginate	Brown algae	NR/ENR	[55, 57]

a Microcrystalline cellulose (MCC), *b* Lignocellulosic refers to those sustainable fillers composed of cellulose and lignin in different proportions (as shown in Table 2).

enhanced hydrogen bond formation and, consequently, improved rubber-filler interaction. Mechanical properties and swelling in water were also studied. Compared to the conventional method (NR previously epoxidized, i.e., ENR), a 20% increase in tensile strength and a 50% increase in modulus at low deformations were achieved with just 10 phr of CNC. They further explored the functionalization of the filler as a reliable strategy to improve interactions with the ENR [77]. For this, carboxylic groups were introduced to the CNC surface by a grafting reaction with maleic anhydride (MAH). With this functionalization, it was possible to form a dual network composed of covalent bonds (from esterification reactions between the carboxylic groups of the filler and the epoxy groups of the matrix) and hydrogen bonds. The combination of both chemical and physical crosslinking networks facilitated uniform

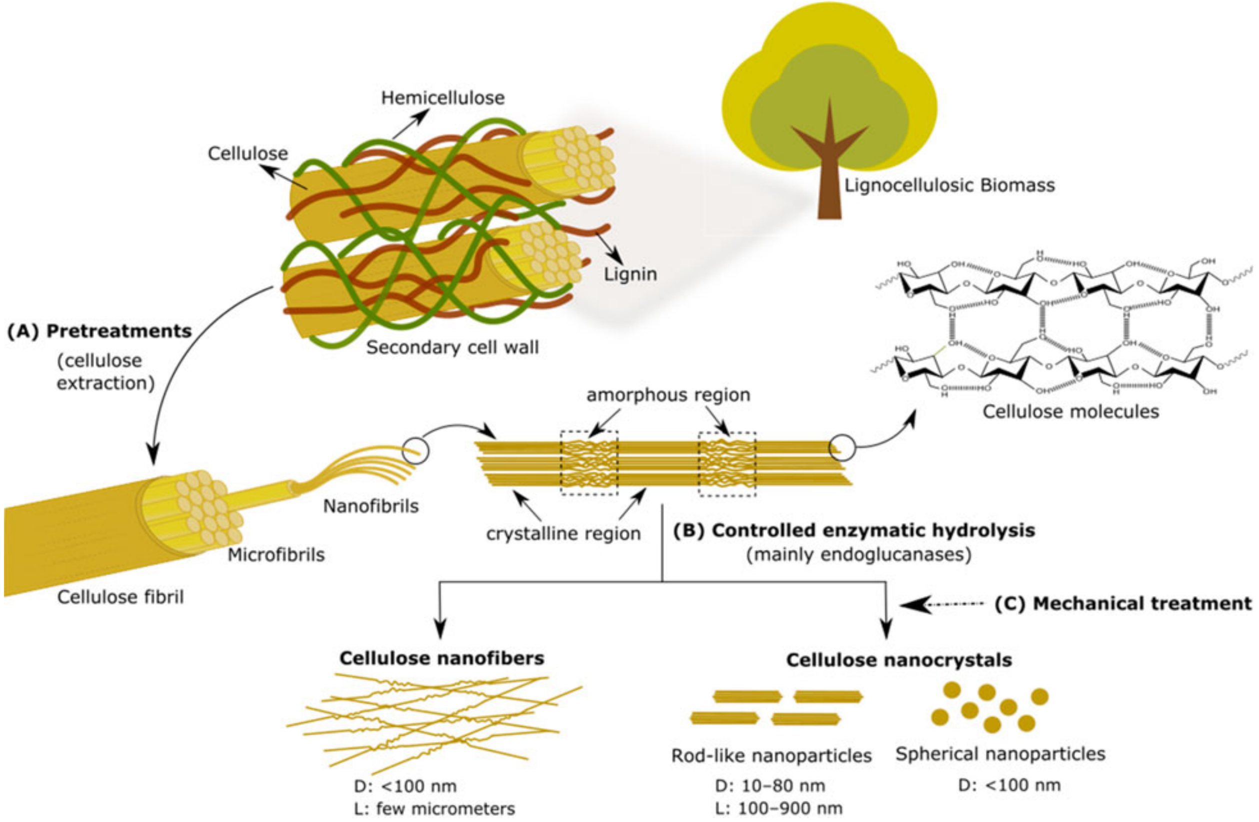

Fig. 9 Process for different types of cellulose production. Reproduced with permission from [124]. 2020, MDPI

dispersion and better interfacial adhesion. The covalent network would be responsible for resistance and elasticity, while the reversible physical network could serve as a sacrificial element during stretching and dissipate energy. The advantages of this development were evident in the increased tensile strength (+172%) and toughness (+158%), as well as decreased hysteresis compared to a single network, crosslinked composites.

Hu et al. presented a strategy for the carboxylation of sulfonated CNCs (CNC-OSO_3H) using citric acid (CA) [78]. Figure 10a, b show the experimental path as well as the chemical reaction, respectively. The grafting of many carboxyl groups on the surface of CNC-OSO_3H enabled the elimination of most of the sulfonate groups and endowed the carboxylated material (CNC-CA) with greater chemical reactivity and thermal stability. The morphologies of the sulfonated and carboxylated material were observed by transmission electron microscopy (TEM) (Fig. 10c, d respectively). The CNC-CA presents a diffuse surface because of the inclusion of small CA molecules. These molecules with carboxylic groups can create intramolecular hydrogen bonds, which would promote the formation of larger agglomerates between the CNC particles. Carboxylated styrene-butadiene rubber (XSBR)/CNC-CA nanocomposites were prepared using polyethylene glycol diglycidyl ether (PEGDE) as the crosslinking agent.

The inclusion of PEGDE enabled the formation of a dual network based on covalent bonds between the epoxy group of PEGDE and the carboxylic groups of the rubber and the filler, in addition to hydrogen bonds (X/mC-D). Compounds without PEGDE with a simple network based on hydrogen bonds (X/mC-H) were comparatively prepared. The coexistence of these networks improved the compatibility between the filler and the matrix and facilitated the homogeneous dispersion of CNC-CA. This dispersion translated into improved tensile strength (Fig. 10e) and dynamic properties (storage modulus, Fig. 10f) with 9 wt% of CNC-CA and $\sim$ 1.8 phr of PEGD. The inclusion of PEGDE and the formation of the covalent network were key for achieving these improvements.

Cellulose can also be used in the form of fibers (CNF). Due to the high aspect ratio that characterizes this shape of fillers, their use as a reinforcing additive is common in multiple composite materials.

Formela et al. presented the use of CNF synthesized in-house from a common parasitic plant from the Kerala region of India, known as *Cuscuta reflexa* [84]. These nanofibers were characterized by showing crystallinity of 67% (by X-ray diffraction, XRD) and diameters between 10 and 30 nm (by TEM observation). Subsequently, the fibers were incorporated into an NR matrix without any functionalization and following two stages: a first pre-dispersion in NR latex and a second conventional mechanical mixing with solid rubber and curing agents in a two-roll mill. The results showed that the incorporation of the filler significantly improved the tensile strength and tear strength of the nanocomposites with only 2 phr of CNF. Through dynamic mechanical, thermogravimetric analysis, and morphology studies, it was concluded that the CNF obtained from this plant are a promising reinforcement for NR.

Despite all the research carried out so far in the field of cellulose, there is still a lot of work to be done to make it a total substitute for conventional fillers such as

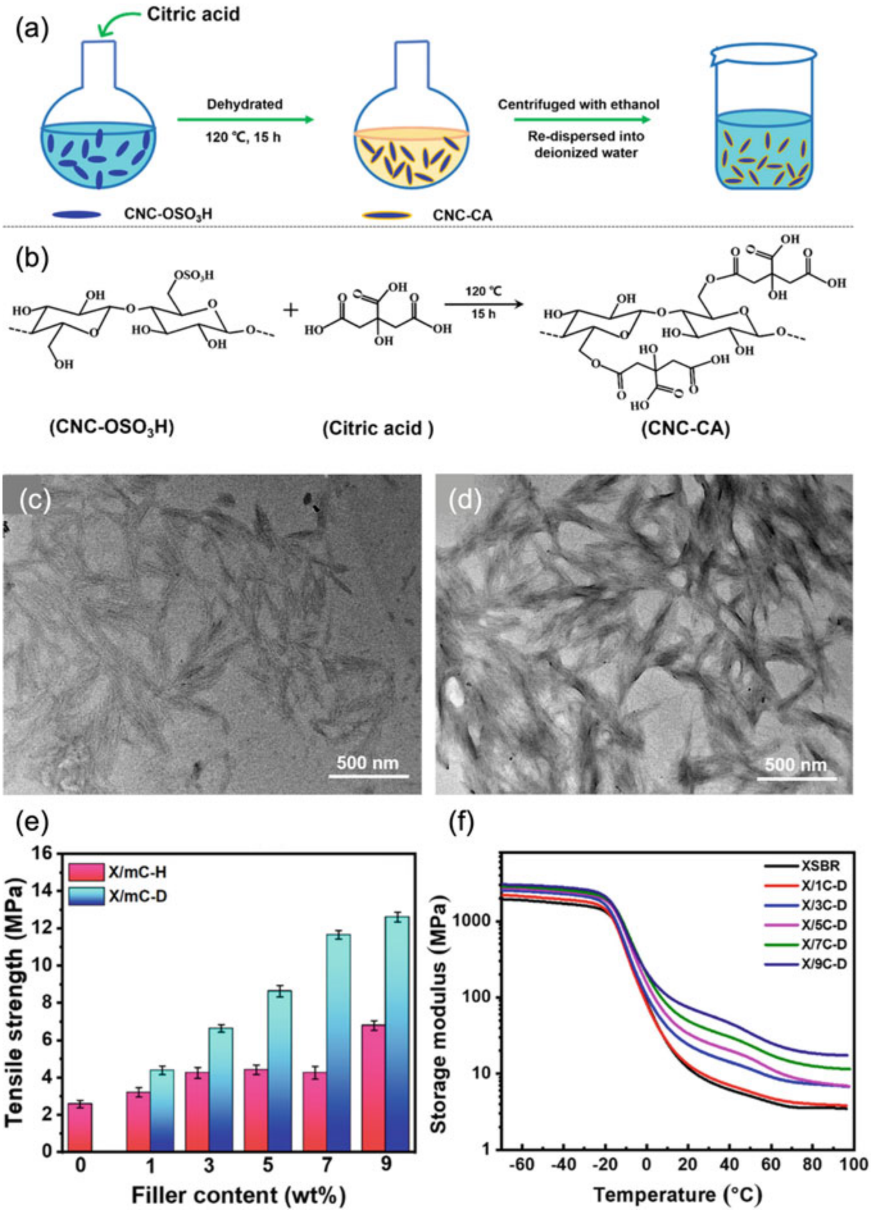

Fig. 10 **a** Experimental path and **b** chemical reaction for CNC functionalization with CA. TEM micrographs of **c** unmodified CNC-OSO_3H and **d** CNC-CA. **e** Tensile strength and **f** storage modulus of XSBR/CNC-CA compounds. Adapted with permission from [78]. 2021, American Chemical Society

carbon black (CB) or silica, mainly due to its poor compatibility with the matrix. To continue to improve this interface between the fiber and the matrix, Kazemi et al. developed a surface treatment using maleic anhydride grafted onto polyisoprene (MAPI) but conceive CNF as partial substitutes for CB, creating a hybrid reinforcing system. Using scanning electron microscopy (SEM), they observed the morphology of unmodified CNF and CNF-MAPI. A change towards a rougher surface is again observed, which would be associated, with the presence of surface MAPI, whose poly(isoprene) portion would be essential to improve compatibility with the NR, due to the chemical similarity. Different contents of CB and CNF (before and after modification) were used in the compounds. The analysis of the Payne effect [127–130] was essential to confirm the effect of the treatment on the improvement of the rubber-filler interfacial adhesion. The compound with 20 phr of CNF-MAPI and 20 phr of CB compared to the compound filled with 40 phr of CB, showed a decrease of only 7% in tensile strength, but the decrease in elongation at break, modulus at 100% of elongation, and storage modulus at room temperature were between 20 and 35% higher.

Lignin is the second most abundant organic polymer in nature, second only to cellulose. It can be considered the largest reservoir of aromatic compounds on Earth, with great potential in several industrial applications [131]. As a low-cost renewable material, it can be used as an antioxidant, reinforcing agent, plasticizer, UV protectant, and coupling agent in rubber matrices [132]. Its use as a reinforcing filler in rubber compounds is long-standing [133, 134] and has been well-reviewed in different articles [132, 135].

The performance of lignin-reinforced rubber compounds is influenced by several factors such as the molecular weight of lignin, the distribution of this molecular weight, the purity, and the concentration of lignin in the matrix, the preparation method, and the compatibility of the rubber with lignin [94, 136]. In terms of results, several authors agree that lignin can be a good alternative to be used as a sustainable filler for the replacement of white fillers (such as silica) in the production of rubber compounds [137]. Additionally, Aini et al. (2020) demonstrated that lignin is a possible alternative substitute for CB.

Ikeda et al. prepared NR composites with lignin obtained from sodium lignosulfonate [94]. They made an important contribution from the point of view of the SIC of NR and the effect of incorporating up to 40 phr of lignin into the rubber matrix. They observed that crystallite formation during SIC is independent of lignin content at least up to a stretch ratio of 5.5. This is an important conclusion from the point of view of preserving one of the most interesting properties of this rubber for mechanical performance. In addition, they made a Payne effect study, observing a marked drop in the storage modulus with deformation, even at low lignin contents (10 phr). This would indicate the formation of a highly interconnected lignin network due to increased filler-filler interactions. These characteristics will be useful to further explore the already promising reuse of lignin as a reinforcing filler in NR.

Recently, lignin has been incorporated as a reinforcing filler in BR, using bis(3-triethoxysilyl propyl) tetrasulfide (TESPT) as a coupling agent (a silane-type molecule, very common in silica-based reinforcement systems) [95]. TESPT would act as a bridge (interface) between the rubber chains and the lignin particles (according to the scheme shown in Fig. 11a), increasing rubber-filler interactions and substantially improving mechanical performance. Three types of lignin were used in the initial stage of the study: Mg-lignin (magnesium salt of lignosulfonic acid) (Mg-L), Na-lignin (sodium salt of lignosulfonic acid) (Na-L), and kraft lignin (softwood kraft lignin, lignosulfonate) (KL). With 50 phr of KL, the best mechanical behavior was obtained (with tensile strength almost twice that of BR rubber), lower hysteresis than equivalent recipes with Mg-L and Na-L, and higher rubber-filler interactions, with a consequent lower Payne effect. For this reason, it was selected for the TESPT incorporation study.

Three TESPT contents were studied (2.5, 5, and 10 phr), reaching a compromise between stiffness and mechanical performance at the breaking point of a tensile test with 2.5 phr TESPT (Fig. 11b). The tensile performance of the KL composite was superior compared to a standard silica-silane and CB system with equivalent filler contents and with a considerably shorter vulcanization time (Fig. 11c).

The third sustainable filler reviewed in this chapter is starch. Although its use as a reinforcement system is much less widespread than cellulose and lignin, recent studies have demonstrated its viability, even in special-purpose elastomers such as ACM. Poikelispää et al. developed ACM composites with different plant-derived fillers such as potato starch, corn starch, garlic, and paprika, using a barium titanium composite as a comparison standard [120]. In this study, the emphasis was not only on the mechanical performance of the materials obtained but also on the dielectric properties for applications as dielectric elastomer actuators (DEA). The results achieved for relative permittivity, loss factor, and conductivity show that all the composites with the vegetable fillers exhibit higher permittivity and AC conductivity at a frequency of 1 Hz than the unfilled ACM, due to a higher interfacial polarization. On the other hand, the actuation studies showed that lower electric fields are required to generate certain actuation forces when potato starch, corn starch, and garlic are used instead of barium titanate, demonstrating potential as DEA. From the mechanical point of view, no substantial improvements in the values at the breaking point of a tensile test were observed; however, all the fillers exhibited higher moduli at low and medium strains. This could be counterproductive for the actuating character; nonetheless, the starch-reinforced composites exhibited moderated module values, reaching a good compromise between stiffness and actuation performance.

The last filler covered in this chapter is alginate. It is probably the least explored, but like its three predecessors has shown promising results with advanced applications. Shen et al. developed an oxidized natural rubber (oNR) film with sodium alginate (SA) [57]. A three-step processing protocol was followed: first, oxidation of NR with potassium permanganate ($KMnO_4$) solution, followed by mixing in solution with SA, and subsequent formation of a film by evaporation of the solvents (Fig. 12). No conventional crosslinking agent was used for this recipe, so the crosslinked network was exclusively constituted by hydrogen bonds between the oxygenated groups of the oNR and the hydroxyl and ether groups of the SA. With 20 phr of SA, a tensile strength of 6.5 MPa was obtained, with a healing efficiency of 60% after only 2 min and 80% after 10 min at room temperature.

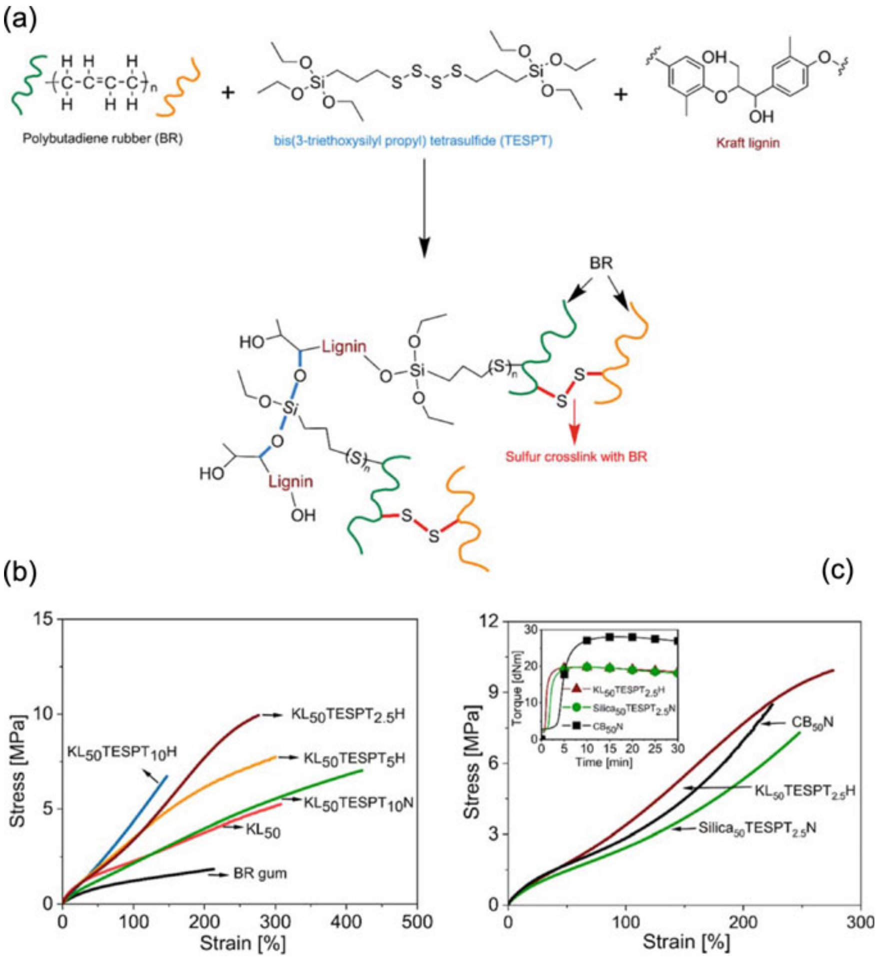

Fig. 11 **a** Scheme of chemical interactions between BR and lignin in the presence of TESPT. **b** Stress–strain curves of BR/lignin compounds. **c** Stress–strain and curing curves (inset) of BR/lignin compounds and BR/conventional fillers compounds. Adapted with permission from [95]. 2021, Elsevier

4 Applications

The use of sustainable fillers in rubbers can be seen as a solution for industries, such as agriculture, forestry, or paper, that produce residues and/or by-products suitable for obtaining this type of fillers. The use of these residues reduces their impact on the environment, and decreases the consumption of conventional fillers (CB, silica, or clays), by replacing them. As an example, the major waste resulting from the exploitation and processing of wood is sawdust, which can be considered an important pollutant because it is stored in uncontrolled conditions [138]. Hence, the

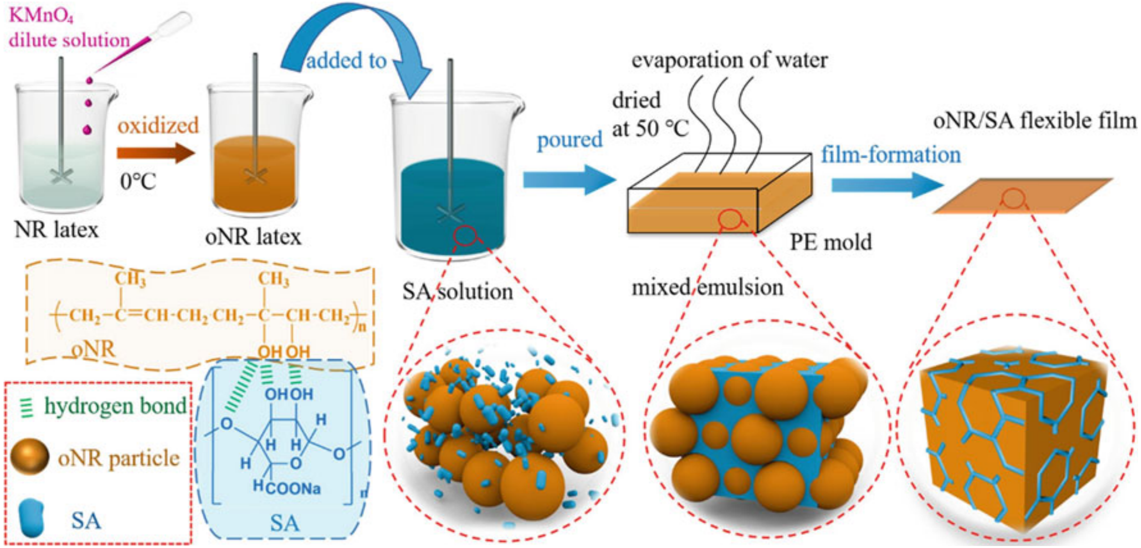

Fig. 12 Schematic illustration of the oNR/SA processing and hydrogen bond interactions. Reproduced with permission from [57]. 2021, Elsevier

use of fillers based on sawdust can be seen as a strategy to decrease the cost of the rubber compounds.

Another approach is to use conventional and/or biodegradable rubber compounds filled with sustainable fillers for obtaining compostable goods that can be disposed of and reprocessed to obtain fertilizers. However, conventional rubbers such as NR, SBR, CBR, and NBR, have slow biodegradation; consequently, the understanding of the biodegradation of rubber matrices, especially that of NR, is also a priority topic for the development of sustainable elastomeric materials [1].

In addition, the degradation of cellulose-based fibers promoted by a specific microorganism can yield compounds, such as itaconic acid, which are chemically useful for the synthesis of polymers and biofuel [139]. The biorefinery used to obtain chemical platforms from biodegradable fillers-based composites can be understood as a circular economy insight of sustainable elastomers. The lignocellulosic biorefinery is based on the use of lignocellulosic feedstock, which yields a wide range of platform chemicals. For instance, the hydrolysis of cellulose renders D-glucose [140].

In conclusion, all the applications of rubber compounds based on biodegradable fillers are addressed to achieve more environmentally friendly goods. Packaging, adhesives, membranes, automotive, civil construction, and biomedicine are some of the industries that can be benefited from these types of materials [141].

5 Outlook and Perspectives

December 31, 2019, changed our societies forever. SARS-CoV-2 coronavirus forced us to rethink our life patterns and joined a long list of actions demanding a redesign of humanity. The arrival of COVID-19 now seems distant thanks to enormous short-term public and private scientific efforts. Now, Ukraine's invasion has once again set off alarm bells about the security of the West, but also about our consumption patterns. The truth is that both events, undoubtedly the most significant of the twenty-first century, are in line with another well-known emergency: Global Warming. The consequences of these three global crises require multiple responses from the social, military, political, and economic points of view. In this last context, the move toward the circular economy model as a sustainable strategy must be definitive.

The circular economy is based on seven concepts, the "7Rs": Redesign, Recover, Reduce, Reuse, Recycle, Repair and Renew. All the research discussed in this chapter is framed in the search for renewable resources ("Renew") capable of replacing conventional ones that generate a devastating environmental impact. This chapter also demonstrates the immense work being done within the rubber science and technology field in search of more sustainable materials that contribute to a real circularity model.

Many other developments have been achieved so far in a wide range of different rubber matrices (natural and synthetic), not only from the point of view of the use of renewable resources but also in Redesign [142], Recovery [143], Reduce [144], Reuse [145], Recycle [146] and Repair [147, 148]. However, there is still a lot of work to be done. The study of the biodegradation of all these new rubber blends is still pending and promises to be an area of significant growth in the coming years, as well as the scaling up from conceptual to more realistic applications for conventional use.

Thus, it is necessary to speed up the transition to full circularity. We affirm that the transition cannot wait for long-term actions [58]. An immediate short-term vision is required, and recent crises have shown us that it is possible. In this sense, science is leading efforts through its research into new sustainable materials and products. Now it is time to demand that companies and governments work together to scale up this research, which will require large investments, but which our descendants and our planet will be grateful for.

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Overview of Natural Nanocomposites and Applications

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Abstract Currently the design, production, application and disposal of natural nanocomposites are of great interest, due to the advantages that present in favor of care and conservation of the environment. This chapter presents a compilation of different studies currently reported on natural nanocomposites. It begins with basic concepts, formulations and different routes and traditional methodologies versus "green" environmentally friendly alternatives, as well as the various and numerous current applications, highlighting the use of natural polymers as renewable natural sources, such as chitosan, chitin, starch (from different sources such as corn, potato, yuca, etc.,) glucose, polysaccharides, cotton, cellulose and derivatives, natural fibers, natural rubber, among others. This chapter presents and discusses the obtaining of different natural nanoparticles, nanofibers and nanostructures, from organic or inorganic nature, which are incorporated into natural, synthetical or semi-synthetical polymeric matrices, to obtain natural nanocomposites. The objective is to present an overview of the different formulations, routes and traditional methodologies versus other alternatives for obtaining, mechanical and chemical properties, biodegradation, biocompatibility, different chemical interactions, applications in different areas, and advantages and disadvantages of using natural nanocomposites.

Keywords Natural nanocomposites · Natural polymer · Application nanocomposites

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1 Introduction

The nanocomposites have various applications in different areas such as: technology, medicine, biotechnology, pharmacy, polymeric materials, ceramics, textiles, paint, automotive, food, among others. There are natural and synthetic nanocomposites, depending on the nature of the phases that form it. Usually the discontinuous phase, is the one that is found in a smaller proportion, when compared to the continuous phase. The discontinuous phase is found on a nanometric scale, and the continuous phase is usually of a different chemical nature, such as polymeric, metallic, ceramic or some combination as hybrid. In this chapter we will focus on natural nanocomposites. Different ways of obtaining them are known, where stand out: in-situ, mixed in solution or melt compounding. The discontinuous phases can be organic or inorganic, where the dispersion plays a very important role. In general, to achieve a good dispersion, the discontinuous phase is superficially modified with a chemical similar to that of the continuous phases, achieving a better incorporation. The nanocomposites are materials with important properties such as biodegradation, biocompatibility, oxodegradation, photodegradation, etc., depending on the chemical nature of the components and affinity with the different fillings or reinforcements organic or inorganic. Specifically, natural nanocomposites are materials that have interesting applications in different areas such as agriculture, food, medicine, pharmacy, among others. Natural nanocomposites are more friendly to the environment, as they are usually of natural origin, and/or renewable and present faster degradation processes [1–6]. However, they often present poor physical, chemical and mechanical properties. The obtaining of natural nanocomposites is subject to the philosophy of green chemistry, prevention is better than clean, based on the twelve principles, affecting all stages of the process, from design, renewable raw materials, use of clean energy, zero waste production, reduction of process times, etc. [7].

Actually, there are numerous reports on obtaining nanocomposites of copper chitosan with important applications in tissue engineering, demonstrating the good activity, presented against pathogens such as *S. aureus* to obtain the nanocomposites of copper/chitosan, used the method of mixing in solution assisting the mixing with ultrasonic energy [8]. In addition to chitosan, there are currently natural nanocomposites based on cellulose, nanocellulose, starch, polylactic acid (PLA), different polysaccharides derived from glucose, with the incorporation of different nanoparticles, such as nanoparticles of Ag, Cu, Ru, Fe, Ti, Au, among others and nanostructures of carbon such as single, double or multiple walled carbon nanotubes, graphene, carbon nanofibers and fullerenes.

The objective is to present in a general way an overview of the different formulations, routes and methodologies traditional versus alternatives for obtaining, mechanical and chemical properties, biodegradation, biocompatible, different chemical interactions, applications in different area, and advantages and disadvantages of using natural nanocomposites.

2 Natural Nanocomposites

Natural nanocomposites are those materials made up of two or more phases, at least one of which has a nanometric scale (generally <100 nm) and which are also obtained from natural sources, such as plants, vegetables, trees, seeds, bacteria, etc. animals, among others [9]. At present, this type of nanomaterials has increased the interest of its research since it has multiple favorable properties such as durability, low cost, low weight, high specific resistance, good mechanical properties and biodegradability [10].

There are different classifications of natural nanocomposites, one of these is regarding their source, which can be animal, mineral and vegetable. The nanocomposites extracted from animal sources are generally taken from the skins or waste produced by animals, those obtained from minerals can be obtained in a natural or modified way and finally the vegetables that are taken from their leaves, stems and roots, being the main one is cellulose [10].

According to Khodakarami et al. in 2021 [11], nanocomposites can also be classified by their size and dimension where we will find four different nanometric scales which are described in Table 1.

In recent years, natural polymer nanocomposites have been widely studied, because the addition of nanometric fillers significantly increases the physicochemical properties, which is attractive for their application in different areas [12]. The union of two or more materials on a nanometric scale can occur in multiple combinations using various materials as shown in Fig. 1. However, to obtain a natural nanocomposite, the addition of at least one component of natural origin will be necessary. An example are nanocomposites based on chitosan, chitin, starch, glucose, cotton, different polysaccharide derivatives, cellulose and derivatives, natural fibers, natural rubber, among others, which are of great interest thanks to their extraordinary mechanical and thermal properties with respect to polymers. synthetics and composite materials with conventional padding [13].

Table 1 Classification of nanocomposites according to their size and dimension

Classification	Dimension	Examples
0D	Nanometer scale from 1 to 50 nm	Fullerenes, nanoclays, nanodiamonds, etc.
1D	Nanometer scale from 1 to 100 nm	Nanotubes and nanofibers
2D	It is found on the nanometric scale and with another dimension on the micron scale	Graphene, nanofilms, and nanocoatings
3D	All dimensions are microscale	Nanostructure

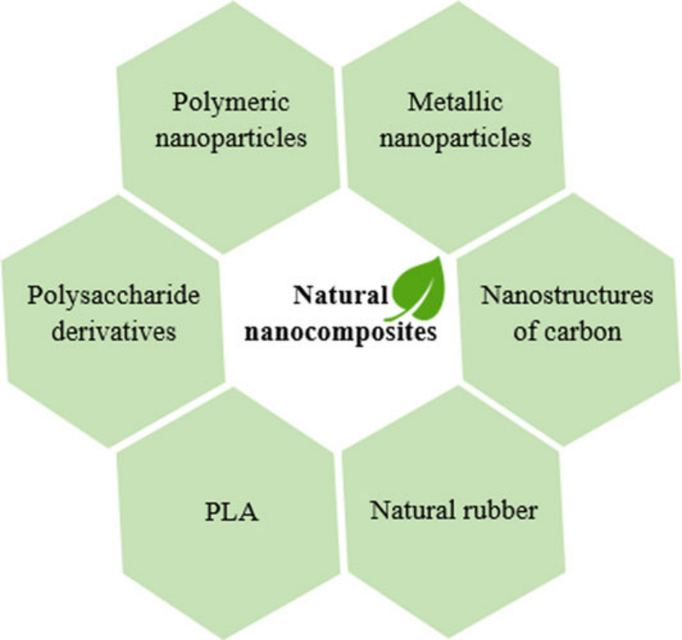

Fig. 1 Examples of natural nanocomposites

3 Obtention of Natural Nanocomposites

There are various methods for obtaining natural nanocomposites which will depend on the properties that are sought to be obtained. However, the most frequently used methods are in situ polymerization, solvent intercalation or exfoliation, and fusion intercalation or exfoliation [14].

In 2017 Maldonado et al. made nanocomposites formulated from chitosan (QS) and copper nanoparticles (nCu) by means of ultrasound-assisted mixing in solution with the aim of increasing the dispersion of the nanometric charge in the biopolymer. They managed to show that the QS formed a mesh with micropores, which facilitated the penetration of bacteria into the nanocomposite and its interaction with the nCu, so they finally obtained a natural nanocomposite with an antibacterial activity of over 90% with possible application in tissue engineering [8].

The cobalt ferrite nanostructures and nanocomposites have been synthesized through different methods in various investigations. However, Ansari et al. in 2018 synthesized $CoTiO_3/CoFe_2O_4$ for the first time using the sol–gel autocombustion technique using glucose, maltose and starch as reducing agents during the synthesis, obtaining a natural nanocomposite with better mechanical properties [15]. Nessi et al. in 2019 made nanocomposites of cellulose/starch plasticized with glycerol to obtain natural nanocomposites by extrusion. The results showed that the incorporation of cellulose nanocrystals up to 2.5% by weight causes mechanical reinforcement and reduces swelling and enzymatic degradation due to the formation of hydrogen and hydroxyl bonds between starch-cellulose [16].

Kamble et al. in 2020 reported the synthesis of thermostable nanocomposites reinforced with cotton fibers extracted from textile waste, which were characterized by their excellent mechanical properties, thermal degradation behavior and water absorption. Finally, they concluded that these natural nanocomposites can have applications in the manufacture of furniture and to develop some visible and non-visible automotive components [17].

Rubber is a natural material with multiple applications throughout history. Natural rubber latex is exceptionally wearing resistant, possesses high tensile strength, and has high hydrophobicity and film-forming character. Hema et al. in 2021 took advantage of these properties for the development of a natural nanocomposite using a mixture of natural rubber latex with xylan nanopowder extracted from sugar cane to develop thin films of good porosity with possible application in the removal of color from sewage water. They finally showed that the films had exceptional dye absorption [12].

Refaee et al. in 2022 carried out a novel multifunctional cotton textile design coated with biointelligent ammonium-salicylidene-chitosan nanocomposites and ZnO nanoparticles which were prepared in situ, where they finally managed to demonstrate that these natural nanocomposites had strong and sustainable antimicrobial impacts on the pathogens *S. aureus, E. coli* and *C. albicans* and that also provided a higher value of the UV protection factor [18].

Akpomie and Conradie in 2021 synthesized natural organic silver nanocomposites for the absorption of oil spills by synthesizing dried and pulverized pineapple (*A. comosus*) skins treated with HNO_3 solution and the addition of $AgNO_3$ solution in a system from a container by green and chemical reduction. They successfully obtained an oleophilic natural organic silver nanocomposite (AgAW) with an efficient application for ultrasonic absorption of oil from an oil-in-water emulsion [19]. Recently, also been studied and reported inorganic natural nanomaterials, in 2020 Lawchoochaisakul et al. published the performed a natural nanocomposite with high adsorbent capacity of montmorillonite intercalated with cationic starch (Mt-CST) for the removal of basic dyes from the reaction of low molar bulk starch with (3-chloro-2-chloride-hydroxypropyltrimethyl) ammonium [20], and Hosseinmardi et al. in 2021 reported a natural nanocomposite using lignin particles (OSL) as nanofiller in a prevulcanized NR latex matrix, achieved that OSL dispersed homogeneously at low loads in the NR matrix, for which they improved by 53% their mechanical properties such as hardness and increased tensile strength and toughness [21].

Other natural material widely used in obtaining natural nanomaterials is cellulose and nanocellulose, there are numerous reports on this type of natural material. Nanocellulose is one of the most widely used plant-based nanomaterials in materials innovation. Obtaining cellulose nanoparticles falls into two general categories. One of them is through top–bottom which includes mechanical, chemical and biochemical methods, while the second, bottom-up, assembles cellulose nanostructures from cellulose molecules and uses biosynthesis processes [22].

Nanomaterials based on metal oxides are recently gaining popularity in ecological issues, especially because it has been discovered that they can be used as adsorbents, photocatalysts and for the manufacture of environmental monitoring devices [23].

In 2020 Nguyen et al. synthesized a ZnO/chitosan nanocomposite using the in-situ precipitation method, obtaining a natural spherical nanocomposite with an average size of 20–25 nm, which has an adsorbent application for the removal of Congo red from aqueous solutions in a single solution monolayer with a maximum adsorption capacity of 227.3 mg/g. This is comparable to other available adsorbents. Therefore, it was concluded that the natural nanocomposite ZnO/chitosan could serve as a promising adsorbent for Congo red in wastewater treatment technology [24].

In the present year, Arockiaraj et al. made iron oxide/chitosan (IOC) nanocomposites using the coprecipitation method with a flaky and sheet-shaped morphology, which showed more than 99% degradation of methylene violet (MV) and 71% degradation of rhodamine B (RhB). Concluding that the iron/chitosan nanocomposite is effective in removing organic contaminants from wastewater [25]. Mehmet et al. for the first time made Fe_3O_4 (NC-Fe_3O_4) nanoparticles (NP) modified with natural cellulose, synthesized by the coprecipitation method. NC-Fe_3O_4 proved to have superior performance both in terms of reuse efficiency and adsorption capacity, so it could be used as a promising and renewable adsorbent for the efficient treatment of VOC contaminants [26].

Among other applications that have been given to natural nanocomposites with metal alloys, are nanocatalysts. Ghourchian et al. studied and published a MOF-chitosan-Fe_3O_4 nanocomposite (NC) for azo dye degradation. To obtain a high degradation efficiency, the effects of different parameters including nanocatalyst dose, temperature and contact time were measured, obtaining a higher degradation efficiency of methyl orange (MO) with the nanocatalyst MOF-chitosan-Fe_3O_4. than 99%. The results showed that the magnetic MOF could be used as a promising recyclable magnetic nanocatalyst for the treatment of colored wastewater [27].

As we can see, there are multiple methodologies and combinations of materials for obtaining natural nanocomposites, however, it is of great importance to know the physical and chemical properties that are sought to be achieved to select the appropriate components [28].

4 Properties of Natural Nanocomposites

Composite materials, by definition, are a type of composite made up of two or more components so that the properties of the final material are superior to those of the separate components. This type of material is mostly composed of a matrix that geometrically configures and gives cohesion to the material and a reinforcement that provides rigidity and strength. Depending on the type of matrix to be used, we have at our disposal materials with organic, metallic, polymeric, and ceramic matrix. And within the most used reinforcements we have carbon fibers, glass, natural fibers, etc. Within the wide range of composite materials, we have a very important group since these materials exist naturally. A couple of examples of this type of natural composite materials are wood and bone. Wood consists of a lignin matrix in combination with cellulose fibers; while bone is a material that is made up of collagen that serves as the matrix and the fibers are made of a mineral called apatite.

In this sense a nanocomposite is a multiphase material where all the dimensions of the material are less than 10 nm. The idea behind this type of material is to use nanometer-scale building blocks to create and design materials with improved properties [29].

Research on these types of nanocomposites has grown exponentially when it was discovered that graphene has excellent desirable properties. The two-dimensional structure of graphene provides it with all these properties of which mechanical strength, thermal conductivity, electrical conductivity among many others stands out. Given these desirable characteristics, graphene is the subject of countless investigations to integrate this material into polymeric matrices. The mechanical properties of graphene are documented, it has a Young's modulus with a value of 1.0 TPa and a tensile strength of 42 N/m and a fracture toughness of 4.0 ± 0.6 MPa. These values confirm the high strength of graphene. Polymer nanocomposites benefit greatly from the use of graphene and its derivatives as fibers to enhance the properties of these composites. An example of this was given by Cheng. et al. this group of researchers tested reinforcing polyvinyl alcohol (PVA) films with 20% graphene, thus obtaining a novel material with a tensile strength of 59.6 MPa more than five times the strength of pure PVA material [30].

This great improvement in mechanical properties can not only be attributed to the integration of graphene into the material, but also to the strength of the matrix and the hydroxyl groups present in the PVA and the functionalities of the oxygen present in the graphene which led to the formation of hydrogen bridges. Another methodology for the synthesis of nanocomposites is hydrothermal carbonization, this type of synthesis represents a green option to the different techniques for the synthesis of carbon-based nanomaterials. Obviously, this synthesis technique is not new, however, hydrothermal carbonization has become an important methodology to produce carbon-derived hybrid materials [31]. The most interesting feature of hydrothermal carbonization is that it is an environmentally friendly, easy to handle and scalable process that allows the production of various hybrid and carbon nanostructures. The materials created from this methodology have applications in the chemical industry as catalysts, water purifiers, CO_2 sequestration among others. In the field of natural fibers, these are subdivided based on their source, whether these fibers come from plants, animals, or minerals. Fibers from plants are mostly made of cellulose, while animal fibers are composed of proteins (hair, silk, wool). In the last decade the research to exploit fibers of natural origin as the main constituents to form nanomaterials, due to their high availability, relative low cost, recyclability and of course improved mechanical and chemical properties [32].

Infected wounds within the biomedical area as well as the longer shelf life of different foods leads to the design of nanomaterials with more specific properties, so currently the main characteristic of the nanocomposites used or oriented within the biological and food areas is to be antimicrobial, When formulating the different multicomponent nanocomposites, researchers focus on different active agents to provide antimicrobial activity, among which different metals stand out, such as silver nanoparticles or zinc oxide nanoparticles, certain transition metals such as Cu [33]. In addition, different natural extracts have been used which can confer these properties to the nanocomposites, the inhibitory activity of antimicrobial agents can vary against different microorganisms due to this, new alternatives are sought to confer this property [34].

Kusmono et al. in 2019, reported the formation of chitosan-based nanocomposite films with different clay loadings (0, 5, 10 and 15%) and crosslinked with glycerol at concentrations of (10, 20 and 30%) by casting technique, Revealing that the addition of clay in the nanocomposites significantly improved the tensile strength and tensile modulus, having the values of strength and stiffness the clay/glycerol nanocomposites 5%/20% respectively However, in no nanocomposite was achieved the formation of inhibition zones only by contact for *E. coli*, proposing it as a suitable candidate for packaging film applications [35].

Cui et al. in 2020, reported the obtaining of porous 3D scaffolds through the freeze-drying technique based on gallium-apatite/chitin/pectin, revealing that through cell viability and biocompatibility tests no cytotoxicity was reported in NIH3T3 cells and MG-63 cells, in addition the cells adhered and proliferated in the scaffolds, Likewise, the implantation of the scaffolds showed the formation of mature bone through the formation of new bone layers and the differentiation of osteoblasts, which is why it is described as a material that meets the prerequisites to be considered for orthopedic applications [36].

In 2021 Nazari et al. obtained a nanomaterial: hydrogel nanocomposite based on arabinoxylan (ARA) and loaded with graphene oxide functionalized with a chemotherapeutic drug fluorouracil (5FU), reporting antimicrobial activity against *S. aureus* and *P. aeruginosa*, as well as activity against the U-87 cell line which is a skin cancer cell line. Therefore, they are reported as a useful nanomaterial for the care and treatment of skin cancer [37].

Li et al. in 2022, performed the obtaining of flexible polyurethane foams added with Cu nanoparticles with sizes between 100 and 130 nm, reporting that the nanoparticles did not modify the chemical structure of the polyurethane foam likewise, they report that it has an excellent antimicrobial activity against different pathogens such as *E. coli*, *P. aeruginosa* and *S. aureus*, which indicates its possible applications in water treatment and medical fields [38].

Environmental pollution has become a global problem in recent decades due to the hazardous effects of different pollutants on the environment and human beings. In that sense the elimination and treatment of different pollutants is vital for the world's population. The biodegradation process is a versatile process for the elimination of pollutants. Biodegradation process is considered as an environmentally friendly method because it does not require higher energy demand, which can save both renewable and non-renewable sources, nanocomposites with unique and specific properties in terms of both chemical and physical stability, high surface area and high adsorption capacity can improve the performance of biodegradation processes [39]. Currently some of the most interesting materials for the study of biodegradation are carbon nanostructures, the main studies are based on enzymatic degradation, cellular degradation, and bacterial degradation [40].

In 2021, Candotto et al. conducted a study to evaluate the environmental biodegradation of graphene using axenic cultures of the basidiomycetes like *Bjerkandera adusta*, *Phanerochaete chrysosporium* and *Morchella esculenta.* reporting that the selected fungi can oxidize graphene to a graphene oxide-like material, however, they propose to continue with times longer than 4 months to determine if the resulting graphene oxide-like material can fully degrade to CO_2 [41].

The properties of natural nanocomposites will depend on the chemical nature of the components that make them. It is known that natural polymers have poor mechanical and chemical properties; however, they have high flexibility and are normally biodegradable. Generally, they have biological properties, where antibacterial and antifungal properties stand out. However, to solve the problem of their poor mechanical properties, different types of additive or fillers are used, where metallic, polymer nanoparticles and carbon-based nanostructures stand out such as multi or single wall carbon nanotubes, nanofibers, graphene, fullerenes, etc., thus improving the properties that the natural polymer presents, making the natural composites very interesting. In the cases of natural inorganic nanocomposites where zeolites, different types of sands, feldspars, and clays are involved and combined with metallic nanoparticles or carbon-based nanostructures, degradation or biodegradation turns out to be more complex. However, as an advantage present excellent properties such as mechanical, corrosion, hardness, etc.

5 Applications of Natural Nanocomposites

Nanomaterials research has grown exponentially in the last decade due to the excellent properties that these materials exhibit as they are in constant development. One of the areas where these materials have gained considerable attention is in regenerative medicine, where nanomaterials are used for tissue regeneration. Tissue engineering is an applied science to create artificial constructs for the regeneration of damaged tissue in different parts of the body [42].

Synthetic polymers have been used as biomaterials in the medical field due to their excellent chemical and mechanical properties and are ideal candidates for use as inert biomaterials to restore the functionality of different tissues [43]. These polymers are crucial when implantations are made in living tissues there may be physiological incompatibility because of the interaction of the synthetic polymer [44]. Due to the important role played by synthetic polymers, research has been directed towards improving the biocompatibility of these materials [45]. This research points out that the adhesion behavior and proliferation of the reconstructed tissue depends on different characteristics such as chemistry, wettability, roughness and stiffness [46].

The material known as layered hydroxide (LDH) nanoclay is a kind of 2D distributed nanomaterial that possesses, this material has been studied for different biomedical applications. According to the publication of Liang et al. LDH is the nanomaterial with the most biocompatible structure among all inorganic materials and is a material of interest for improving biocompatibility capabilities [47].

The positive aspects of nanocomposites are evident, however, there is always a potential risk due to the nature of the size of the nanoparticles when penetrating tissue pores as these materials may present some type of toxicity which is of great concern as the specific area and specific energy of the material in question may cause an increase in biological activity which may lead to the formation of some type of tumor. In addition, most nanocomposites are considerably smaller in size than some cells or cellular organelles present in the body, as a consequence, these materials can be absorbed by these cells and interfere with biochemical processes fundamental to life [48].

Another factor to consider is the toxicity of the metals present in these types of molecules. Research carried out by the Cortese-Krott et al. [49], working group indicates that silver ions in different nanocomposites induce oxidative stress. As a second example, there are cases where concentrations higher than 0.36 ppm cause the release of Zn ions from TiO_2 nanotubes which can lead to stem cell death [50].

In the modern era, nanocomposites of natural origin are in full bloom not only because of their different and wide-ranging properties, but also because of the different areas where these materials have practical applications, ranging from medicine to energy generation. Graphene-based nanomaterials are very popular for research due to their multiple applications in different fields of science. One of these applications is the use of graphene nanomaterials for the shielding of electronic components by EMI shielding, which is an acronym for electromagnetic interference, where graphene-derived nanomaterials possess these superior shielding properties [51].

Also has been reported research in medicine about natural nanocomposites has led us to different applications with ceramic nanomaterials since these types of compounds are the most demanded for this type of applications, for example, dental and/or orthopedic implants based on zirconium (ZrO_2) or alumina (Al_2O_3). Dental implants are usually made of zirconium nanomaterials because of their color and translucency characteristics, which are purely aesthetic properties. While alumina composites are ideal candidates for the manufacture of orthopedic implants due to their hardness, low wear and of course their excellent biocompatibility [52].

The college of food science and technology in Wuhan synthesized $Fe_3O_4^-$ based magnetic nanomaterials not only for the detection of pesticide residues in food, but also for the removal of toxic metals, radioactive ions, or the capture of pathogens. Their nanomaterials are suitable for preconcentration and purification of non-polar pesticides. These materials are used as adsorbents due to their good separation capacity, excellent stability, and ease of workability. Magnetic nanomaterials compared to traditional extraction technology have a higher enrichment factor which means that they have an even lower detection limit of the pesticide. This feature promises to be a breakthrough for the food industry. This small essay tries to demonstrate the different applications that nanocomposites of natural origin can have, emphasizing that these applications can mean a breakthrough for human development not only in the field of technological growth but also promise materials made based on methodologies that are in many cases sustainable and above all environmentally friendly [53].

The chitosan is a natural polymer, is used in applications where very specific properties such as biodegradability and zero toxicity. It also has antimicrobial, antioxidant, anti-inflammatory, antitumor properties and its use has been shown to accelerate wound healing in humans. It is a polymer highly used in the medical area, this type of polymer is currently used to obtain natural nanocomposites [54, 55]. Chitosan can be obtained from fungal sources, presenting important food, pharmaceutical or biomedical applications, for example, as antimicrobial agents, coating material, water purification or biopesticides. Hu et al. in 2022, published a detailed article on the chitin extraction from fungi and different fungal source, also showing applications in commercial chitosan products. Finding that chitosan extraction form fungi cell wall by fermentation is economically feasible and is considered as a green synthesis process [56]. Chitosan is an excellent natural polymeric matrix for obtaining natural nanocomposites, presenting numerous applications and uses [57–61].

In addition, it has been reported that chitosan with inorganic material is used in medical applications of great interest, as it has properties antibacterial, anti-inflammatory and cell motility activity [62].

Recently it was published a detailed study of obtaining of sustainable hydrogels-based chitosan Schiff base and their potential applications by Abdalla et al. in 2022, reporting that chitosan was chemically modified converting it into chitosan hydrogels with Shiff basses, highlighting the unique biological properties that chitosan presents. They were synthesized using isonictinic aldehyde in the presence of epichlorohydrin, obtaining chitosan hydrogels with Shiff bases, finding the high efficiency of adsorption of cobalt and mercuric ions presented by hydrogels based on chitosan-Shiff bases, outstanding results were also found against (*B. subtilis*) Gram-positive [63]. In 2021 Qu et al. reported a study of the preparation and characterization of nanocomposite films-based chitosan-clay and coatings for food packaging applications. This nanocomposite was easily obtained for the purpose of being used in combination with essential oils, metals and metal oxides [64].

Another natural polymer highly used as matrix in natural nanocomposite is starch, it is a natural polysaccharide widely used due to its biodegradation properties. However, mechanically it has poor properties, its structure is made up of chains of sugar or glucose, which depending one are linear are called amylases, or amylopectin if the chains are branched. These sugar chains are organized into little particle called starch grains or granules. Currently numerous publications have been reported don obtaining natural nanocomposites, in 2022 Martins et al. published a detailed study on the impact of starch nanocrystals on the physicochemical and thermal properties and structural characteristics of starch-based films, in this publication emphasizes the need to replace synthetic polymers with environmentally friendly polymers such as biodegradable ones, as well as the alternative of producing nanomaterials from low-cost biodegradable resources, in order to be used in food packaging, in the study they reported the results of obtaining films based on native and hydrolyzed reinforced with nanocrystals of starch and potato starch, finding important changes in the physical and structure properties, finding that adding 0.1% of the nanocrystals of native starch strongly influences the physicochemical properties, reducing the solubility by 18%, likewise a significant increase in the percentage of crystallinity of the films was found [65].

Cellulose, its derivated, as well as different polysaccharides derived from glucose, have also been widely studied and reported as raw materials for natural nanocomposites, due to their biodegradability properties, it has been reported that when combined with different nanoparticles or nanostructures based of carbon, can increase the mechanical properties, thus preserving its biological properties [66–69].

6 Conclusions

It is concluded that nanotechnology is a science of a great scope. The interest in the study of natural nanocomposites is due to its numerous applications in different areas. This type of material can be composed of organic and inorganic phases. It can be either natural polymers such as chitosan, chitin, starch, cellulose, nanocellulose, polysaccharides and derivatives; synthetic polymer such as PLA (polylactic acid), polyesters, among others and combination of these can form polymeric matrices with unique properties, by incorporating organic or inorganic nanoparticles, natural nanocomposites with special properties are obtained. The methods of obtaining are varied and are currently based on different factors where the properties and costs stand out, the applications of this type of materials are an area of great interest, from primary applications, such as food, health and well being, as well as communication, and energy. This chapter shows a general overview of natural nanocomposites, presenting novel studies, where organic raw materials such as polymer or inorganic raw materials such as montmorillonite, metals, zeolite, etc., are used highlighting that are a viable and friendly alternative for obtaining natural nanocomposites.

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High Performance Thermoplastic Starch/Vermiculite Bionanocomposites

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Abstract Thermoplastic starch (TPS)/vermiculite (VMC) bionanocomposites having exceptional high tensile properties were prepared by a one-step extrusion process using pre-dispersed clay particles. For predispersion of clay, VMC was oxidized and dispersed in water suspension using a combination of ultrasound (US) and hydrogen peroxide (H_2O_2) treatments. Oxidized and dispersed VMC particles were organo-modified using aminosilane. The degree of oxidation, silanization and dispersion of VMC particles were evaluated by DRX, SEM, titration of OH and NH2 groups and the viscosity of VMC suspensions. TPS/VMC bionanocomposites were prepared in a twin-screw extruder using a suspension of starch/glycerol/water/VMC in which the starch-glycerol ratio was 64:36 in the water-free TPS and TPS/VMC bionanocomposites. The dispersion, morphology and mechanical performance of TPS/VMC bionanocomposites was evaluated using DRX, TEM and tensile tests, respectively. The combination of both US and H_2O_2 treatments resulted in VMC suspensions having pseudoplastic behavior. Oxidized and silanized VMC particles were melt-blended with TPS and the stiffness of bionanocomposite increased almost exponentially with the incorporation of the oxidized VMC. The water-free TPS had Modulus of 1.08 MPa and the TPS/VMC bionanocomposites compounded with 1, 2 and 5% VMC had values of 8.2, 118.6 and 223.8 MPa, respectively, and elongation at break above 150%.

Keywords Thermoplastic starch · Vermiculite · Bionanocomposites · Dispersion · Mechanical properties

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1 Introduction

Biodegradable materials made from renewable sources have been studied during years as potential replacements for oil-produced plastics. Starch is a promising biopolymer because of its low-cost, biodegradability and annual availability from many plants [1]. Starch is obtained from plants in the form of granules, which can be transformed in thermoplastic starch (TPS) in the presence of water, plasticizers, heat and shear [2]. An almost water-free TPS has been prepared by extrusion in a process in which a starch/glycerol/water suspension was fed into a twin-screw extruder equipped with a venting zone for the extraction of water and other volatiles [3]. The presence of water produced the formation of bubbles at the processing conditions of synthetic polymers (above 150 °C) and no bubbles were observed in TPS when processed at such high temperatures [4]. It has been reported that thermal and rheological properties of water-free TPS [2] and the tensile properties of TPS are dependent of plasticizer content [1]. Both viscosity and Tg of TPS decrease as the glycerol content increases [2]. Shi et al. reported that TPS plasticized with 40 wt% or more glycerol were soft and ductile while the TPS formulated with 30 wt% glycerol has a tensile resistance more than 7 times higher than the ones with high-glycerol content [1].

It is well-known that starchy materials have poorer mechanical properties than synthetic polymers and that many works have been devoted to improve the mechanical performance by melt blending with other polymers or by the incorporation of reinforcement materials such as cellulose or clay [3, 5–11]. Melt blending TPS with low density polyethylene (LDPE) resulted in just a slight reduction of tensile resistance of the synthetic matrix and almost no effect on ductility, which was a consequence of the fiber-like morphology produced by the control of glycerol content, TPS concentration and processing conditions [3]. The improvement of the tensile properties of starch/natural fiber biocomposites has been reported to be dependent of the type, nature and content of the fiber, orientation of fibrillary particles, type and content of starch plasticizer, and blending processes [12]. As well, reinforcement of TPS matrices with mineral clays has been mainly carried out using montmorillonite, kaolinite and vermiculite (VMC) which are composed of silicate layers that are 1 nm thick and several nm to few μm in the lateral dimension [13, 14]. The reinforcement efficiency has been relied to the nanoparticle geometry and surface area, which is highly related to the dispersion state. It is generally postulated that a good dispersion state is the consequence of the increasing of both the exfoliation of individual clay laminates and the intercalation of the liquid media (polymer molecules or dissolvent) between silicate layers [15]. Mineral clays are composed by exchangeable cations such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Na^{+}, K^{+} in the interlayer [14] and those cations could be interchanged by organic surfactants having alkylammonium and alkylphosphonium cations to produce organo-modified clays [15]. Organo-modification of clay is commonly carried out by the exfoliation/adsorption process but novel processes has been proposed such as the shear induced clay organo-modification [16]. Organo-modification processes have been used to change the hydrophilic nature of clay in hydrophobic and to increase the interlayer distance of clay (reduction of the diffraction angle in 2θ), which can allow the intercalation of hydrophobic polymer chains [15]. TPS/clay bionanocomposites have been prepared using both the hydrophilic sodium-montmorillonite and the commercially available hydrophobic organo-modified clays [5–7, 9, 10, 16–18].

The preparative methods that have been reported for the development of polymeric nanocomposites are the intercalation of polymer or prepolymer from solution, the in-situ intercalative polymerization and the melt intercalation method [15]. TPS/clay bionanocomposite films have been prepared by the intercalation of polymer from solution method where starch or modified starch have been gelatinized in the presence of plasticizer and clay to form filmogenic solution and film formed by casting [7, 9, 13, 18]. On the other hand, TPS/clay bionanocomposites have also been prepared by the melt intercalation method using two or one-step processes. In the two-steps process, TPS is prepared in a first step and clay is dispersed in the TPS matrix in a second process [5, 6]. In a variation of the two-step processes, Zhang et al. combined the preparation of TPS/clay bionanocomposites by the intercalation of polymer by solution method (starch, water, glycerol and clay were gelatinized at 110 °C overnight to form TPS and TPS/clay bionanocomposites) with the melt intercalation method where the materials were homogenized by extrusion at 160 °C in a second step [19]. In the one-step process, starch, glycerol, clay and, optionally, water were mixed manually or in high-speed mixers before being fed to the melt mixing system, such as roll mill, internal batch mixer, single-screw extruder or twin-screw extruder [10, 16, 17, 20–22]. All components have been mixed and used immediately [10, 20–22] or stored for 1 h or overnight [16, 17] to allow the equilibration of all components. Clay has been incorporated in the formulations in powder form and with no further treatment, however, in some cases, clay has been swollen with water or glycerol before being mixed with TPS components [16, 20].

Dispersion of clay nanoparticles in TPS matrix has been studied as an alternative to reduce water absorption and to improve barrier, thermal and mechanical properties of starch-based materials [17]. However, research for new biodegradable, low-cost starchy materials have been mainly focused on how the tensile properties of TPS/clay bionanocomposites could be affected by the concentration and hydrophobicity of clay. In processes where clay was dispersed in a polymeric solution and TPS/clay bionanocomposite films were prepared by the solution casting method, the effect of natural clay content [18] or the combined effects of starch source (corn, potato wheat), clay cation (MMT, Cloisite®30B, citric acid-modified MMT), glycerol content (10, 20 and 30 wt%), mixing mode (mechanical, ultrasonic, combined) and clay content (3–15 wt% citric acid-modified MMT) [9] have been reported. In the former, it was reported that the addition of sodium montmorillonite (MMT) to a TPS plasticized with 30% glycerol decreased slightly the elongation at break (ε_b) but increased both stress at break (σ_b) and Young's modulus (E) where, remarkably, the values of E increased up to 500% for the bionanocomposites containing 5 wt% of clay [18]. In the later, it was not reported the mechanical properties of the TPS plasticized with 10, 20 and 30 wt% glycerol and it was not possible to determine the improvement of bionanocomposites compared to TPS, but it was reported that

TPS/clay bionanocomposites that showed the highest E values were those formulated with the citric acid-modified MMT, those where clay particles were dispersed by a combination of mechanical and ultrasonic treatment and those having 6 wt% of the citric acid-modified MMT [9].

TPS/clay bionanocomposites have been obtained by melt intercalation in a two-steps process using an internal batch mixer [5, 6]. They studied the effects of the hydrophilicity of 5 wt% of clay nanoparticles [5], and the concentration of a natural clay (Na-MMT) and an organo modified clay (Cloisite®30B) on the tensile properties of a TPS prepared from a premixture containing starch/water/glycerol (5/2/3 by weight ratio) [6]. In those works, it was reported that tensile properties showed the highest performance when the hydrophilic Na-MMT was dispersed in the TPS matrix [5] and that maximum stiffness and ductility balance at a concentration of 5 wt% of the natural clay [6]. They related the best performance of TPS/Na-MMT bionanocomposites compounded with 5 wt% of Na-MMT to the formation of an intercalated nanostructure [6]. In a similar way, TPS/clay bionanocomposites have been also prepared by melt intercalation in internal batch mixer but in a one-step process [16]. Chivrac, Pollet and Averous [16] evaluated the effect of 6 wt% of Na-MMT and two OMMT modified by the exfoliation-absorption or the shear induced clay organo-modification processes on the tensile properties of TPS/clay bionanocomposites. Conversely to the observations of Park and coworkers, they observed that the incorporation of 6 wt% of OMMT increased more than 50% the stiffness without decreasing the ductility of TPS, while 6 wt% of Na-MMT increased just about 30% of E value but decreased 30% the ε_b with respect to TPS. In order to improve the mechanical performance, TPS/clay bionanocomposites have also been obtained in continuous one-step processes using single [10] and twin-screw extruders [17, 21, 22]. Natural and organo-modified clays were dispersed in concentrations between 3 and 6 wt% in TPS matrices having between 20 and 41% glycerol. Müller, Laurindo and Yamashita reported increments of the values of E of 5.62 and 3.12 times with respect to TPS, without effect on ε_b, for the TPS/clay bionanocomposites formulated with 3 and 5 wt% of Na-MMT, respectively [10]. Dispersion of organo-modified clay had almost no effect on E and ε_b. Conversely, Gutiérrez and Alvarez reported that the dispersion of 4 wt% Na-MMT or OMMT surprisingly resulted in a reduction of E of around 50% with respect to TPS [21]. From the above discussed literature, it is seen that improvements in stiffness above 500% can be produced using both the intercalation of polymer from solution [18] or the melt intercalation methods [10]. Therefore, the purpose of this work is the evaluation of new strategies for improving the dispersion of clay particles in a TPS matrix using pre-dispersed clay particles in an aqueous medium and a combination of solution and melt intercalation in a continuous one-step process.

2 Preparation and Characterization of TPS/VMC Bionanocomposites

2.1 Abbreviations

ε_b	Elongation at break
E	Young's Modulus
H_2O_2	Hydrogen peroxide
MMT	Montmorillonite
Na-MMT	Sodium montmorillonite
NaOH	Sodium hydroxide
OMMT	Organo-modified montmorillonite
σ_b	Stress at break
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
Tg	Glass transition temperature
TPS	Thermoplastic starch
TPS36	Thermoplastic starch plasticized with 36% of glycerol which is almost water-free
TPS/VMC30-2%	Bionanocomposite of thermoplastic starch and 2% of VMC30
TPS/VMC30-5%	Bionanocomposite of thermoplastic starch and 5% of VMC30
US	Ultrasound treatment
VMC	Raw vermiculite
VMC0	Vermiculite treated without H_2O_2 and sonicated during 60 min
VMC30	Vermiculite treated with 30% H_2O_2 and sonicated during 60 min
VMC400	Vermiculite milled and sieved that passed 400-meshes
VMCS	Vermiculite silanized, which was synthesized from a VMC30
XRD	X-ray diffraction

2.2 Materials

Potato starch was supplied by Penford Food Ingredients (Monterrey, México). Thermoplastic starch was plasticized using glycerol (purity 99.5%) from INSUMA (Saltillo, México). VMC b MIC (5) was supplied by Termolita S.A.P.I. de C.V. (Santa Catarina, México). Hydrogen peroxide (H_2O_2) at 30% purified (from Fermont Lab, Monterrey, México) was used as oxidant agent. *N*-(2-Aminoethyl)-3-aminopropylmethyldimethoxysilane, Geniosil GF95 from Wacker de México, was used for the modification of VMC.

2.3 Methods

2.3.1 Modification of VMC Particles

VMC was pulverized using a Fritsh Pulverisette 6 planetary mill at a rotation speed of 300 rpm during 5 min. Pulverized VMC was screened using a RO-TAP model RX-29 having screens of 40, 100, 200 and 400 meshes. Dispersion and oxidation of the fraction of ground VMC having particle size below 40 μm (VMC400) was performed by a combination of US and H_2O_2 treatments at 750 W, amplitude of 25% and 25 °C using an ultrasound probe (Cole-Parmer, model CP500). Parameters controlled during dispersion and oxidation of VMC were sonication time (20 and 60 min) and peroxide content in the solutions (0, 5, 10, 15 and 30%). Oxidized VMC was washed with deionized water (two times) and with ethanol (one time) and dried during 24 h at 80 °C. Grafting of aminosilane groups was performed on the oxidized clay sonicated for 60 min in the presence of 30% peroxide solution (VMC30) using the method reported by Fernandez [23]. For the silanization of 50 g of vermiculite, a suspension at 20% of solids of VMC30/ethanol was sonicated during 60 min and then 12.5 g of aminosilane were added and reacted by refluxing at around 75 °C during 6 h. The silanized vermiculite (VMCS) was filtered and washed with ethanol and dried during 24 h at 80 °C.

2.3.2 Preparation of TPS/VMC Bionanocomposites

Preparation of TPS/VMC bionanocomposites was carried out in a 30 mm of diameter twin-screw extruder ZSK30 de Werner & Pfleiderer having 6 heating zones. TPS was prepared from a suspension of starch/glycerol/water, which has a starch-glycerol ratio of 64:36 (TPS36), such as reported by Rodriguez [2]. Dry VMC30 and VMCS were added to the suspension in concentrations of 1, 2 and 5 wt% with respect to starch + glycerol content. Suspensions were fed in zone 1 (25 °C) at a feeding rate of 48 g/min using a peristaltic pump (Masterflex L/S). Gelatinization and plasticization of starch occurred in zones 2 and 3 (80 °C), while water extraction and pressurization of the water-free TPS was performed in the zones 3, 4 and 5 (120 °C). The 6th heating zone was a 3 × 50 mm rectangular die (120 °C). Screw rate was maintained at 150 rpm and the extruded TPS ribbons were cooled with a 3-roll calendar system (Killion Extruders Inc.). For the evaluation of tensile properties, TPS extruded ribbons were compression molded (Carver press model 3895.4NE1000) in 3 × 150 × 150 mm sheets at 120 °C and 5 ton of pressure during 2 min.

2.3.3 Characterization

The crystalline structure of VMC (in powder or aqueous suspensions) and the TPS/VMC bionanocomposites (in films) were evaluated by X-ray diffraction (XRD) using a Bragg–Brentano geometry at room temperature and radiation CuKα radiation ($\lambda = 1.54183$ Å) in a diffractometer Ultima IV (Rigaku) equipped with a D/tex detector. XRD pattern were collected from 4° to 80° (2θ) in 0.02° steps at 10°/min. The interlayer distance of VMC was calculated by the 001-peak position, using the Bragg's equation ($d = n\lambda/2\text{sen}\ \theta$).

The hydroxyl content on VMC surface was determined by the ASTM E-222 method. 1 g of VMC was suspended in 5 mL of a 3:1 mixture of pyridine-acetic anhydride (reagent solution) in a 250 mL round-bottom flask and sonicated at room temperature during 1 h. Flask was connected to a condenser and the solution refluxed during 1 h. The unreacted acetic anhydride was hydrolyzed with 10 mL of distilled water and the whole refluxing system was rinsed with 25 mL of ethanol. Unreacted acetic acid was titrated at room temperature with a 0.5 N NaOH solution using an automatic system 848 Titrino Plus. The hydroxyl number was determined using the equation: $OH = ((A - B) * N * 40)/W$, where: A = mL base used to titrate a blank, B = mL of base used to titrate the VMC sample, N = Normality of NaOH solution, W = g of VMC sample.

The amine content on the VMCS was determined by titration. 1 g of VMC was suspended in 5 ml of a 3:1 mixture of pyridine-acetic anhydride (reagent solution) in a 250 mL flask and sonicated at room temperature during 1 h. After sonication, the reaction continued at room temperature during 15 min and then the unreacted acetic anhydride was hydrolyzed with 5 mL. Hydrolysis of unreacted acetic anhydride continued for 10 min and 10 mL of n-butanol were added before titration. The produced acetic acid was titrated at room temperature with a 0.5 N NaOH solution using an automatic system 848 Titrino Plus. The amine number was determined using the equation: $\#NH_2 = (A - B) * N$, where: A = mL base used to titrate a blank, B = mL of base used to titrate the VMCS sample, N = Normality of NaOH solution.

Morphological changes of treated VMC samples were observed by SEM using a microscope JEOL (model JCM 6000) operated at 10 kV. Morphology of cryogenically microtomed TPS/VMC bionanocomposites was evaluated using a TEM FEI-TITAN 30–800 kV working at 300 kV. Both VMC and TPS/VMC bionanocomposites samples were coated with a Au/Pd alloy before SEM observation.

Rheological properties of VMC suspensions were performed in steady state using an Anton Paar rheometer (MCR 501). Suspensions were evaluated at 25 °C and shear rate from 1 to 1000 s^{-1} using a double gap geometry C-DG26.7.

Thermal properties were evaluated by differential scanning calorimetry using an equipment TA Instruments Discovery Series DSC 2500. Thermal transitions in the interval between −90 and 200 °C were evaluated at 10 °C min^{-1} under nitrogen atmosphere. Values reported were recorded from the second heating scans.

Tensile properties were evaluated according to the ASTM D638 standard method using an Instron (model 4301) universal machine. Compression molded plaques were cut into type IV samples and tested at a crosshead speed of 50 mm min^{-1} using a load cell of 5 KN.

3 Results and Discussions

3.1 *Modification of VMC*

The purposes for modification of VMC were: firstly, increase the surface area of clay particles via reduction of both thickness and size of platelets [24], secondly, oxidation of clay surface for having more hydroxyl groups available for silanization [25, 26] and, third, silanization of VMC particles to improve the exfoliation of clay [27]. VMC400 was treated with ultrasound and 0, 5, 10, 15 and 30% H_2O_2 during 60 min, and the effect of the treatment on clay dispersion was evaluated by viscosity measurements (Fig. 1). Distilled water was also evaluated for comparison purpose and showed a viscosity of almost 1 mPa s. The suspension of VMC0 (20%) depicted a Newtonian behavior and a viscosity around 3.38 mPa s. On the other hand, all the VMC suspensions sonicated in the presence of H_2O_2 showed pseudoplastic behavior and viscosities above 1 and 0.01 Pa s at shear rates from 1 to 1000 s^{-1}, respectively. These results confirmed that the combination of sonication and H_2O_2 treatments are very powerful for the improvement of the dispersion of clay particles by increasing the surface of clay particles that interact with aqueous medium [24, 25, 28, 29]. Moreover, the viscosity of the oxidized VMC suspensions seemed to decrease as the H_2O_2 content was increased.

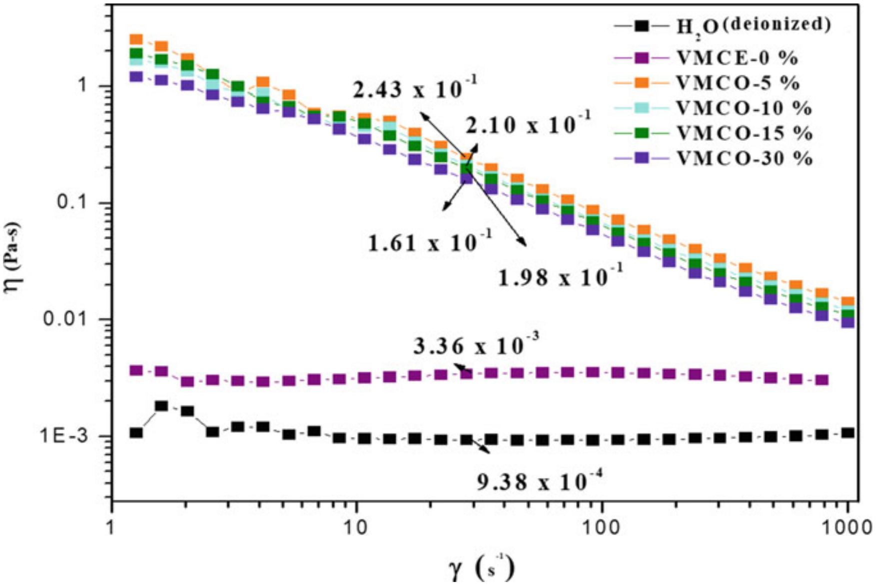


Fig. 1 Rheological measurements of VMC treated by sonication with 0, 5, 10, 15 and 30% of H_2O_2 at 25 °C

Table 1 Effect of H_2O_2 content on the hydroxyl number of VMC suspensions treated at 25 °C

Sample	OH (meq/g)
VMC0	16.6
VMC5	19.8
VMC10	23.2
VMC15	35.6
VMC30	44.8

It has been reported, on one hand, that the hydroxyl number in chemically treated clays increased due to the breakup of VMC particles which resulted in the increasing of the edges of clay layers [26] and, on the other hand, the ultrasound treatment using H_2O_2 produced the reduction of VMC particle size [24, 25, 28, 29]. Table 1 shows that the hydroxyl number of VMC0 was 16.6 meq/g. The values of hydroxyl number increased to 19.8, 23.2, 35.6 and 44.8 meq/g with the addition of 5, 10, 15 and 30% of H_2O_2, respectively. The trend observed for the hydroxyl number in clay suspensions was inverse to that observed for the viscosity in which the viscosity of clay suspensions decreased as the content of H_2O_2 increased. These results suggest that as the content of H_2O_2 increased during the treatment there was a higher number of breakup of clay layers, but as the size of clay platelets decreased the viscosity of VMC suspensions decreased.

The combination of sonication and oxidation with H_2O_2 improved the dispersion and hydroxyl number of VMC due to the breakup and delamination of clay particles. In the SEM images at 300× (Fig. 2a), it can be observed the morphology of VMC400 where there was a population of thick platelets having particle sizes ranging between 10 and 40 μm and a population of particles smaller than 10 μm that seemed to be thinner than the big ones. Moreover, the surface of clay particles appeared to be smooth and compact as that observed by Kehal and coworkers [28] and Ali et al. [30]. After sonication (Figs. 2b–d), the population of large particles decreased and the surface of clay platelets changed to a woolen-like structure, as reported by Ali et al. [30]. They reported that structure of clay particle was related to the exfoliation produced by the combination of the mechanical energy supplied by ultrasound and the decomposition of H_2O_2 in $O_2 + H_2O$ into the VMC interlayers. Moreover, exfoliation of clay fundamental particles resulted in the scrolling of the borders and the formation of tubes (Fig. 3). Similar observations were reported by Wiewióra et al. after more than 10 h of sonication of clay suspensions [31]. The dramatic increasing of the viscosity of VMC suspensions confirmed that the treatment of VMC with ultrasound and H_2O_2 at 25 °C gave place to an extraordinary dispersion due to the breakup and exfoliation of clay particles.

TPS/VMC bionanocomposites have been generally formulated using milled, sieved and dried clay [5–10]. In this work, VMC was pre-dispersed into the starch/glycerol/water suspensions. In order to simulate the state of dispersion for determining the effect of water intercalation on the interlayer distances, clay/water suspensions having 20% of solids were evaluated by DRX. Figure 4 shows a 4–8°2θ region of the untreated VMC and the treated VMC observed by SEM (Fig. 2).

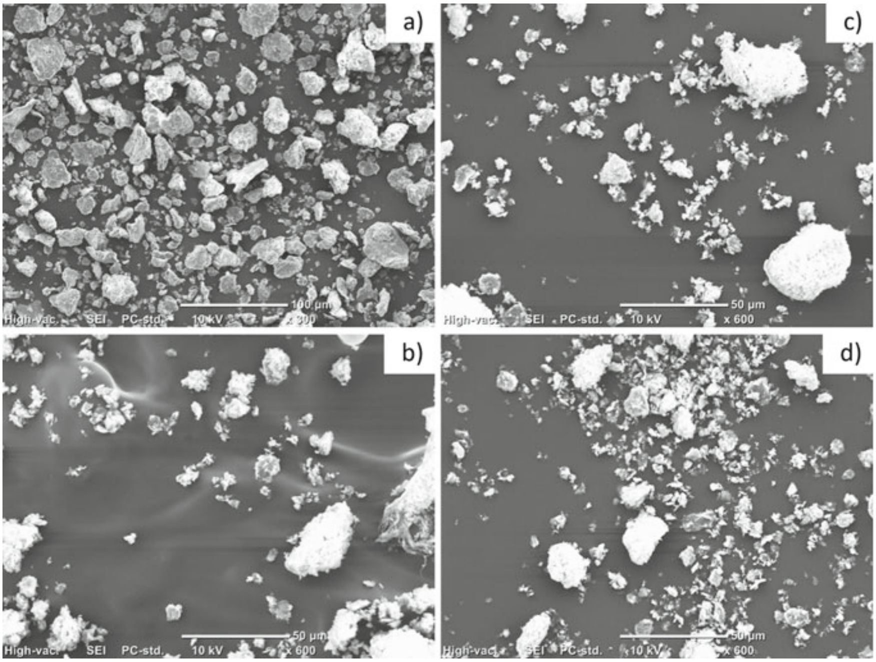


Fig. 2 SEM micrographs of vermiculite **a** before modification (VMC400), modified by sonication and **b** 0% (VMC0) and **c** 30% H_2O_2 (VMC30), and **d** silanization (VMCS)

The dry powder of VMC400 showed a signal at 6.15°2θ, which represented an interplanar distance of 14.370 Å. On the other hand, the interlayer distances of VMC0 and VMC30 increased to values of 14.488 and 14.905 Å due to the swelling effect of water in clay suspensions. These results suggested that the interlayer distance increasing produced by the intercalation of water could be related to the smaller particle size and higher content of hydroxyl of clay particles. Conversely, VMCS (which is a silanized VMC30) showed an important reduction of the interlayer distance to 12.899 Å in the water suspension produced by the hydrophobic nature of silanized clay.

3.2 TPS and TPS/VMC Bionanocomposites

The thermal properties of TPS/VMC bionanocomposites as a function of the type and content of VMC were studied in this work. The thermal behavior of TPS36 is depicted in Fig. 5. The second heating scan clearly showed only one thermal transition at −57.09 °C, which corresponds to Tg and was 3 °C lower than that reported by Rodriguez et al. [2]. No thermal transitions above room temperature were observed for TPS36 as reported in other works [17, 32]. Thermal properties were evaluated

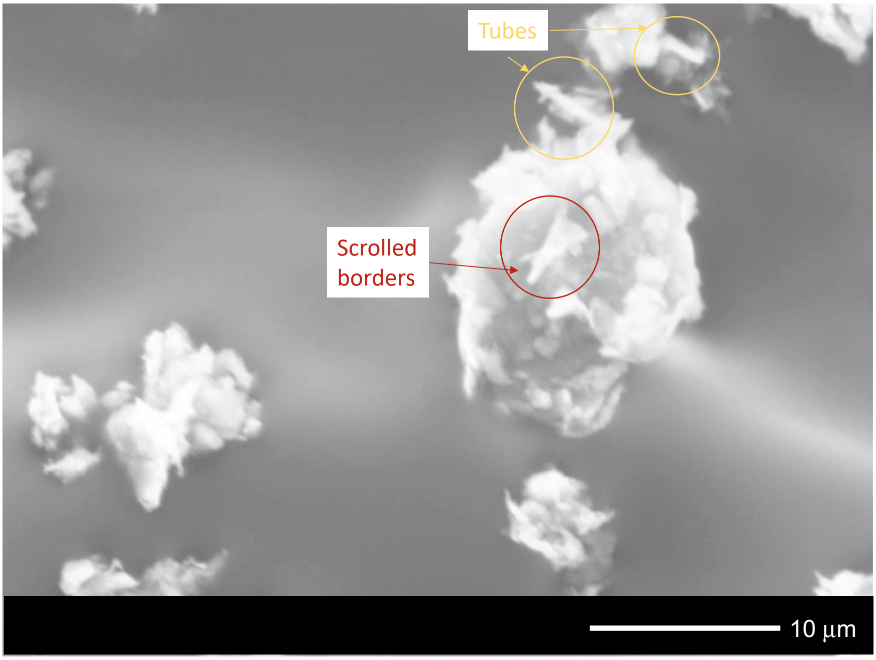


Fig. 3 SEM micrograph VMC-0 at 1500X

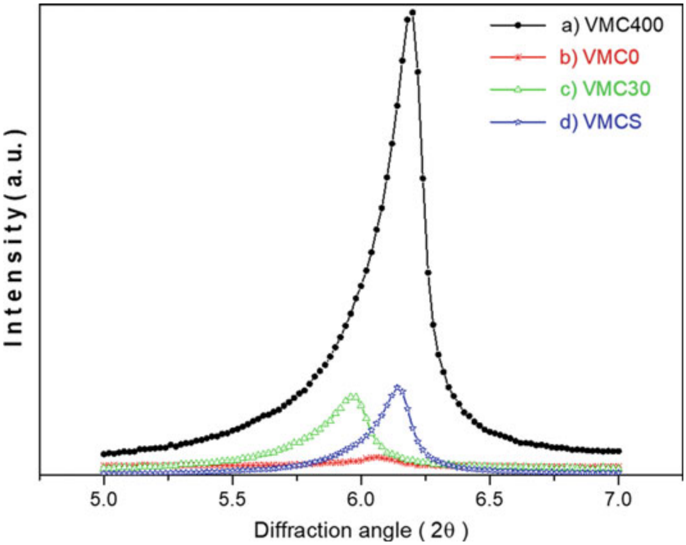
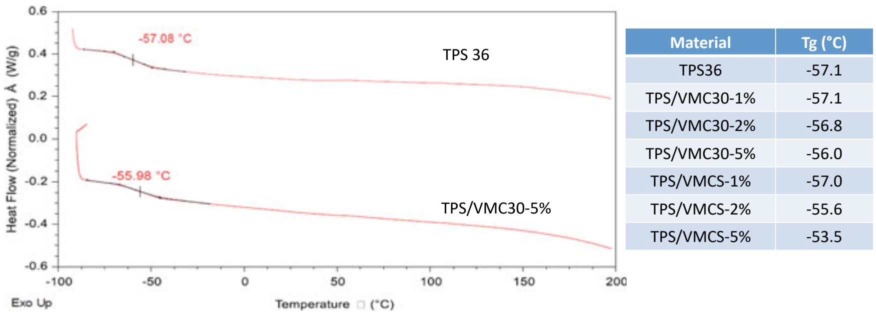


Fig. 4 XRD of vermiculite **a** before modification (VMC400), modified by sonication and **b** 0% (VMC0) and **c** 30% H_2O_2 (VMC30), and **d** silanization (VMCS)



Material	Tg (°C)
TPS36	-57.1
TPS/VMC30-1%	-57.1
TPS/VMC30-2%	-56.8
TPS/VMC30-5%	-56.0
TPS/VMCS-1%	-57.0
TPS/VMCS-2%	-55.6
TPS/VMCS-5%	-53.5

Fig. 5 DSC thermograms of TPS36 and TPS/VMC30-5% evaluated within the 48 h after extrusion and the effect of the type and content of VMC on the Tg of TPS/VMC bionanocomposites

before retrogradation of starch as demonstrated by the lack of signals above room temperature.

TPS/VMC30-5%, and the other bionanocomposites, depicted a similar thermal behavior to that of TPS36 but the values of the Tg of the bionanocomposites changed as a function of VMC type and content (Fig. 5). The Tg of the TPS/VMC30 bionanocomposites having 1 and 2% of clay showed a very similar Tg as TPS36, while TPS/VMC30-5% had a slight increase of 1.1 °C, which suggested that almost no glycerol was migrated out of starch domains to VMC particles. Unexpectedly, the bionanocomposites prepared with the hydrophobic VMCS showed a higher increasing on the Tg of TPS (1.5 and 3.6 °C for the bionanocomposites compounded with 2 and 5% VMCS, respectively). Nigago and coworkers observed an increase of 4 °C with the addition of 5% of bentonite to a TPS formulation [32]. Tg of TPS is highly dependent on glycerol content and the values of Tg increase as the content of plasticizer decreases [2]. These results suggested that some glycerol could migrate to VMCS domains resulting in the increasing of Tg.

TPS was prepared from a suspension of starch/glycerol/water (48.5:27.5:20) and for TPS/VMC bionanocomposites, 1, 2 and 5% of VMC30 or VMCS, with respect to starch + glycerol, were added to the suspension. The dispersion of mineral particles into polymeric matrices can be improved by dynamic and thermodynamic ways. The incorporation of VMC in the water/glycerol medium had the purpose of increasing the interactions between clay particles and starch molecules using a common dissolvent. X-ray diffractograms in the range between 3 and 10 °2θ of TPS36 and TPS/VMC30 bionanocomposites are shown in Fig. 6. The whole biocomposites showed diffraction peaks about 6.3 °2θ, which are higher than that of the original VMC400 (6.15 °2θ). This result evidenced, by one hand, that cations were replaced by protons from the surface of VMC during the oxidation process (Table 1) and, by the other hand, almost no intercalation of starch molecules or glycerol occurred during the melt mixing of TPS and VMC particles (Fig. 5).

Figure 7 shows the TEM micrographs of the TPS/VMC30 bionanocomposite (5% clay) at different magnifications. At low magnifications (Fig. 5a, b), it can be observed a good distribution of clay tactoids and agglomerates. There was one agglomerate of

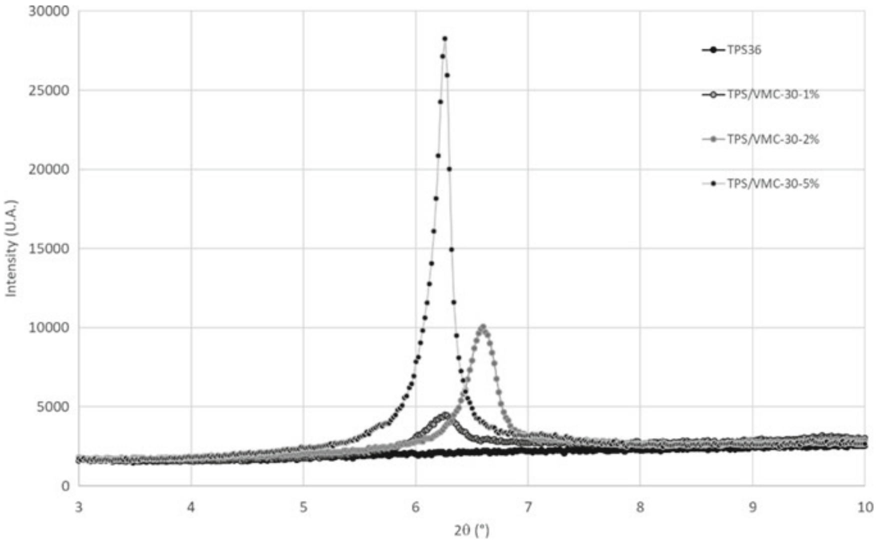


Fig. 6 XRD of TPS 36 and TPS/VMC30 bionanocomposites compounded with 1, 2 and 5% of clay

around 600 nm in Fig. 5a and another of more than a 1000 nm (Fig. 5b), but lots of tactoids of less than 100 nm (Figs. 5c, d), which indicated an excellent dispersion of clay particles in the TPS matrix. From micrographs of Figs. 5c, d it seemed that there was not intercalation of starch molecules into clay galleries, but just an extraordinary interfacial contact. These results confirmed the efficiency of pre-dispersion of VMC in the starch/glycerol/water suspension and the combination of solution and melt dispersion during extrusion.

Tensile properties of TPS have been reported to be dependent on plasticizer content, i.e., at low plasticizer content TPS is stiff and brittle and at high plasticizer content TPS is soft and ductile [1, 33]. The stress–strain curves of TPS and TPS/VMC bionanocomposites are shown in Fig. 8. As expected, the water-free TPS36 was soft and ductile having average ε_b strain and σ_b and E of 335 ± 35%, 1.14 ± 0.4 MPa and 1.08 ± 0.18 MPa, respectively. The addition of 1% of VMC30 increased more than 7 times the stiffness of TPS36 matrix (E = 8.23 MPa) with no effects on ductility. The incorporation of 2% of VMC30 to the TPS36 matrix produced a dramatic change of the tensile behavior of TPS/VMC30-2% bionanocomposites showing a yielding point at low ε followed by an increment of tensile strength, similar to that observed in semi-crystalline polymers [34]. The change of the stress–strain curve behavior of the bionanocomposites compounded with 2% of clay was produced by an increment of the values of E of more than 100 times compared to TPS36 (E = 118.66 MPa). It is well known that an increasing of rigidness results in a reduction of ductility as observed in the TPS/VMC bionanocomposites compounded with 2% of clay, where the values of ε dropped from above 300% to around 200%. Increasing the clay content to 5%, TPS/VMC30-5% resulted in a further increment of E values to 223.79 MPa,

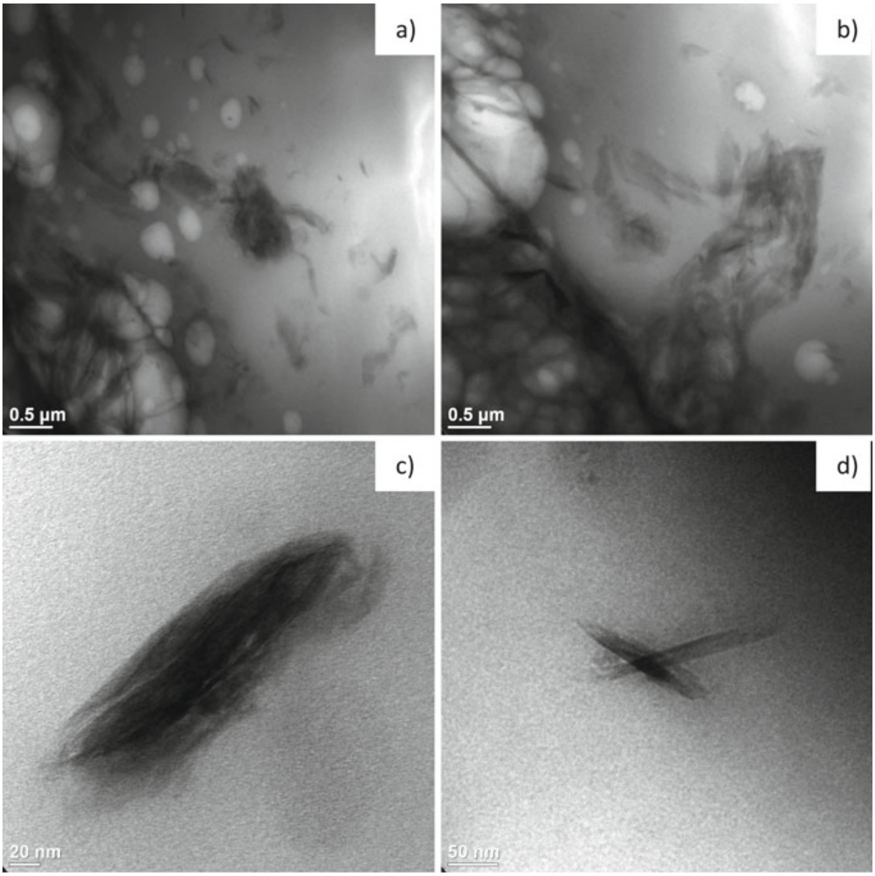


Fig. 7 TEM micrographs of microtomed TPS/VMC30 bionanocomposites

which is more than 200 times or 20,000% higher than the water-free TPS36, but a reduction of ε to around 150%. It is important to highlight that the elastic modulus of the TPS/VMC30-5 bionanocomposite is above the low range of E for LDPE which varies from 150 to 520 MPa according to MatWeb database [35]. Several works have reported on the development of TPS/clay bionanocomposites [5–7, 9, 10, 16, 36]. In most of those works, TPS was formulated with low glycerol content showing a stiff and brittle behavior with ε values far below 100%. In a outstanding work published in the art, Katerinopoulou and coworker reported that E increased almost 2 and 5 times with respect to a TPS plasticized with 30% glycerol [36]. Therefore, the results discussed in the present work showed for the first time that super tough TPS/clay bionanocomposites can be developed by the combination of solution and melt dispersion using pre-dispersed VMC particles in the starch/glycerol/water suspension during extrusion in a one-step process.

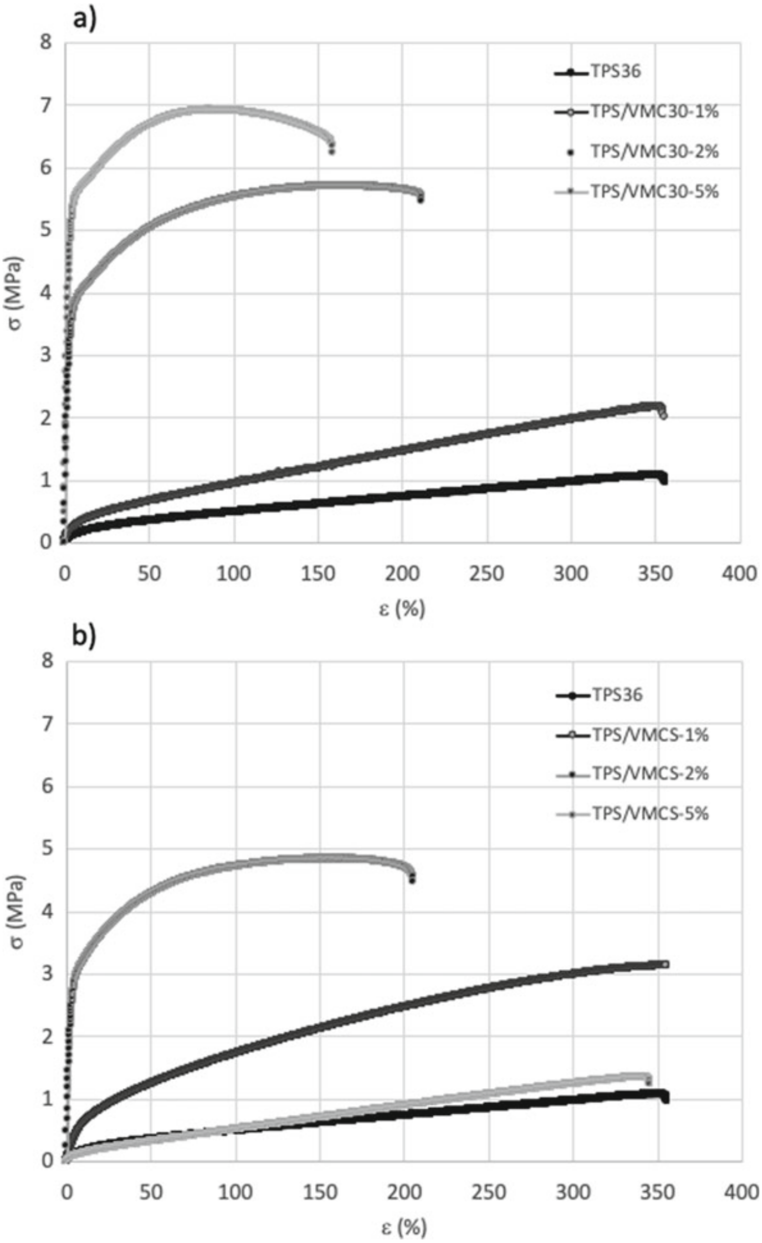


Fig. 8 The stress–strain curves of TPS and TPS/VMC bionanocomposites compounded with **a** 1, 2 and 5% VMC30, and **b** 1, 2 and 5% VMCS

The addition of 1 and 2% of VMCS showed a similar trend as TPS/VMC30 bionanocomposites (E = 8.14 and 108.26 MPa, and ε_b of *ca.* 340 and 200%, for TPS/VMCS formulated with 1 and 2% of the hydrophobic clay, respectively). However, the incorporation of 5% of VMCS to the TPS36 matrix produced a dramatic decrease of stiffness (E = 0.53 MPa) but the recovery of ductility to the values observed in TPS36. It seemed that the hydrophobic nature of VMCS resulted in the re-agglomeration of particles at high clay contents. Similar results of hydrophobic clays have been reported in the art [5–7, 9, 10].

4 Conclusions

High performance TPS/VMC bionanocomposites were prepared using starch/glycerol/water suspensions containing pre-dispersed clay particles. VMC particles were successfully pre-dispersed using a combination of US treatment and H_2O_2 and this was evidenced by a pseudoplastic rheological behavior and high viscosity. Conversely, clay suspensions treated without H_2O_2 showed Newtonian behavior and low viscosity, which demonstrated poor dispersion. It was proven that good dispersion of VMC treated with both US and H_2O_2 was produced by the breakup, deagglomeration, delamination and exfoliation of VMC particles. The analysis of the concentration of VMC30 on the tensile properties of TPS/VMC bionanocomposites showed that the addition of 1% of oxidized clay increased more than 7 times the stiffness of TPS36 matrix with no effects on ductility while the incorporation of 2 or 5% of VMC resulted in super tough TPS materials showing increments of the values of E of more than 100 and 200 times compared to TPS36, and ε_b of 200% and 150%, respectively. After the silanization of VMC, the hydrophilic clay changed to a hydrophobic nature which showed a reduction in the interlayer distance when suspended in water and produced the re-agglomeration of clay particles in the TPS/VMCS bionanocomposites compounded with 5% of VMCS. It was proved in this work that the combination of solution and melt dispersion of the pre-dispersed VMC during blending in a one-step extrusion process results in a super tough TPS that has a stiffness similar to LDPE.

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Chitin and Chitosan Nanocomposites: From the Synthesis to the Application

E. Oyervides-Muñoz, M. A. Oyervides-Muñoz, and M. A. Garcia-Lobato

Abstract Chitin is a bio-based and biodegradable polymer since it is the second most abundant natural polysaccharide on earth after cellulose. It is composed of *N*-acetyl-D-glucosamine, and it is found in different organisms such as in the exoskeleton of crustacean, insects, annelids, mollusks, plants, and some microorganisms. On the other hand, Chitosan is generally obtained by the controlled deacetylation of chitin in the presence of alkali, composed of randomly distributed β-(1–4) linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Chitin and chitosan are different based on the degree of deacetylation. These polysaccharides possess different physicochemical and biological properties which has been modified and/or improved thanks to the development of chitin and chitosan nanocomposites. There are various methods for the development of chitin and chitosan-based nanocomposites, including solvent casting, spray coating, layer-by-layer, and extrusion. Also, it has been combined with different nano compounds to obtain these nano composites. This chapter summarizes some of the most important technologies involved in synthesizing chitin and chitosan nanocomposites, as well as the study of its physicochemical and biological properties, its production costs and ending in the analysis and discussion of the applications that these materials have in various fields.

Keywords Chitin · Chitosan · Nanocomposites · Biopolymer

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1 Introduction

1.1 Generalities of Chitin

Chitin is the second most abundant biopolymer in nature after cellulose. It is predominantly found as the building material that gives strength to the exoskeletons of different species of mollusks, crustaceans, insects, and in the cell walls of fungi, algae, and related organisms. The term chitin is derived from the Greek word "chiton" which means "a coat of mail" (a jacket composed of metal rings serving as armor). Chitin is bio-renewable, environmentally friendly, and biocompatible. It is composed of *N*-acetyl-D-glucosamine and its chemical structure is very closed to cellulose with the difference that chitin has acetamide groups ($–NHCOCH_3$) at the C-2 positions [1]. Chitin was first discovered in fungi, in 1811 by Henri Braconnot and later named by Auguste Odier in 1823. Chitin is primarily obtained after refining crustacean shells derived from marine industries, the obtention of this biopolymer is through the demineralization and deproteinization generally of shrimp and crab skeletons. Vallejo-Domínguez et al. investigated a modification to a chemical method of chitin synthesis from shrimp shells using high-frequency ultrasound obtaining a more efficient deproteinization process [2]. Figure 1 shows the chemical structure of chitin. Although in its crude form, chitin has poor solubility and low reactivity [3], it is insoluble in traditional solvents, limiting its applications. It exhibits distinct properties, which are highly desired for specific applications related to biomedical, pharmaceutical, and similar industries, i.e., it has been used as chelating agent, additives for water treatment, drug carriers, wound-healing agents, etc. [4].

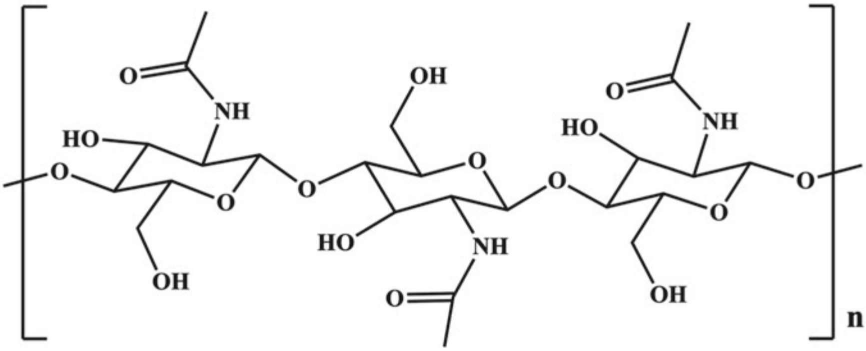

Fig. 1 Chemical structure of chitin

1.2 Generalities of Chitosan

Chitosan $(C_6H_{11}NO_4)_n$ is a derivative copolymer usually obtained by chemical or enzymatic deacetylation of chitin. The alkaline method of deacetylation is carried out by refluxing chitin in 40–50% of sodium hydroxide solution for a specific period. Unfortunately, this method is not environmentally friendly. The enzymatic method involves treating the chitin with a chitin deacetylase enzyme extracted from various bacterial strains [5]. Nevertheless, an investigation has dealt with the preparation of chitosan from γ-irradiated prawn shells and the comparison of molecular weight, alkali concentration for deacetylation and reaction time of the chitosan obtained from γ-irradiated prawn shell with those of chitosan prepared using the conventional method. It was found that the cost of extraction of chitosan can be minimized by irradiating the starting raw materials. Chitosan with higher deacetylation degree can be prepared by applying γ-radiation, thus minimizing the use of concentrated NaOH solution and heating time during the deacetylation process [6].

Chitosan is composed of *N*-acetylglucosamine and glucosamine, and its physical, mechanical, and biological properties depend on the degree of deacetylation which is usually between 70 and 95% [7]. The degree of deacetylation of chitosan can be measured by using acid–base titration, conduct-metric titration, spectroscopic methods such as infra-red spectra, UV and proton or carbon NMR, as well as gel permeation analysis. If this degree of deacetylation is higher than 50 mol%, the product is designated as chitosan. Figure 2 shows the chemical structure of chitosan as partially deacetylated chitin. This polysaccharide is soluble in dilute aqueous acidic solutions below its pKa (~6.3), in which it converts the amine units ($-NH_2$) into the soluble protonated ($-NH_3^+$). In the solid state, chitosan is a semicrystalline polymer. The main commercial production of chitosan is estimated to be 10^{10} tons per year by eco-friendly and economically viable processes. This biopolymer is totally eco-friendly, antibacterial, antifungal and provides many possibilities for food, biomedical, cosmetology, pharmacy, textile, paper industry and other industrial purposes [8]. The antimicrobial activity of chitosan has been related to the free amino group of the glucosamine residue. This fraction of chitosan can be easily protonated, and these groups disrupt the cell membranes of microbes. Another plausible mechanism by which chitosan is expected to inhibit microbial cellular growth is the interaction of the chitosan molecules with the microbial cell´s genetic material inhibiting DNA transcription interacting with the essential nutrients and trace elements that are required for cell growth and toxin production by the pathogenic microbes. Additionally, the chitosan molecules can interact with the spore of the microbes, preventing its growth [5]. The production of chitosan generally involves three steps (demineralization, deproteinization and deacetylation). Chemical extraction involving acids and alkali is considered the best for the industrial production of this biopolymer because of its shorter reaction time and capacity to remove complete organic salts and proteins from the starting material [9].

Chitosan is the only positively charged polysaccharide [10], it possesses high thermal stability and its rich in OH groups [11]. Chitosan has excellent film-forming

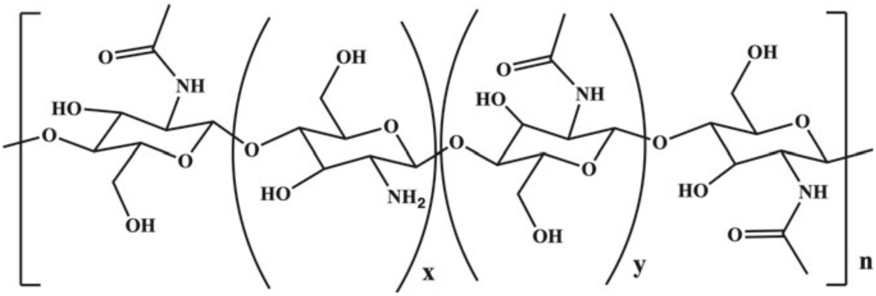

Fig. 2 Chemical structure of chitosan

properties, hydrophilic/moisturizing effect [12] and films of chitosan are generally nonporous, biologically adhesive, capable of chelating ions and have low ion conductivity with hemostatic effect [13]. These films can be prepared by solvent casting or spin coating [14]. Chitosan is also non-toxic, non-immunogenic and non-carcinogenic [15], it can be used as excellent starting agents for demulsifiers since they feature a broad range of large molecular weights, active hydrogen groups, branched structures, good rheological properties and can occupy a large area of the oil–water interface [16]. Chitosan is a natural antioxidant; this property is related to its free radical scavenging activity. The antioxidant property come from the lone pair of electrons on the nitrogen atom located at the C-2 position. The lone pair of electrons allows the nitrogen atoms to accept a proton to form NH_3^+ group, which immediately reacts with the free radicals to generate stable molecules. It can be easily processed in diverse forms, such as films, threads, tablets, membranes, and nanoparticles, allowing the design of a variety of devices adaptable to end purposes. In agriculture, it is used as a carrier for controlling the release of pesticide, herbicide, macro and micronutrient, and plant hormones [17]. From a biopharmaceutical viewpoint, chitosan has the potential of serving as an absorption enhancer across intestinal epithelia prolonging the residence time of delivery systems at absorption sites and has the ability to open the thigh junctions of cell membranes [18]. Chitosan has been found to interfere with the growth of several plant pathogenic fungi, e.g., *Fusarium solani*, *F. oxysporum*, *Puccinia arachidis*, *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Rhizoctonia solani* [19]. In addition to this, chitosan is reported to act as enhancer of plants growth and yield in several crops, it is easily absorbed to plants surface, and it emerged as a valuable carrier for controlled delivery of agrochemicals [20]. Chitosan is considered safe by the Food and Drug Administration (FDA) and is widely used in the medical field as treatment of obese, to decrease high cholesterol, for the treatment of kidney failure, loss of appetite, insomnia, and other diseases. Its anti-fibroblastic activity may aid in wound healing [21, 22].

Due to its properties, chitosan can be used in battery systems as membrane, polymer electrolyte, separator, and binder in lithium ions battery electrodes. Salleh et al. assembled a biopolymer made of chitosan binder in a graphene electrode to fabricate chitosan binders with good properties [23]. Ikram et al. developed chitin and chitosan/graphene-based bio-nanocomposites for energetic applications, including biosensors, batteries, fuel cells and solar cell systems [24].

This biopolymer presents some disadvantages, for example, the industrial application of pure chitosan is limited due to its weak mechanical resistance, low chemical stability [25, 26]. Another problem concerning the industrial use of chitosan is that it is difficult to dissolve it in water and at neutral pH [27].

2 Synthesis and Applications

Nanotechnology is considered an important area of research concerning the design, preparation, and manipulation of molecules at the nanoscale. It is aimed to produce nanostructures with unique properties that put them in good stead over their single particle types. These materials show a wide range of novel biological, optical, electrical, magnetic, and catalytic activities with a great variety of industrial applications [28]. Biopolymer-based nanocomposites have received considerable interest because of their superior thermal, mechanical and biodegradable properties [29]. For all the above, the synthesis of chitin and chitosan-based nanocomposites have offered a wide field of studies.

2.1 Chitin Nanocomposites

Waste from seafood industry is a great source of chitin, on a global level, about 6 mega tons of crustacean shells are discarded per year. For this reason, many researchers evidenced the possibility of exploiting this resource to obtain valuable materials such as chitin nanofibrils which consist of colloidal nano-rods. Chitin nanofibrils possess good antimicrobial properties, but they also act as indirect antimicrobial, stimulating the immunomodulatory activity of skin cells. Chitin nanofibrils have an average size of 240 × 7 × 5 nm and their shapes are like small splinters with the characteristics of being able to be absorbed by the human body and promote cell proliferation. It has been demonstrated that chitin nanofibrils possess antimicrobial activity as function of the pH and acetylation degree [30]. Consequently, they can be used in cosmetic and biomedical sectors, but their potential can also be exploited in sanitary and food packaging sectors. The dispersion of chitin nanofibrils in polylactic acid was attempted by several techniques. Coltelli et al. prepared nanocomposites consisting of a polylactic acid matrix and dispersed chitin nanofibrils by extrusion and they incorporated these nanofibrils to polyethylene glycol. These chitin-based nanocomposites can be used in bioplastic items such as in biomedical and pharmaceutical fields due to its intrinsic antimicrobial and skin regeneration properties [31]. Also, they prepared skin compatible films by flat dyer extrusion by using plasticized polylactic acid, poly (butylene succinate-co-adipate) and chitin nanofibrils as functional fillers. These materials did not show any antimicrobial activity and it could be explained considering that the chitin nanofibrils are not on the surface of the material. Nevertheless, the mechanical properties such as flexibility and resistance were improved [32]. The use of natural nanofillers, including nano-chitin, on composite materials is not recommended as it is not compatible with thermoplastic matrices. Following the interest in the preparation of chitin nanofillers-based nanocomposites, Salaberria et al. improved the compatibility of chitin nanocrystals with polylactic acid matrix via acylation using anhydride acetic and dodecanoyl chloride acid. The nanocomposites obtained showed enhanced antifungal activity and could be used as active food packaging [33]. Smirnova et al. optimized the mechanical and physicochemical properties of film matrices based on chitosan by adding chitin nanofibrils. The introduction of chitin nanofibrils increased the mechanical stability obtaining an optimal balance of physicochemical properties and bioactivity [34]. Also, Patel et al. evaluated the effect of different concentrations of triethyl citrate as a dispersing agent of chitin nanocrystals in a polylactic acid matrix to perform the biological and mechanical properties, here, it was observed an increase of the melt viscosity, as well as enhancing the antibacterial activities [35].

Chitin whiskers are another kind of materials that can be obtained from chitin, these can be prepared from hydrolysis in a strong acid aqueous medium. These whiskers have been used as a reinforcement in polysaccharide composite films. Carsi et al. developed chitin-based whiskers loaded with carrageenan nanocomposites, and they studied the dynamic behavior for possible applications [36].

Nanoscale chitin is of great value as an efficient functional and reinforcement material for a wide range of applications. The use of nano chitin to produce nanocomposites offers a good opportunity to prepare bioplastic materials with enhanced functional and structural properties. Some applications of nanoscale chitin are: high strength paper for bioassay applications, flexible packaging coatings, adsorbent for water purification, conductive materials applications, active food packaging materials and biomedical applications [37]. Further, chitin-based nanocomposites exhibited antiproliferative properties against human lung cancer cells which made it a promising candidate in the biomedical field [38].

2.2 Chitosan Nanocomposites

The development of new polymeric materials based on chitin and chitosan represents a particularly interesting and promising strategy within the context of new sustainable and environmental-friendly products derived from renewable resources. Most of the applications for chitin and chitosan nanocomposites came from the emergence of novel pathogens and rise in antimicrobial resistance.

There are many studies on the antibacterial properties of nanoparticles, but the main problem with bacteria is that they create antibiotic resistance, giving rise to strong and dangerous infections. Because of this, different nanoparticles have been utilized in biomedical, cosmetics and food fields as innovative antibacterial agents with excellent results. Bharathi et al. prepared chitosan with iron (II) oxide nanocomposites using *Sida acuta* leaf extract by a facile and eco-friendly green chemistry route. These nanocomposites showed the potential bactericidal activity against *E. coli*, *B. subtilis*, and *S. aureus* pathogens. There are different methods to prepare chitosan nanocomposites i.e., Salari et al. dissolved chitosan into 2% (v/v) glacial acetic acid aqueous solution and mixed it with nanocrystals of cellulose or silver nanoparticles under sonication and then these nanocomposites where mixed with glycerol as plasticizer to obtain chitosan-based nanocomposite films [39], it can be used as encapsulating agents of food packaging, for tissue regeneration, drug carriers [40]. Other applications of the chitosan nanocomposites are using it with carbon nanofibers for chemical and electrochemical catalysts [41], Salari et al. prepared nanocomposites of chitosan with silver nanoparticles and cellulose nanocrystals to improve the antibacterial activity against food borne pathogens, this nanocomposite increased the shelf life of packaged foods [39]. Mujtaba et al. combined the physiological and biological properties of chitosan with copolymerized cellulose nanofibers obtaining a material with enhanced mechanical properties, hydrophobicity, and antioxidant activities. The incorporation of cellulose nanofibers and diatomite to chitosan made it suitable for food packaging applications [42]. Jamróz et al. prepared chitosan-furcellaran nanocomposite films using the casting method. The obtained materials showed good thermal, mechanical and water barrier properties which made them good candidates for food packaging applications, but it may be disadvantageous for packaging food with high humidity [43].

The most intensive research on chitosan-based nanocomposites is focused on blending this biopolymer with nanoparticles. Silver nanoparticles have attracted significant interest due to their broad antimicrobial, antifungal, and antiviral properties and these has been used by combining them with chitosan. Kizioł et al. Deposited chitosan layers embedded with silver nanoparticles and other nanoparticles to reduce the peri-implant infections and other bacteria complications thar are the major concern for failure of arthroplasty operations [44]. Wang et al. blended silica nanoparticles with chitosan to make electrosporum nanofibers that can be applied for in vitro detection of Cu^{+2} ions in human blood, drinking water and biological systems [14]. Saleem et al. investigated chitosan coated polylactid-glycolic acid nanoparticles which exhibited antigen adsorption efficieny and immunogenicity which could be used as vaccine formulations [45]. While Meshkini and Rafie prepared chitosan nanocomposites containing Folic Acid-Magnesium Oxide-Zinc Oxide (F.MgO:ZnO) for regenerative medicine and wound healing purposes. In this study, they found this material can provide a suitable microenvironment for fibroblast cells to maintain their ability to proliferate and migrate which is suitable for skin regeneration [46]. Aljuhani et al. syntesized a copper-oxide-chitosan nanocomposite throught the solution casting method. The prepared nanocomposite film was an excellent ecofriendly heterogeneous basic catalyst for regioselective 1,3-dipolar cycloaddition of hydrazonoyl halides with enamine to produce 1,3,4-trisubstituited pyrazoles which can be used as nanocomposite-based catalyst for the synthesis of various toxic heterocycles like piperidine, pyridine and triethylamine [28]. Liang et al. synthesized casein phosphopeptides-chitosan nanocomposites to encapsulate quertin by multi-frequency power ultrasound which exhibited good stability in simulated gastrointestinal digestion, and an enhancement of the antioxidant activity of the formed nanocomposites. It could be applied in the pharmaceutical and food industry. However, further cell experiments and in vivo experiments are needed for better understanding of the bioavailability of quertin ternary nanoparticles prepared [47]. Currently, the applications of metal nanoparticles and their nanocomposite materials have been majorly reported depending on their structural and surface characteristics, these have various applications such as biological, pharmaceutical, chemical manufacturing, catalyst, environmental technology, and energy storage and conversion.

A water crisis is one of the most challenging global problems and affects human survival and economic development. Membrane technology for water treatments has been widely developed to overcome the problems of water shortages and water pollution. Khoerunnisa et al. prepared chitosan-based nanocomposite membranes with benzalkonium chloride incorporated. This material has a great potential in wastewater treatments for minimizing the water pollution's problem [48]. Li et al. developed through various steps of solubilization and mixing with nanoparticles a porous nanocomposite cross-linked chitosan bead-supported $MnFe_2O_4$ nanoparticles as an efficient adsorbent to remove metalloid (As(III)) and heavy metals like cadmium, copper and plumb from contaminated water [49]. Olajire and Bamigbade synthesized a chitosan-Fe/Ag nanocomposite by a simple green synthetic method by a coagulation-flocculation process to obtain a natural coagulant aid in effluent treatment plant used for wastewater treatment with lowest risks of organic release and it can serve as best substitute to conventional synthetic polyelectrolyte used in effluent treatment plants [50]. In search to use by-products and wastes as a new and renewable source for advanced materials, soot particles (one of the main byproducts of combustion process of all hydrocarbons) have been used in combination with chitosan to develop monolithic aerogels by the addition of soot powder to an aqueous chitosan/acetic acid solution and then this solution was subjected to bath sonication, then glutaraldehyde was used as a crosslinker agent. Finally, this nanocomposite aerogel was freeze-dried and heated to allow complete crosslinking. This material can be used as a new low cost and sustainable material for effectively remove organic contaminants from water and environmental remediation [51].

Within biomedical applications, there are various chitosan-based nanocomposites with different biological and mechanical properties. Cheng et al. prepared bionanocomposites containing chitosan with fluorohydroxyapatite nanoparticles with biocompatible properties in human skin fibroblasts. These nanocomposites could be applied in the regenerative medicine field [52]. Abadehie et al. prepared a biodegradable and absorptive wound dresses with antibacterial activity using nanofibrous mats based on chitosan/polyethylene oxide fibers containing various ratios of lawsone, the fibers were fabricated by electrospinning for wound dressing materials. These nanocomposites showed antibacterial properties and low platform's cytotoxicity and promoted the cell viability of normal human fibroblast cells [53]. Mishra et al. encapsulated *Ocimum basilicum* L. extract in a cellulose nanofiber/chitosan nanocomposite using sonication and used for encapsulating the extract. This loaded nanocomposite presented an enhancement of the antioxidant and antibacterial activity against *S. mutans* and *S. typhimurium.* Due to these biological activities and the low-cost of production, this material could be used for wound healing, food packaging, health care materials, and pharmaceuticals purposes [54]. To take advantage of the inflammatory, antimicrobial, and wound-healing properties of the hydrophobic polyphenol curcumin, Lin et al. prepared nanocomposite nanoparticles with chitosan-poly-γ-glutamic acid and pluronic using a simple ionic gelation technology, this material promoted neo-collagen regeneration. The In vivo experiments demonstrated that chitosan wound dressing containing nanoparticles promoted tissue reconstruction processes [55].

Other applications for chitosan-based nanocomposites are for preservation of food, chitosan-clay nanocomposite films have been prepared for three different methods. The first is the solution casting method which due to its low-cost and convenience in preparation. Typically, chitosan-clay nanocomposites are prepared by dissolving chitosan with various amounts of nano clays in a dilute acid solution, then this is dispersed, immersed in sodium hydroxide, and dried. The second technique is layer by layer self-assembly which produce thin coatings by incorporation of active agents within the matrix of individual polyelectrolyte or between each layer. The third method is coating, which is widely used as active packaging system in preserving the quality of fresh products and extending shelf life of food. Spray coating and dipping are two main methods to apply coatings onto food products to preserve and protect them from bacteria and fungi [29]. Electronics is another not common application for chitosan-based nanocomposites. Chitosan/PVP/Bi_2O_3 nanocomposite films were synthesized by Elabbasy et al. via laser ablation. Its antibacterial activity was improved with the laser ablation time against *E. coli* (Gram negative) and *S. aureus* (Gram positive), and the electrical conductivity parameters were enhanced giving the possibility to use this material in electronic and antibacterial applications [56].

For the agro-industrial applications, nanotechnology proves an effective means for enhancing agronomic potentials. This technology uses the most negligible concentration and utmost in pest control to decrease the cost of pest control due to its small size. The nanoencapsulation of fungicide in biopolymers, such as chitosan and gum acacia, has been applied to commercial fungicides, and is capable of enhanced disease potency due to controlled release, site-direct delivery, and reduced toxicity. Thus, Kumar et al. formed mancozeb-loaded chitosan-gum acacia nanocomposites via ionic gelation and polyelectrolyte complexation methods. This material showed great suppression against *Alternaria solani,* with a good efficiency in pathogen-disease control. This is an eco-friendly material that could help to combat the soil and water pollution menaces of commercial pesticides [57].

With the aim of improve the mechanical properties of chitosan-based nanocomposite films, Rodrigues et al. prepared nanocomposite films using self-assembly association between chitosan and functionalized single-walled carbon nanotubes to study its mechanical properties. Here it was observed the improvement in tensile strength at break property, a rise in the hydrophilicity and decrease in thermal stability after the functionalized single walled carbon nanotubes was noticed [58]. Fernandes et al. prepared nanocomposite films by a simple and green procedure consisting of casting a water-based suspension of cellulose nanofibers in chitosan. The obtained nanocomposite films showed considerably improved mechanical properties with substantial increases in the Young's modulus. The superior mechanical properties of these nanocomposite films were better than pure chitosan films [59].

Nanocomposites can be utilized to maximize the porosity of paper using nanopores silver/chitosan nanocomposites on the cellulose surface, this increases the number of hot spots and provide a filtration function for small molecules enhancing its mechanical and thermal stability. To fabricate the silver/chitosan nanocomposite, chitosan was diluted in acetic acid and combined with a silver nitrate solution and reduced with different reductant solutions. Then, the paper immersed and rinsed in water under magnetic stirring and dried. This low-cost silver/chitosan nanocomposite substrate was used to detect a low concentration of a model small chemical, 4-aminothiophenol. Combining the general traits of paper celluloses and the functionality of this nanocomposite offers potential for novel biosensing applications, such a real-time monitoring during microbial cultivation and lateral flow sensing. Additionally, this multilayered paper could serve in detection and monitoring of various disease factors in human body fluids, including blood and tears [60].

Progress in developing new chitosan-based nanocomposites with improved mechanical and biological properties has encouraged researchers to produce chitosan-based hydrogels nanocomposites. The first application of hydrogels in biomedical field was reported in 1960 by Wichterle. Although it has long been a topic of interest to the scientific community and industry, there is still a growing focus on this area due to the versality of these structures and since they have not yet reached the high level of expectations in various domains. Generally, hydrogels are defined as three-dimensional networks of polymeric chains that are chemically cross-linked, insoluble in water but with a great ability to uptake large volumes of water. A very important feature of most hydrogels is their good biocompatibility, which relies mainly on the high-water content that determines the rubbery and soft properties, as well as on the overall physicochemical resemblance to the native extracellular matrix. The research interest in this regard is focused towards developing hydrogels with tunable properties, desired shapes, suitable porosity, an adequate degradation rate and a controlled release of active principles [61]. Regarding these investigations, Jafari et al. designed montmorillonite-incorporated pH-responsive and magnetic k-carrageenan/chitosan nanocomposite hydrogels for controlled dosing of anticancer drug in the presence of magnetic montmorillonite nanoplatelets. These hydrogels can be considered as a promising drug carrier in the treatment of damaged cancerous tissues [62]. The use of low-cost and eco-friendly adsorbents is one of the best ways for heavy metals removal from wastewaters and surface water. Nanocomposites based on chitosan-g-(acrylamide-co-itaconic acid) and titanium dioxide or silicon dioxide nanoparticles were synthesized by Banivahed et al. to remove Cd^{+2} from aqueous solution. These hydrogel and nanocomposites were synthesized via in situ polymerization within TiO_2 and SiO_2 nanoparticles separately. Also, this material showed antibacterial activity against *E. coli* and *S. aureus* [63]. Ohemeng-Boahen et al. developed chitosan/hematite nano-composite hydrogel capsules via anionic surfactant gelation simultaneously with the immobilization and impregnation of α-Fe_2O_3

nanoparticles within the gel matrix. This nanocomposite hydrogel was highly stable in acidic media and showed tremendously enhanced adsorptive capacity for Congo red in aqueous solution. Compared to other absorbents reported in the literature, the absorptive capacity of this nano-composite hydrogel is among the highest reported. The adsorption process was however found to proceed via both physical and chemical adsorption. These results indicate that, the nano-composite hydrogel provides a viable alternative bio nanocomposite adsorbent with stupendous adsorptive capacity suitable for consideration as an adsorbent for wastewater treatment while curbing the potential toxicity they may be associated with the direct use of nanoparticles in water treatment [64].

With the advances in technology, 3-D extrusion-based printed materials appeared and might offer advantages compared with randomly porous scaffolds. The rheological properties of these materials play a vital role in this field because they must have an adequate viscosity and the printed filaments must withstand the stresses between layer and layer of material to avoid warping of printed objects. Marapureddy et al. studied the rheology of graphene-oxide-filled chitosan hydrogels and improve the strength and print fidelity of chitosan by simply varying the concentration of graphene-oxide without requiring additional polymeric support. The rheological characteristics of these nanocomposite hydrogels made them good candidates for direct-write printing technique. Also, these materials support cell adhesion, proliferation, and differentiation with neurite extensions. The addition of a small amount of graphene-oxide to chitosan hydrogel nanocomposites improved the strength of these materials [65]. Jayaramudu et al. synthesized chitosan stabilized copper nanoparticles via facile chemical reduction process using ascorbic acid as reducing agent. The developed nanoparticles were successfully incorporated into blended chitosan-pluronic F127 polymer solution and made their nanocomposite hydrogels by solution casting technique. The results demonstrated that these chitosan-pluronic F127 nanocomposite hydrogels can be used effectively in antimicrobial applications and wound dressing applications because they inhibited both *E. coli* and *S. aureus* strains [66].

Among the biomedical applications, therapeutic hyperthermia procedure, as an impressive and complementary method has been applied alongside of surgery, radiotherapy, chemotherapy, and immunotherapy in order to threat advanced and recurrent cancers. Some studies have shown that cancer cells are vulnerable to high temperature. In this regard, applying magnetic nanoparticles and their activation by alternating magnetic field have been converted to a new strategy for the therapeutic heating. Eivazzadeh-Keihan et al. fabricated three-dimensional cross-linked chitosan hydrogel by the chemical cross-linked interaction between chitosan polymeric chains and synthetic terephthaloys diisothiocyanate as a cross-linker. These chitosan-based nanocomposite hydrogels were evaluated by magnetic fluid hyperthermia procedure under alternating magnetic field. These materials can influence on heating capacities and could be applied in therapeutic heating as an alternative to cancer treatment [67].

Approximately, one billion people lack drinking water and every year almost five million people die because they are forced to drink polluted and unsafe water. Also, textile industry is the major source of industrial water pollution. Trying to minimize this great impact, Mittal et al. functionalized graphene-oxide nanosheets via grafting with vinyltriethoxysilane to form an adsorbent dye. This material showed an excellent regeneration and reusability efficiency where it was used consecutively for twenty cycles of adsorption–desorption, and it could be used for the remediation of both anionic as well as cationic dyes [68]. Water pollution by industrial compounds is considered as important issue addressing by researchers. As one of main industrial polluted compounds, nitrophenol gained much attention, owning to its toxicity and persistency nature. This is an intermediate in production of important products such as pesticides, pharmaceuticals, and dyestuffs, it has been reported to be carcinogenic, mutagenic, and teratogenic. Due to the above detailed problem, Danish et al. developed a simple synthesis of an efficient material based on CeO_2–Co_2O_3 chitosan nanocomposites hydrogel with high performance for sensitive detection and complete removal of nitrophenol, which will be beneficial for clean water purposes [69].

In the search of new chitosan-based hydrogel nanocomposites, new forms of synthesis have also been sought. Synthesis under microwave conditions require less time, more yield, and require less space as compared to an air condition synthesis. Kaur et al. synthesized chitosan hydrogel nanocomposites by microwave-assisted synthesis, this material showed negligible tendency to degrade cationic dyes from the effluent because of its cationic polyelectrolytic nature. The hydrogel networks were modified to nanocomposite ion exchangers by incorporating zirconium selenerphosphate inorganic ion exchanger and made the polymer network highly selective for cationic dyes. Also, this can act as a wonderful remedy to ameliorate the negative impacts of the environmental issues like heavy metal ion removal and photodegradation of dyes from the contaminated part of the hydrosphere [70].

The preparation of thermosensitive chitosan hydrogels can be achieved using neutralization process without any use of organic solvent crosslinker. The mechanism of gelation is attributed to the formation of physical inter-chains junctions. When chitosan is dissolved in acetic acid aqueous solution occurs the stoichiometric protonation of the $-NH_2$ sites. Then, the mixing of the chitosan solution to a base as $NaHCO_3$, produce CO_2 and increase the pH value. The gel matrix is a result of the proton transfer from the amine moieties of chitosan to the base. Using this method, Santos et al. developed a chitosan-based nanocomposite hydrogel for potential treatment of herpes infection. The combination of nanoparticles and the hydrogel formed stronger and elastic gels. These properties are advantageous to prolong residence time and reduce leakage of the dosage form, when applied on mucosal layers. These materials could be considered a promising thermosensitive system for delivery of flavonoids to improve properties for innovative applications for the thermosensitive hydrogel [71]. Hydrogel systems have been explored as a great variety of applications due to their excellent mechanical performance and facile fabrication processes. These materials can rapidly transmit the applied stress, consume the energy, and delay the crack propagation through the breaking or desorption of polymer chains form the nanoparticles surface, to improve the mechanical performance of conventional hydrogels. Considering the above, Li et al. prepared a robust nanocomposites hydrogel with excellent electrical conductivity reinforced by chitosan grafted with magnetite nanoparticles combined with multiple ionic covalent interactions based on a facile and effective strategy. The obtained hydrogel exhibited balanced mechanical properties and water absorption potential. The strain sensor fabricated with this nanocomposite hydrogel demonstrated successful applications for monitoring various human activities. The resultant nanocomposite hydrogels exhibit sensitive strain-induced resistance change as well as outstanding stability and repeatability under various strains, making them promising candidates for monitoring human activities. As a result, the obtained hydrogel-based strain sensor could accurately and repeatedly detect and distinguish the bending of finger, wrist and elbow, the change of facial expressions and walking frequency. This hybrid nanocomposite hydrogel holds promising potential application in novel electronic devices for complicated human/machine motion detection [72]. The entrapment of silver nanoparticles into hydrogels leads to the obtaining of antimicrobial nanocomposite materials with biomedical applications in wound and burn treatment, or periodontal regeneration. Meanwhile, chitosan provides a synergistic antibacterial effect in combination with silver nanoparticles. In addition, chitosan can reduce Ag^+ ions to Ag^0 and also to stabilize the silver nanoparticles. Popescu et al. obtained composite hydrogels with antibacterial properties containing a well-determined amount of silver nanoparticles, uniformly distributed in the polymeric network, using eco-friendly methods both in the preparation of silver nanoparticles and hydrogels. These materials have low cytotoxicity and its antibacterial activities against *S. aureus and K. pneumoniae* were tested. Due to the antibacterial efficacy of the nanocomposite hydrogel, it is recommended for wound and periodontitis treatment [73].

3 Conclusions

In conclusion, the synthesis, processing, mechanical and biological properties as well as its applications of chitin and chitosan-based nanocomposites have been examined. This examination included a detailed review of recent advances in novel chitin and chitosan-based nanobiocomposites. Specific structures and subsequent properties are controlled by many specific factors such as filler shape, size, surface and chemistry of the nanocomposites, processing conditions and environmental aging. Large improvements in the material performance were found with chitosan-based hydrogels. The different structures, which are obtained, can fulfill the requirements of different applications, such packaging or other agro-industrial, water treatment or electronic applications where long-lasting polymers are not entirely adequate. In most of the cases, these materials find biomedical applications linked to their intrinsic biological properties.

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Composites Based on Chitosan and Inorganic Materials for Biomedical Applications

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Abstract Chitosan, a linear and semicrystalline polysaccharide composed of randomly distributed β, 1–4 linkages of *N*-acetyl-D-glucosamine and D-glucosamine, obtained from partial deacetylation of chitin, is a natural polysaccharide present in shells of marine crustaceans and cell walls of fungi, as well as other invertebrate organisms. This biopolymer is a polyelectrolyte with reactive functional groups, high adsorption capacity, mucoadhesive, biodegradable, bioresorbable, innately biocompatible and non-toxic to living tissues as well as having antibacterial, antifungal, and antitumor activity. These features highlight the suitability and extensive applications of chitosan in the biomedical field, such as implant coatings, wound healing devices, tissue engineering, and drug delivery systems. However, its limited solubility in water and other organic solvents, poor colloidal stability, poor mechanical strength, and high swelling ratio limits its full exploitation for some biomedical purposes. To overcome these limitations, chitosan-inorganic composites have been formulated to enable the adjustment of the desired properties for intended applications. This chapter focused on the fundamentals of chitosan and its composites and how the chitosan-inorganic materials, called "green composites" or "bionanocomposites," can be utilized in biomedical applications.

Keywords Chitosan · Biocomposites · Tissue engineering · Wound healing · Drug delivery

1 Introduction

Chitosan is a natural biopolymer that can be obtained from fungi, yeasts and skeletons of insects, crustaceans and marine animals from the deacetylation of chitin [1]. The molecular structure of chitosan and chitin are similar, being differentiated only by the presence of 2-amino-2-deoxy-D-glucopyranose groups in chitosan [2]. The degree of deacetylation of chitin and the molecular mass of chitosan directly influence

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its physicochemical and biological properties [3]. The properties of chitosan that arouse interest in the use of this biopolymer in biomedical applications are mainly its biocompatibility, biodegradability, bioactivity, nontoxicity, filmogenic capacity, antimicrobial activity, bioadhesiveness and hemostatic power [4].

Due to having these biological properties, chitosan has been used in several devices in the biomedical area [5]. However, some applications are limited because chitosan has low solubility in water, low colloidal stability, low mechanical strength and high expansion rate [6]. To overcome these limitations and achieve the desired properties, the formulation of biocomposites based on chitosan and inorganic material is suggested. The incorporation of inorganic fillers in the chitosan matrix can provide improvement in mechanical properties, adjustments in degradation and swelling rates, improvement in drug release control, bioactivity and binding with hard tissues, osteoconduction and osteoinduction, improvement in guided tissue regeneration, and triggering some unique properties like hemostasis and antibacterial activity, essential for the treatment of wounds [7–12].

The benefits of fillers, combined with the ease of processing of chitosan, make it possible to obtain different structures, such as films and membranes, scaffolds, nanofibers, nanoparticles, spheres and microspheres, and hydrogels, among others [12–19]. A combination of moldability and processability expands the applications of chitosan-based biocomposites in, e.g., controlled drug delivery systems, skin and bone tissue engineering, wound treatment and other applications such as gene therapy [15, 17, 20–25].

This chapter provides an overview on the fundamentals of chitosan-base composites and how chitosan-inorganic materials, called “green composites” or “bio-nanocomposites”, can be used in biomedical applications.

2 Chitosan

Chitosan is a natural polysaccharide, composed of glucosamine and *N*-acetylglucosamine, obtained from the deacetylation of chitin. It is biocompatible, biodegradable, non-toxic, non-immunogenic and non-carcinogenic and presents healing, hemostatic, antimicrobial, anti-inflammatory, antioxidant, mucoadhesive and analgesic properties. In addition, it accelerates rapid dermal regeneration and re-epithelization and can be degraded in vivo by several enzymes, mainly lysozymes, generating non-toxic degradation products like oligosaccharides that stimulate deposition of collagen fibrils in the extracellular matrix components. Moreover, clinical tests had shown that chitosan-based biomaterials do not result in allergic reactions in the human body after implantation, injection, topical application or ingestion [26–31]. All of these properties make chitosan an excellent candidate for biomedical applications.

Although chitosan occurs naturally in some fungi and green algae, it is mainly obtained from the chemical treatment of chitin using alkali [32]. In the chitin deacetylation process with concentrated sodium hydroxide treatment for a few

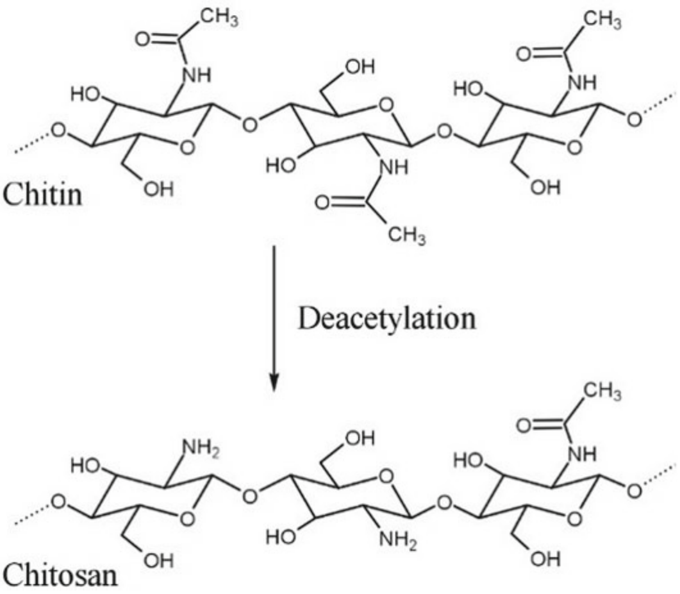

Fig. 1 Molecular structure of chitin and chitosan

hours, acetyl groups are removed from the chitin molecular chain, resulting in chitin deacetylation up to about 85–99% [33]. Chitosan is a polymer constituted by a linear sequence of monomeric sugars β-(1–4) 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine) and, structurally, similar to chitin, differing only by the presence of 2-amino-2-deoxy-D-glucopyranose groups in chitosan [34], as shown in Fig. 1.

Chitosan is a partially crystalline polymer that has two main crystalline polymorphic forms; hydrated crystals (type II crystals) and anhydrous crystals (type I crystals). In both polymorphic forms, the hydrogen bonds are responsible for stabilizing the crystal structure, which is in the form of a double helix with two antiparallel molecules. In the X-ray diffraction analysis, chitosan presents two crystalline peaks $2\theta = 10°$, characteristic of type I crystals, and $2\theta = 20°$, typical of type II crystals [35]. The physicochemical (tensile strength, solubility, surface area, viscosity, conductivity, porosity, and flexibility) and biological (absorption enhancer, biodegradability, antioxidant, bioavailability, and biocompatibility) properties of chitosan depend on the degree of deacetylation (DD), crystallization and molar mass (MM) of the polymer [4, 35].

The degree of deacetylation (DD), molar fraction of repeat units with free amino groups, and the degree of acetylation (AD), percentage of *N*-acetylated repeat units, present a relationship that can be described as $\text{AD} + \text{DD} = 100\%$. Chitosan DD can be determined by several techniques such as: hydrolysis of acetamide groups, titration of free amine groups, dye adsorption, spectroscopy in the infrared region, spectroscopy in the ultraviolet and visible region or magnetic resonance imaging. The clearness, solubility, ability to absorb metals and interchain aggregation are physicochemical properties influenced by the DD of chitosan. The higher the DD, the greater the number of free amine groups in the molecular structure of chitosan after hydrolysis. These free amine groups are responsible for chitosan's affinity for metal cations such as copper, nickel, lead and iron; its solubility in water, and interchain aggregation [35]. The protonation of its amino groups allows chitosan to be soluble in dilute solutions of many organic (acetic, lactic, maleic, tartaric and citric acids) and inorganic ($pH < 6$) acids. The protonated amino groups are also responsible for the biological activity of chitosan, as they interact with the target sites of organisms [36].

Considering the molar mass (MM), chitosan can be divided into low MM, medium MM and high MM chitosan. The methods commonly used to calculate polymer MM are chromatography and viscosimetry. The MM of chitosan also influences many physicochemical and biological properties of the polymer [33, 37]. Chitosan with low MM has a low viscosity, higher solubility and better biological effects, such as biodegradability, biocompatibility and bioactivity. The relationship between the modification of these properties and the variation of the MM of the polymer can be explained by the correlation between the configuration of its chains and MM. When chitosan has low MM, the polymeric chains relax and extend in an almost linear chain exposing a greater amount of amino groups, responsible for most of the interactions performed by the polymer. However, with the increase in MM, the polymeric chains begin to coil around the amino groups and keep them inside the chains, thus reducing the possibility of interactions carried out by these groups. Thus, low MM chitosan behaves more like a polycationic polymer than high MM chitosan [37].

Due to its biological properties, chitosan has been widely used in the development of various types of devices in the medical field such as drug delivery systems, tissue engineering, dressings, among others. However, some medical applications of this polymer are limited by its low ability to solubilize in water, low colloidal stability, low mechanical strength and high expansion rate [5, 38, 39]. One possibility that allows the adjustment of these properties is the formulation of biocomposites based on chitosan and inorganic material.

3 Biocomposites

Two or more components form composite materials to achieve better properties in relation to the constituents. In this sense, inorganic materials are added to chitosan to improve its properties. The chitosan-inorganic composite materials can be processed into different forms, such as sponges, hydrogels, membranes/films, nanofibers and nanocomposites, and have aroused interest for applications in several fields, such as wastewater treatment, agriculture, textile and food industries [16, 40, 41]. Furthermore, these materials are extensively studied in the biomedical field, for tissue engineering [42–44] wound care [23, 45] and controlled drug delivery systems [23, 46–48].

The diversity of structures and applications of these materials is due to the properties of chitosan, especially its polycationic nature, biological properties and multifarious physicochemical properties [14]. On the other hand, the incorporation of inorganic fillers makes it possible to modify different aspects of chitosan, such as mechanical properties, thermal stability, barrier properties, rates and mechanisms of degradation and drug release, providing adaptation to new needs [49]. From this perspective, a class of materials based on chitosan [49] incorporated with an inorganic solid phase, with at least one dimension in the nanometric range (1–100 nm). This nanoscale dispersion of fillers is responsible for modifying and improving chitosan properties [14]. The good interaction at the interface between the nanoparticles incorporated as filler and the matrix occurs through the amine (NH_2) and hydroxyl (OH) groups present in the molecular structure of chitosan, which enable the formation of intra and intermolecular hydrogen bonds, in addition to interactions with the reinforcement [49].

Chitosan nanocomposites with enhanced properties show favorable results, such as acceleration of bone regeneration, targeted drug/cell delivery, improvement of the wound healing process, enhancement of angiogenesis and osteogenesis [23]. Various types of inorganic, metallic or semiconducting materials are dispersed as micro or nano-additives in chitosan, as listed in Table 1.

One of the main classes of inorganic materials used as fillers in chitosan is calcium phosphates, generally intending to promote and/or improve the bioactivity and mechanical properties of the composite [24, 58]. Additionally, calcium phosphates are reabsorbed in vivo after releasing calcium and phosphate ions, which act in the formation and regulation of bone formation through osteoinduction [59]. The combination of polysaccharides with calcium phosphates, especially hydroxyapatite, also triggers osteoconductivity, meaning that bone-like precipitates grow on the surface of the composites [24, 50].

In the literature, studies are toward adding inorganic components to chitosan, either on a molecular scale (hybrid) or as the dispersed phase (composite), to promote some properties in addition to those inherited from the initial constituents. For example, the combination of hydroxyapatite and wollastonite as a dispersed phase improved the thermal stability of chitosan films without significantly affecting the cell viability of the composites [10]. Silk fibroin was also used in conjunction with hydroxyapatite as a reinforcement phase in the chitosan matrix, enabling bioactivity in simulated body fluid (SBF), improving mechanical properties and antibacterial activity [60]. In addition, other biopolymers can be used together with chitosan in the matrix to form a blend, such as an alginate, capable of forming a polyelectrolytic complex with chitosan, allowing the mechanical stability of the blend in the form of a hydrogel. The dispersion of hydroxyapatite in the chitosan/alginate blend for 3D bioprinting promoted post-printing structural stability without compromising the hydrophilicity of the samples [61].

In addition to calcium phosphates, other materials are also used as the dispersed phase in chitosan, such as carbon-based materials (nanotubes and graphene oxide) [44, 48], and clays [13, 62–65]. In this sense, incorporating inorganic fillers into chitosan allows the regulation and adequacy of chemical, physical and biological

Table 1 Chitosan-based biocomposites with inorganic filler for biomedical applications

Applications	Matrix	Dispersed phase	Form	References
Tissue engineering	Chitosan	Graphene oxide, hydroxyapatite and gold nanoparticles	Film	[43]
	Chitosan	Hydroxyapatite and wollastonite	Film/membrane	[10]
	Chitosan	Hydroxyapatite and silk fibroin	Film/membrane	[50]
Drug release system	Chitosan	Montmorillonite—5-fluorouracil	Microsphere	[51]
	Chitosan	Hydroxyapatite—dexamethasone	Sphere	[15]
	Chitosan	Ag-doped 45S5 bioglass	Membrane	[11]
	Chitosan	Zeolitic imidazolate framework-8 (ZIF-8)—vancomycin	Scaffold	[52]
	Chitosan	Montmorillonite—ibuprofen	Film	[53]
Dressings—wound treatment	Chitosan/Gelatin	Tripolyphosphate nanoparticles	Film	[45]
	Chitosan	Aluminum chloride, aluminum sulfate hydrates or iron(III) sulfate	Membrane	[54]
	Chitosan	Montmorillonite	Film	[8]
	Chitosan	Bio-glass	Hydrogel	[55]
Other applications	Chitosan	Materials based on carbon, polymers, metals or metal oxides	Dental implant	[56]
	Chitosan/gelatin	Hydroxyapatite—stem cell	Scaffold	[57]

properties in biomedical applications. In the following topics, the applications of these hybrid materials in targeted drug delivery, tissue engineering, wound care and other biomedical applications are addressed.

4 Drug Release System

Controlled release systems reduce the frequency of drug administration, maintain the therapeutic level of drug, improve administration control and provide the least possible side effects [66]. The mechanisms by which the drug is released from a polymeric delivery system are: (I) swelling, as a result of water absorption by the device, the drug is repelled from the matrix; (II) diffusion, the drug migrates from the site with the highest concentration to the one with the lowest concentration; and (III) degradation or erosion, the matrix can undergo degradation or erosion and release the drug [51, 67].

Chitosan has been widely used as a matrix in producing isolated controlled release systems [53, 68–73] and biocomposites [20, 52, 62, 64, 74, 75] for various biomedical applications. In order to develop a drug delivery system for the treatment of cancer, chitosan loaded with 1,4-naphthoquinone and ionically cross-linked with different pentasodium tripolyphosphate could inhibit the proliferation of cancer cells, melanoma, in vitro [72]. Another drug that has been added to chitosan for the treatment of cancerous lesions is 5-Fluorouracil (a drug commonly used in the treatment of breast, pancreas, brain, liver, colorectal and gastric cancer), which enabled the formation of chitosan/5-Fluorouracil beads for oral administration of the drug with sustained release for 48 h in an in vitro assay ($pH = 1.2$) [69]. The chitosan/5-Fluorouracil emulsion for transdermal administration also showed controlled release of the drug for 1500 min [73]. Chitosan-based composites: (I) with montmorillonite for the formation of microspheres loaded with 5-Fluorouracil, was proved to be suitable for drug release in the gastrointestinal environment with sustained release controlled by diffusion and swelling mechanisms, the drug was released for 1440 min [64]; (II) with polyvinylpyrrolidone (PVP), chitosan was used as a filler to obtain chitosan/PVP/5-Fluorouracil nanofibers which, at a concentration of 10 mg/mL, were effective in killing epithelial adenocarcinoma cells human lung (A549) [76]; (III) and with polyacrylic acid (PAA) and Fe_2O_3, a magnetic nanomaterial, for the development of a magnetic nanohydrogel that was able to release a higher amount of 5-Fluorouracil under tumor tissue conditions (pH 4.5) than in physiological environments (pH 7.4) [74].

Aiming to treat bacterial infections, chitosan, due to its antimicrobial potential, was used as a matrix or a filler for the formation of composite devices with antimicrobial activity. Clove and tea tree essential oils were incorporated into chitosan to form films and emulsions that demonstrated antimicrobial activity against gram-positive, gram-negative and yeast strains [53]. A high-density polyethylene (HDPE) composite that hosted chitosan filler demonstrated that the incorporation of chitosan produced a bacteriostatic effect against *Escherichia coli* [52]. Biocomposites made of chitosan/45S5-silver (Ag-doped Bioglass) showed antibacterial activity against gram-positive and gram-negative bacteria and were non-cytotoxic and degrading at physiological pH in 28 days [62]. pH-sensitive scaffolds were obtained by 3D Printing from chitosan loaded with crystals of zeolitic imidazolate frameworks (ZIF-8) impregnated with vancomycin for the treatment of osteomyelitis; the encapsulation efficiency of vancomycin by the monocrystals of ZIF-8 was 99.3%, approximately 77% of the vancomycin present in the crystals of ZIF-8 incorporated in the chitosan scaffolds was released at pH 5.4 in 48 h and showed antimicrobial activity against strains of *Staphylococcus aureus* in vitro. Other scaffolds prepared by lyophilization for the treatment of osteomyelitis were chitosan-based calcium phosphate composites loaded with moxifloxacin hydrochloride; the device almost completely released the drug in three days, promoted proliferation and differentiation of osteoblasts, reduced bacterial infection, inflammation and intramedullary fibrosis in an animal model in which osteomyelitis was induced [77].

Controlled delivery systems of several anti-inflammatory drugs were produced based on chitosan and its biocomposites aiming for several medical applications. Absorbable chitosan suture threads loaded with *N*-acetyl-D-glucosamine (GlcNAc) had the properties required by the American Pharmacopoeia (USP), and showed a sustained release of GlcNAc for 28 days [68]. In other analyses, chitosan-na/GlcNAc suture threads demonstrated controlled release of GlcNAc for 49 days, the process controlled by diffusion (up to 21 days) and erosion (from 21 to 49 days). They showed that the fibers were not toxic to cells of the L929 lineage. Chitosan/montmorillonite nanocomposite films were produced to promote the controlled release of Ibuprofen; the films containing 10% (m/m) of montmorillonite showed greater loading capacity and encapsulation efficiency than the other films (20 and 50% of montmorillonite), in addition to promoting a sustained release for 100 h [53]. Chitosan/hydroxyapatite/dexamethasone granules cross-linked with sodium tripolyphosphate demonstrated sustained release of dexamethasone for 24 h and the release mechanism was diffusion and relaxation of polymeric chains [15].

Controlled release systems of chitosan and its biocomposites in the form of nanoparticles have been introduced into neural guidance channels, by chemical and physical immobilization or loaded into hydrogels and injected into the lumen of these channels, for the treatment of nervous system injuries. In general, chitosan nanoparticles preserved drug bioactivity and sustained release profile [65]. Due to its biodegradability and biocompatibility properties, chitosan has been used in the production of composites for the formation of microneedles (adhesive with hundreds of micron-sized needles that pierce the stratum corneum) for drug release, e.g., a transdermal patch of peptides and proteins (hydrophobic macromolecules). Chitosan was combined with natural or non-natural polymers and inorganic compounds to improve its properties in the formation of microneedles where hydrophobic macromolecules were incorporated, thus helping the transposition of the stratum corneum and promoting the release and absorption of macromolecules through the skin [76].

5 Tissue Engineering

Tissue engineering seeks the regeneration of living organs and tissues through the culture of the patient's cells on biological or synthetic supports, called scaffolds (supports, three-dimensional matrices, chests, etc.). The starting point for the repair of an organ or tissue is the selection of support for cell growth, which must take into account the type, location and extent of the lesion [77]. Scaffold function provides support for cell growth, serving as an extracellular matrix (ECM) for cell growth, assisting in the fixation, proliferation and differentiation of seeded cells while acting as a temporary mechanical and structural substitute for the original tissue until the formation of the new tissue and complete scaffold bioabsorption [78].

Different natural and synthetic materials were used as cell support materials. However, current choices alone present limitations regarding the degradation rate and mechanical properties [15]. To be effective in their application, tissue engineering supports must have fundamental properties: biocompatibility with tissues; biodegradation rate compatible with the rate of formation of new tissues; non-toxicity and non-immunogenicity; adequate mechanical strength; porosity and morphology that allow the transport of cells, gases, metabolites, nutrients and signaling molecules both within and across the support and host environment [15, 79].

Different biocomposites based on biopolymers and inorganic fillers have been developed to meet tissue engineering requirements and overcome challenges [80, 81]. Among biopolymers, chitosan has stood out as a promising bioactive material for engineering various tissues, such as bone, skin, cartilage, intervertebral discs, blood vessels, etc. [15]. Due to the different structures in which chitosan can be processed and the similarity with glycosaminoglycans in the ECM, it is particularly important in regenerating epithelial and cartilaginous tissues [82]. It shows the ability to contribute to or stimulate the synthesis of the ECM components [68, 83].

Different chitosan-based structures have been developed for skin tissue engineering [78] such as titanium dioxide loaded membranes, promoting mechanical stability and antibacterial activity [9] hydrogels with high water absorption capacity [84], sponges with interconnected three-dimensional porous structure [85], and fibrous materials to support cell growth and proliferation [86]. Each structure presents a favorable set of properties and, on the other hand, some limitations according to the application of the tissue to be repaired [80].

In bone tissue engineering, the main requirement of scaffolds corresponds to the properties of the tissue they will replace, in addition to biocompatibility, osteoconductivity and osteoinductivity [87]. And in some places, the properties of chitosan scaffolds are inferior to those of natural bone; thus, they are unable to meet the load-bearing requirement of bone implants. In addition, chitosan alone is not osteoconductive, making it impossible to mimic the properties of the host bone. An alternative to improve mechanical strength and structural integrity is the incorporation of hydroxyapatite and other bioceramics at the nanometer scale, consisting of a material that resembles natural human bone [15, 87]. Hydroxyapatite can improve the mechanical properties and osteoconductivity of implants and be one of the most stable forms of calcium phosphate and naturally occurring as a component of the inorganic phase of bone [88]. In this way, chitosan and hydroxyapatite composites can mimic the organic and inorganic phases of natural bone tissue [89]. The strontium-doped hydroxyapatite (SrHA) is commonly recommended for bone regeneration due to its good osteoconductivity and high alkaline phosphatase (ALP) activity. However, it lacks mechanical strength and reliability. Wu et al. [90] used a facile hydrothermal method to synthesize graphene oxide (GO)-reinforced SrHA nanoparticles. Then, a scaffold was developed using a leophilization method by incorporating SrHA/GO nanoparticles into chitosan (CS) and quaternized chitosan (QCS) mixed solutions. The compressive modulus of the CS/QCS/SrHA/GO scaffold reached 438.5 kPa, fourfold higher than that of the CS/QCS scaffold. The composite showed significantly higher in vitro mineralization levels and ALP activity. In vivo rat skull repair indicated that the CS/QCS/SrHA/GO scaffold can significantly promote bone regeneration. The bone density of the CS/QCS/SrHA/GO scaffold after 4 weeks of implantation reaches 0.34 mg/cm^3, significantly higher than that in the blank group (0.12 mg cm^{-3}). After 12 weeks, all scaffolds were observed to promote bone regeneration. The CS/QCS/SrHA/GO scaffolds showed the greatest bone mass and bone density of 0.83 mg cm^{-3}, higher than that of the CS/QCS/SrHA scaffold (0.68 mg/cm^3).

Another alternative for hard tissue reconstruction is chitosan and bioactive glass composites. These materials also provide organic and inorganic compositions/structures similar to native tissue. However, due to limited mechanical strength, they are used in applications where mechanical forces are minimal or non-existent, such as cancellous bone tissue [91]. The main advantage of bioglasses compared to other bioceramics is adjusting their degradation rate to match the formation of new tissue through small changes in chemical composition and doping them with various uniques therapeutic ions [92].

Scaffolds for tissue engineering and regenerative medicine have been obtained by different techniques, including solvent evaporation, lyophilization and electrospinning [21, 93]. However, these techniques are not very precise and have limitations in terms of architectural design. In this sense, additive manufacturing (3D printing) stands out for being promising because it allows the manufacture of scaffolds with detailed and adjustable geometry to serve as a substrate for guided tissue regeneration, enabling the manufacture of complex parts and better reproducibility, in addition to the possibility of incorporating different inorganic fillers [23, 94].

Tissue engineering plays a fundamental role in the effective repair and healing of wounds resulting from physical trauma or burns by enabling the development of grafts and bioactive dressings [95, 96] This topic is discussed in the next section.

6 Dressings—Wound Treatment

A wound is characterized when there is an interruption in the continuity of the epithelial lining of the skin or mucous membranes, resulting from physical, chemical or thermal damage. According to the time and nature of the healing process, wounds are classified as acute or chronic [97]. Acute wounds are injuries that occur suddenly, due to accidents or surgical injuries, with a predictable healing period, between 8 and 12 weeks, depending on the size, depth and extent of damage to the dermis and epidermis. While chronic wounds, on the other hand, do not progress through the normal stages of healing and cannot be repaired in an orderly and timely manner, and often originate from ulcers or burns [98, 99].

Wound healing involves a complex process of tissue regeneration and growth that occurs in four distinct phases: (I) the coagulation and hemostasis phase, which occurs immediately after injury; (II) the inflammatory phase, right after tissue injury, when local swelling occurs; (III) the period of proliferation, where new tissues and blood vessels are formed and (IV) the maturation phase, in which the remodeling of new tissues takes place [100]. These steps occur in an orderly fashion, overlapping each other, in a well-connected cascade. How these phases take place largely depends on the type of wound and its associated pathological conditions and the type of dressing material [101, 102].

With the advancement of technology, different types of dressing materials are available for all types of wounds, and the selection of this biomaterial is an important step in achieving faster healing. According to Dhivya et al. [100], the choice of the ideal dressing for each type of wound must be performed according to its ability to: (a) provide or maintain a moist environment; (b) improve epidermal migration; (c) promote angiogenesis and connective tissue synthesis; (d) allow the exchange of gases between the injured tissue and the environment; (e) maintaining the appropriate tissue temperature to improve wound bed blood flow and epidermal migration; (f) protect against bacterial infection; (g) does not adhere to the wound and is easy to remove after healing; (h) provide debridement action to enhance leukocyte migration and support enzyme accumulation; (i) be sterilizable, non-toxic and non-allergenic. Thus, the materials commonly used in dressings are textile materials, films, sponges, fibers or hydrogels of natural or synthetic polymers and their combinations. It requires a thorough understanding of wound types and healing mechanisms and knowledge of the interactions that are available between the dressing and the wound. Wound dressings can be passive, bioactive or interactive. Where passives simply provide coverage, the bioactive or interactive dressings can modify the physiology of the wound environment [103, 104].

Traditional dressings such as sterile gauze, nonfat medical cotton, etc., can only serve as a physical barrier as they can effectively promote healing, as well as being easier to adhere to the wound, causing secondary damage when changing the dressing [105]. In this sense, polymers of natural origin, such as chitosan, are generally chosen for wound management over synthetic polymers, as they present biocompatibility, ability to stimulate the wound environment and similarity with the chemistry of ECM. In addition, chitosan promotes antimicrobial activity and healing stimulation, fluid absorption, preventing the accumulation of exudates, also serving as an autograft bed in wound treatment and helping gas exchange, all essential to the healing process [106–109]. Chitosan is also known for its hemostatic properties and fungistatic behavior, which are very useful properties for wound care. In addition to their positively charged portions, they act to block nerve endings, promoting an analgesic effect. During its biodegradation, chitosan releases *N*-acetyl-β-D-Glucosamine and D-Glucosamine, which stimulate the proliferation of fibroblasts, assist in the deposition of collagen and increase the natural synthesis of hyaluronic acid at the site of the wound. All of these processes help to improve wound healing and decrease scar formation [54, 110, 111]. Different materials for the treatment of wounds based on chitosan have been reported, either with the polymer alone or associated with other substances.

Silva et al. [86] developed woven meshes using chitosan filaments coated with the same biopolymer. The materials developed showed good mechanical properties in dry and wet conditions, high swelling capacity and adjustable degradation rate according to the configuration of the filaments. The authors suggested, among others, the application as dressings for wound healing. Nanofibers [112], nanoparticles [19], hydrogels [113], films [24, 35] and chitosan scaffolds [22] are other forms demonstrated with high potential as dressings.

Bioactive antimicrobial dressings play an important role in the management of wound infections, as the injured tissue often exudes a large amount of fluid, which together with body temperature and the presence of nutritional components, serves as the ideal site for bacterial growth. The most effective way to control the spread of bacteria from the wound is to incorporate antimicrobial agents into the chitosan matrix. In this sense, metallic oxide nanoparticles, including zinc oxide (ZnO) and titanium dioxide (TiO_2), have been applied as antibacterial materials [25]. Behera et al. [9] developed chitosan/TiO_2 membranes with antibacterial activity and improved cell proliferation as a potential healing material. Silver nanoparticles are widely used in wound care because of their broad antibacterial spectrum and lower drug resistance [114].

Inorganic materials can be incorporated into bioactive dressings to regulate drug release and facilitate wound healing. For example, Ambrogi et al. [8] developed composite chitosan/montmorillonite films loaded with chlorhexidine (CLX), an antimicrobial agent that may be cytotoxic to human fibroblasts. The clay was able to provide a prolonged release of CLX, and the composites did not show a cytotoxic result. Sundaram et al. [55] developed an injectable chitosan/nano-bio-glass composite. Sol–gel method was employed to synthesize nano bioactive glass (SiO_2–CaO–P_2O_5) particles with average particle size around 14 ± 3 nm. 2%Chitosam-5%bio-glass hydrogel was then prepared and studied. The material provided rapid blood clotting, with great potential for effective control of bleeding during critical situations [55]. In vivo blood clotting property of the hydrogel was evaluated on the liver in a rat model. Without the application of composite bleeding from liver injury took 132 ± 5 s to achieve stable blood clot formation and the mass of blood loss because of the injury was 880 ± 17 mg. Chitosan alone could stabilize blood clot formation within 75 ± 3 s and the mass of blood loss was reduced to 543 ± 10 mg. However, through the application of chitosan-glass composite blood clot formation was achieved within 54 ± 3 s and the mass of blood loss was further decreased to 350 ± 8 mg [55].

7 Other Applications

Among the biomedical applications, chitosan-based composites have been used in several areas such as genetic engineering, stem cell treatments, dentistry and biosensor manufacturing.

In genetic engineering, the aim is the targeted delivery of a small, double-stranded interfering RNA (siRNA) of about 20–30 nucleotides that mediates the specific enzymatic cleavage of the target messenger RNA (mRNA) sequence through complementary base pairing and silencing the gene. In this case, each siRNA is designed for a specific protein [115]. Chitosan-based nanocarriers are used to transport siRNA into cancer cells in order to (I) inhibit cell proliferation, (II) prevent metastasis and (III) silence genes that promote resistance to drugs used in the treatment of cancer [116]. An example is the research aiming at the treatment of osteosarcoma using nanocomposites of oxidized chitosan, folic acid and propargyl focal point poly(L-lysine) dendrons (PLLD) incorporated with astrocyte elevated gene-1 siRNA (siAEG-1); graphene oxide chitosan nanoparticles incorporated with siRNA; and mineral-coated chitosan and alginate capsules incorporated with gfp, LacZ and Sox-9-bearing plasmid genes, efficiently transported the genes into osteosarcoma cells promoting inhibition of tumor cell proliferation [117].

In addition to gene transport aimed at cancer treatment, chitosan nanocomposites are being used to promote stem cell differentiation aimed at treating type I diabetes mellitus. Chitosan is able to activate and deliver stem cells; in addition to differentiating them into cells similar to β cells of the islets of langerhans, making treatment with stem cells safer for type I diabetes mellitus [57].

Chitosan composites with materials based on carbon, polymers, metals or metallic oxides are being developed for application in dental implants and dental engineering because they present biodegradability, biocompatibility, antibacterial activity and minimal foreign body reactions, in addition to the possibility to control the molding and porosity of the composite formed, favoring osteoconduction and cell growth [56].

Chitosan has been used to reduce gold III and produce biocompatible nanoparticles of the chitosan/AuIII composite (NpAu) for use in innovative biomedical applications: (I) biosensors, normally detect clinical parameters or monitor concentrations of biological molecules in tissues and organisms, the NpAu commonly detects biological molecules in liquids; and (II) cancer treatment, due to the reduced size (10–100 nm) NpAu diffuses through the extracellular matrix reaching cancer cells, where they act as photothermic agents converting infrared light into heat and inducing death of cancer cells [118]. Furthermore, the nanocomposite internalizes in the microbial cells, disrupting biofilms. The chitosan-capped NPsAu@2-mercapto-1-methyl imidazole composite links with gelatin and tannic acid support durable hydrogel membranes. A study in vivo reveals that the material has excellent antibacterial activities against *S. aureus*. The composite membranes have been tested as wound dressings and scaffold devices because they have antimicrobial activities and anti-biofilm formation.

8 Conclusion

Chitosan is a biopolymer with unique properties for biomedical applications; on the other hand, its high hydrophilicity and low mechanical properties limit its applications. Composites/hybrids of chitosan and inorganic materials solve most of these problems, giving the biocomposite characteristics that combine the properties of its organic and inorganic components and improve interactions with living tissues, not altering the biocompatibility and biodegradability characteristics of chitosan. In this sense, biocomposites formed by chitosan and inorganic fillers become important and promising alternatives, with increasing attention to developing controlled release systems, tissue engineering, and wound repair materials. However, much remains to be researched in this exciting field. Most studies are at an early stage of research and others are in the early phase of clinical trials (phase I/II), designed primarily to determine the safety and not efficacy.

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Biodegradable Acrylic Polymers and Nanocomposites

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Abstract This chapter describes a recent literature analysis on the synthesis, applications, and biodegradation of green-based grafted acrylic copolymers and nanocomposites. It presents an updated review of the different polymers, such as natural gums, starch, cellulose, polylactic acid, chitosan, and alginate, modified through this methodology. In all cases reviewed, these materials are copolymers of polyacrylic acid (PAA), polyacrylamide (PAM), or sometimes both simultaneously, grafted to other natural polymers with a polysaccharide chain. Copolymerization is normally carried out in aqueous solutions of the biodegradable polymer adding acrylic acid (AA) or acrylamide (AM) and a radical initiator to start the in-situ polymerization process. In some cases, depending on the final application, small concentrations of methylene bisacrylamide (MAB) is added to the polymerization solution as a crosslinking agent. The biodegradation analysis of the prepared copolymers is conducted under three conditions and tests: the first one is by composting; the second one is the soil burial method; and the third one, normally used in copolymers used for controlled release agents of drugs, is carried out through in-vitro tests using standardized buffer solutions or pancreatic solutions in pH ranges, simulating the conditions of stomach gastric juices.

Keywords Biodegradable · Acrylic polymers · Ecofriendly · Green-based polymers

1 Introduction

Due to the physicochemical characteristics of acrylic polymers and their versatility in different applications, the annual production reported in 2021 was more than 9 million tons, and it is estimated to continue growing. However, it is well-known

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that these polymers are not easy to degrade, so they are normally known as non-biodegradable materials. They become a concerning problem due to the crescent degree of plastic pollution that our planet suffers. In that sense, Gaytan et al. recently presented an exhaustive review of the possible routes of biodegradation of acrylic polymers, describing the microorganisms capable of biodegrading them through their enzymatic systems, as well as the metabolic process that implies [1].

Regarding the biodegradation of PAM, Joshi et al. presented a detailed review and proposed a very explicit metabolic route for the enzymatic degradation process of PAM and hydroxylated polyacrylamide (HPAM). However, their conclusions still leave several doubts about the knowledge of the route involved in the enzymatic biodegradation process and the metabolic mechanism in aerobic and anaerobic conditions [2].

In a more recent review done by Nyyssölä and Ahlgren [3], they present, similarly to Joshi et al. [2], a historical review since 1994. Different authors present the microbial species that degrade polyacrylates and PAMs under aerobic conditions. It is mentioned that the degradation of PAM is catalyzed by amidases and leads to the generation of ammonia, which is a food source for bacterial growth. Although they do not clearly describe how the enzymatic degradation occurs, they suggest several hypotheses. Moreover, they affirm that the degradation in the presence of white-rot fungi begins with an indirect radical mechanism catalyzed by oxidative enzymes.

This review presents an analysis of the most recent developments in the synthesis and applications of the so-called biodegradable acrylic polymers, presented as an eco-friendly solution to the plastic pollution in our planet. Nevertheless, these polymers are in fact, graft copolymers or mixtures with truly biodegradable polymers, with a main chain of polysaccharides combined with non-biodegradable acrylic polymers, which can be presented in the form of physical mixtures or core–shell type structures. Hence, the biodegradable material tends to disappear due to hydrolysis, favored by the presence of enzymes released by microorganisms. The last situation also occurs in graft copolymers, where the truly biodegradable part of the polymer normally has a chain that is sensitive to hydrolytic cleavage by enzymatic effect.

Although some studies present experimental evidence of biodegradation of the graft copolymers or their mixtures, there is no evidence that the acrylic part is digested or depolymerized by the enzymatic reaction. Only exceptional cases such as the studies carried out by Sarmah and Karak [4], in which only total weight loss results are shown in the presence of different microorganisms, even in gastric juice-type digestion systems, or the results reported by Jing et al. [5].

In addition, nanoparticles used to prepare nanocomposites based on biodegradable acrylic matrices are an issue to consider. From this point of view, it should be considered that many studies based on the development of biodegradable nanocomposites, depending on the application to which these investigations are heading, use non-biodegradable nanoparticles. Nevertheless, in the case of nanocomposites, the level of environmental pollution caused by the synthetic nanoparticles during their preparation compared to conventional composites is much lower (in many cases reaches over 60 wt%, against 3–5 wt% in the case of nanocomposites). Therefore,

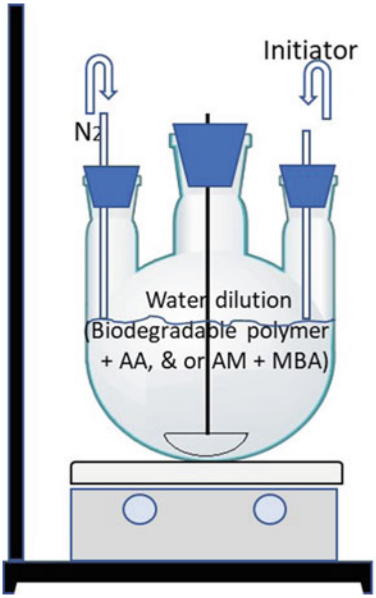

Fig. 1 Schematic representation of biodegradable polymers graft modified with acrylic monomers

in the case of nanocomposites, the biodegradable fraction is exclusively from the biodegradable matrix used.

Under the considerations exposed so far, this book chapter deals with the synthesis, characterization, applications, and biodegradability of acrylic copolymers and nanocomposites derived from six of the most known biodegradable materials grafted with acrylic polymers of the PAA and PAM types (a schematic representation of this process is shown in Fig. 1). Firstly, copolymers based on natural gums of different origins are presented. Next, starch and its copolymers are analyzed, followed by the most relevant studies on the copolymerization of cellulose, chitosan, polylactic acid (PLLA) and alginate with acrylic monomers. Some of the systems described are crosslinked to obtain hydrogels with high moisture absorption capacity or for encapsulation of nanoparticles with specific applications.

2 Natural Gums with Acrylic Polymers and Nanocomposites

As mentioned above, one of the varieties of biodegradable acrylic copolymers is in the form of graft copolymers. This section will describe natural gums and extracts to which sections of acrylic monomer have been grafted. One example is the case of the work performed by Kumar et al. [6]. They modified the gum tragacanth to develop biodegradable hydrogels and improve the water retention capacity of the soil and the controlled release of agrochemicals. In this case, a grafting reaction of AM is carried out on the chemical structure of gum tragacanth. Subsequently, crosslinked gels are prepared using glutaraldehyde. The crosslinked polymer reacts with a solution of methacrylic acid, which is reacted by adding an acid initiation system. Glutaraldehyde is then added to generate a second network, resulting in the formation of interpenetrated networks. These interpenetrated networks are used as matrices for the controlled release of urea and calcium nitrate. A schematic representation of this type of materials is shown in Fig. 2.

The biodegradability of these interpenetrated networks derived from this natural rubber is carried out under composting and burial in garden soil. Under composting, a complete biodegradation is observed at 77 days, while under the second biodegradation process, only 81.26% of degradation occurs in the same period.

Recently, Choudhary et al. [7] presented a study using gellan gum, a polysaccharide obtained from the bacterial fermentation of starch grafted by in-situ free radical polymerization with PAA. The graft copolymer is crosslinked with N,N'-methylenebis(acrylamide) to develop moisture-retaining gels for agricultural applications. The measurements of moisture retention as a function of water salinity show that the salt content is crucial since it significantly reduces the degree of swelling. The biodegradation of the gels obtained was carried out through composting and burial in soil, finding that after 22 days, the degradation percentage was 89.95 and 86.71%, respectively.

Mittal et al. [8] obtained an in-situ graft copolymer by preparing an aqueous solution of gum ghatti (also called Indian gum, a natural polysaccharide with a complex structure widely used in foods and pharmacy) in the presence of AM monomer by microwave-assisted technique, obtaining a gum ghatti-cross-linked-polyacrylamide (Gg-g-PAM) hydrogel. This graft copolymer was used as a flocculant and adsorbent for dyes such as brilliant green (BG), rhodamine B (RhB), congo red (CR), and methyl orange (MO) to evaluate the effectiveness of this material in water treatment of polluting effluents. The effectiveness of this material was demonstrated. However, it presented a selectivity dependence on pH and the type of dye used. This copolymer was also evaluated as a superabsorbent hydrogel, obtaining a swelling degree up to 2117% at neutral pH and 50 °C. Biodegradability tests of Gg-g-PAM were carried out under composting conditions and determined through spectroscopic techniques. It was demonstrated a degradation degree up to 93% in 60 days.

One of the most widely used natural polymers in the food and pharmaceutical industry is xanthan gum, a high-molecular weight polysaccharide normally obtained by fermentation of corn carbohydrates. Kaith et al. [9] studied the free radical copolymerization of xanthan gum with AA and potassium persulfate (KPS) as initiator by microwave method. Like previous cases, glutaraldehyde was also used as a crosslinking agent to obtain a three-dimensional network. This copolymer was used for the controlled release of fertilizers in the agrochemical industry, obtaining high efficiency in the dosage of urea in *Phaseolus vulgaris* plants crops. Furthermore, an efficient bactericidal capacity was demonstrated when this copolymer is formulated with amoxicillin against *Bacillus subtilis* and *Salmonella enteritis* using Agar-Well Diffusion Method, which makes this graft copolymer a potential material for agrochemical applications. Biodegradability tests were carried out using the soil burial

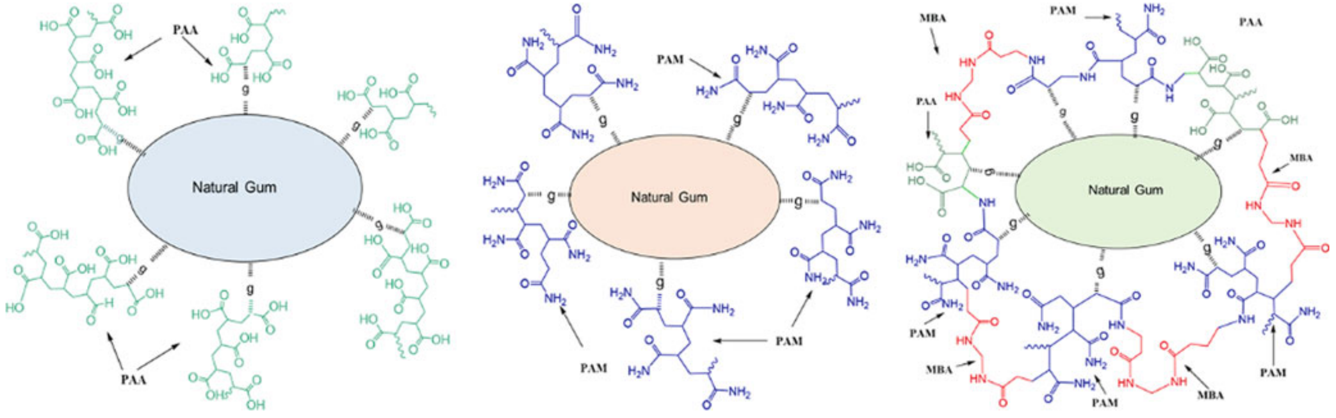

Fig. 2 Schematic representation of natural gums graft-modified with acrylic polymers. Modification with AA (left), with AM (center), and with AA and AM cross-linked with MBA (right)

method, and degradation of 78% was determined after 70 days of testing, which was confirmed using microscopic, spectroscopic, and gravimetric techniques.

Singh et al. [10] studied the modification of AA-modified xanthan gum in the presence of MBA as a crosslinking agent. In this case, an aqueous solution of the different reagents used was prepared. Ammonium persulfate (APS) was added as a polymerization initiation agent, and the solution was placed in a microwave oven. Once the reaction is finished, the polymer was washed, dried, and characterized by different instrumental techniques. A nanocomposite was prepared with the crosslinked copolymer and amoxicillin, a well-known antibiotic. Controlled release tests of this drug were carried out under different pH conditions at 37 °C. It was found that release of amoxicillin is favored in a basic medium rather than in acid medium. Biodegradation tests were carried out through the soil burial method, measured by weight loss, and confirmed with spectroscopic methods. The weight loss of the nanocomposite was 75% in 75 days, while the biodegradation of the crosslinked copolymer was 84% in the same period.

Another natural gum modified with acrylic polymers is myrrh gum (guggul), made by Kaith et al. [11]. In this study, two materials were prepared: On one side, aqueous gum solutions and monomers of AA and AM; on the other side, aqueous gum solutions, monomers, and MBA as crosslinking agent. Once the mixtures were homogenized, they were polymerized with APS as initiator to obtain two types of copolymers, one crosslinked as an interpenetrated network and the other without crosslinking. The polymers were washed, dried, and finally characterized. The water absorption capacity of these materials was measured, finding that the percentage of swelling of the non-cross-linked material was fivefold greater than the crosslinked material. These copolymers were combined with bismuth ferrite nanoparticles and were evaluated as absorbers of inks used in the textile and paper industry (Coomassie brilliant blue G-250 and fuchsin basic). Both polymer nanocomposites, with or without crosslinking, demonstrated high efficiency in the adsorption of selected inks. Biodegradation tests were carried out by composting and the soil burial method. It was found that the weight loss in composting was greater than underground. Moreover, the non-cross-linked showed degradation in a higher percentage than the crosslinked material, reaching a mass loss of up to 90.64% and 82.38%, respectively, after 70 days of testing.

Gou et al. [12] modified Konjac glucomannan, a natural polysaccharide composed of β-D-glucose and β-D-mannose. In this study, an aqueous solution composed of this gum, the monomers AA, AM, and allyl polyoxyethylene ether was prepared. The solution was vigorously stirred, and ceric ammonium nitrate was added to initiate the polymerization reaction under an inert atmosphere. At the end of the reaction, the polymer was purified, dried, and characterized using different instrumental techniques. Biodegradation tests were carried out using the OECD 301 methodology, which allows the evaluation of the biodegradability of chemical substances under an aerobic aqueous medium. It was found that the copolymer obtained is susceptible to biodegradation by increasing the biochemical oxygen demand.

3 Grafted Starch with Acrylic Polymers and Nanocomposites

Starch is another recurrent biodegradable natural polymer modified by acrylic monomers by in-situ polymerization. Analog to natural gums, the modification is done by grafting different acrylic monomers in aqueous starch solutions depending on the type of material and properties required, as shown in Fig. 3.

Ganguly et al. [13] synthesized an AA/AM grafted starch by free radical polymerization under an inert atmosphere using KPS. Sodium metabisulphite was employed as redox initiator. Ethylene glycol dimethacrylate (EGDMA) is added to obtain a crosslinked polymer. In this case, different concentrations of AA/AM and EGDMA were studied, obtaining a grafted polymer of AA and 2-acrylamido-2-methylpropane sulfonic acid (AMPS) on the starch macromolecule. The material obtained was characterized by spectroscopic techniques, scanning electron microscope (SEM), and thermogravimetric analysis (TGA), confirming that the starch modification increases the thermal stability of this material. A composite of this polymer was prepared with memantine, a specific drug for the treatment of Alzheimer's. Controlled release tests were carried out at different temperatures, confirming the promising feasibility of this material as a vehicle for this drug. Biodegradation of this material was studied in hen egg lysozyme medium. It was shown that degradation occurs enzymatically on the starch bonds, producing a mass loss of up to 60% during the first 7 days of testing.

Similar to natural gums, grafted starch has also been used for the recovery of dyes in aqueous effluents. In a recent study presented by Junlapong et al. [14], gelatinized cassava starch is modified by grafting with AM via radical polymerization to obtain a superabsorbent polymer capable of absorbing more than 8000% of humidity. This polymer was evaluated as an absorbent of methylene blue (MB), with a removal effectiveness of 2000 mg/g of grafted polymer, confirming this material as an excellent candidate for wastewater treatment. Biodegradation studies were carried out in soil burial tests. Although they do not present numerical results, the authors affirm that modified starch has a biodegradability of up to 80% in 30 days.

Sarmah and Karak [4] reported an exhaustive study on synthesizing a superabsorbent polymeric hydrogel based on starch modified with AA, with a water absorption capacity up to 700-fold its mass weight. As in previous studies, a slightly alkaline starch solution, a solution of AA, and MBA as crosslinking agent were added under constant stirring and inert atmosphere. Then, once the mixture was well homogenized and transparent, an APS solution was added as an initiator of the polymerization process. The copolymer prepared was used for the encapsulation of fertilizing urea. The same methodology for the grafted and crosslinked copolymer was followed but with a certain concentration of urea added to the solution. Controlled release tests of this fertilizer were carried out on chickpea crops, observing good germination and growth results for these vegetable plants compared to crops without using these superabsorbent hydrogels.

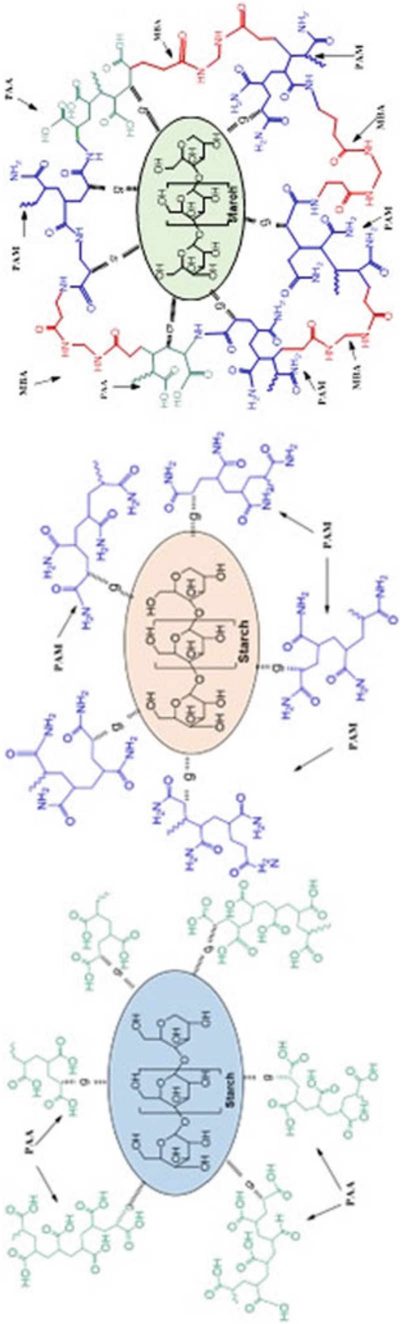

Fig. 3 Schematic representation of graft-modified starch with acrylic polymers. Graft modification with AA (left), with AM (center), and with AA and AM, cross-linked with MBA (right)

Biodegradation tests of the crosslinked graft copolymer were carried out at the laboratory level in the presence of two specific bacteria, *Pseudomonas aeruginosa* (gram negative) and *Bacillus subtilis* (gram positive). Degradation was evaluated using the McFarland turbidity method, observing an increase in turbidity as a function of time due to the decomposition of the polymeric samples by the presence of these bacteria. The results showed that the prepared medium favors bacterial growth and biodegradation of the synthesized copolymer. The authors carried out rigorous biodegradation tests under the soil burial method of their materials. They found that the biodegradation process occurs in three stages. The first stage occurs during the first three months, where 40 wt% of the copolymer degrades due to the decomposition of the biobased part of the synthesized material. In the second stage, the superabsorbent effect of the remaining material does not allow air to enter. As a result, the microbiological attack by aerobic batteries does not occur. Finally, during the third stage, the hydrogel breaks and becomes susceptible to microbial attack. It is interesting to analyze the results obtained in this study, where it is clearly observed how the bacterial growth at laboratory level is directly proportional to the weight loss of the samples exposed to biodegradation underground.

Another study was carried out by Kolya et al. [15]. They present the modification of starch through the use of AM by radical polymerization using KPS as an initiator, through a technique used by this same research group and previously reported in other studies. The purpose of the synthesis of this material was to evaluate it as a flocculant for iron ore slime, kaolin, coal, and silica particles, for its possible application in the treatment of waste effluents from the textile, paper, and municipal industries. Biodegradation tests were carried out under enzymatic media using amylase enzymes. Despite the fact that they do not show conclusive results, they showed the feasibility of the hydrolysis of amylopectin and the graft copolymers obtained in different studies reported by the same research group [16–19].

A methodology for the preparation of Ag-Au nanocomposites based on a biodegradable material was reported by Tripathy et al. [20]. In this study, the biodegradable copolymer was prepared in an aqueous solution of hydroxyethyl starch, AA, AM, and KPS used as initiator at 70 °C under a nitrogen atmosphere. Once the polymerization was finished, the copolymer was precipitated with methanol and purified, eliminating the homopolymers that could have formed. The product was then dried and characterized.

To prepare the bimetallic nanocomposites, solutions of chloroauric acid tetrahydrate and silver nitrate were added to an aqueous solution of the copolymer. The reagents were mixed vigorously, the temperature was increased to 80 °C, and a solution of NaOH was then dropped into the system drop by drop for 1 h. The nanocomposite prepared is presented in the form of a fine dispersion, which was later used for the reductive catalysis of chemical compounds with high effectiveness. The biodegradation of the materials obtained was evaluated by enzymatic degradation with α-amylase, a specific enzyme that breaks the 1–4 α-glycosidic bond of the starch macromolecule. The density tests results by coloration change showed that the polysaccharide changes its color by the effect of glycolysis.

There are other systems in literature in which natural polymers such as rubber are used. Tanan et al. [21] report the obtention of a biodegradable hydrogel based on modified natural rubber and polyvinyl alcohol mixed with cassava starch grafted with PAA. They propose this type of material to be used in agricultural applications for controlled dosage of fertilizers and pollution control. A gelatinized starch system was prepared in a slightly alkaline aqueous solution where APS and AA were added simultaneously under intense stirring. Then, MBA is added as a crosslinking agent, keeping the system under a nitrogen atmosphere during the entire reaction. In another flask, an aqueous solution of polyvinyl alcohol with NR latex was prepared. An emulsifier was added to prevent coagulation of the latex. In a third step, both solutions were mixed and vigorously stirred at 50 °C for 3 h. Finally, the prepared materials were washed, dried, and characterized using different instrumental techniques. Biodegradability tests were carried out using the soil burial method for 120 days, observing a mass loss of 72.7% at the end of this experiment.

An apparently more straightforward technique for preparing grafted starch is the one proposed by Quia et al. [22]. In their study, they used a chamber of an internal mixer with Haake-type rollers as their reactor. AA and the selected starch were added, and MBA was selected as the crosslinking system. Water is then added, and the entire mixture is stirred at 80 °C at 80 rpm until gelatinized. After that, a ceric ammonium nitrate solution and a NaOH solution are added and stirred until the end of the polymerization, maintaining an inert atmosphere during the entire preparation process. The material was then purified, dried, and characterized. This material proved to be superabsorbent of humidity and was used to prepare compounds for controlled release of fertilizers in agriculture. Although no biodegradability tests were done, they assume that the starch part of the prepared copolymer must be sensitive to the enzymatic degradation of microorganisms.

4 Grafted Cellulose with Acrylic Polymers and Nanocomposites

Cellulose is undoubtedly one of humanity's most employed natural polymers in several daily applications. It is a homopolysaccharide composed of β-glucose molecules on its main chain. Considering the cellulose limitations in some specific applications, or in order to improve some desired characteristic of this material, different studies have been performed where cellulose is modified by grafting with acrylic polymers. AA is the most employed monomer using MBA as a crosslinking agent. Some researchers report the synthesis of environmentally friendly biodegradable cellulose-acrylic materials since cellulose is very sensitive to a microbiological attack. In this sense, some of these reports are particularly analyzed. A representation of the different types of modifications with acrylic cellulose monomers is shown in Fig. 4.

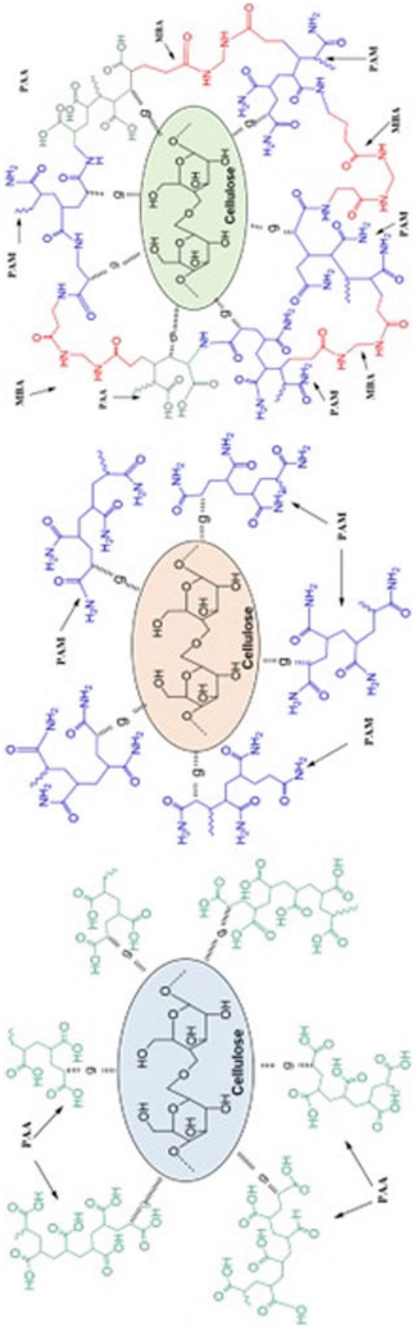

Fig. 4 Schematic representation of graft-modified cellulose with acrylic polymers. Modification with AA (left), with AM (center) and with AA and AM, crosslinked with MBA

In a study performed by Rop et al. [23], hyacinth-cellulose (water hyacinth) nanocomposites were prepared in a reactor where a mixture of nano-hydroxyapatite, different concentrations of AA, and small amounts of MBA were added as crosslinking agent. APS was added to this solution as a generator of free radicals to start the graft polymerization process. Once the material was obtained, it was characterized by spectroscopic and microscopic techniques. Water retention capacity was measured, obtaining a retention of up to 120 g of water per gram of synthesized material. The authors propose this grafted and crosslinked copolymer as a potential material in agriculture. Due to the presence of hydroxyapatite, the material can supply phosphorus to the crops in which it is used. In 2019 the same authors prepared a copolymer with two different types of monomers, AA and ammonium acrylate. The results also demonstrate that the material can also be used in agriculture as water retainers like their 2020 study [24].

Luo et al. [25] used a bacterial cellulosic material and developed a cellulosic material grafted with AA and crosslinked with small concentrations of MBA, using KPS as initiator. In this study, the methodology was similar to the study made by Rop [23, 24] but with cyclohexane as the selected solvent. The material water absorption capacity was evaluated under different salinity, temperature, and pH conditions, finding that the moisture absorption capacity is inversely proportional to the concentration of salt in water and the temperature. Interestingly, water absorption exhibited two main peaks at pH values 6.0 and 8.0, which are slightly acid and alkaline conditions. The maximum capacity of the synthesized materials is above 300 g of water per gram of crosslinked grafted polymer.

Guan et al. [26] used cyclohexane as a solvent to obtain a superabsorbent polymer based on carboxymethyl cellulose. Similar to Luo et al. [25], they added certain concentrations of AA, MBA, and KPS to the cellulose solution to start the polymerization. Once the product was obtained, it was washed, dried, and characterized by FTIR. This research group also used other crosslinking systems such as ethylene glycol, diethylene glycol, propylene glycol, and chloropropane epoxy. The water absorption capacity was determined for all these materials under different conditions of temperature, salinity, and even in the presence of oil, finding results comparable to those obtained by Luo et al. [25]. Biodegradability tests were not performed in this work. However, they assume that due to the cellulose section and its glycosidic bonds, glycolytic degradation may occur due to the effect of enzymes of microorganisms present in the environment.

Modified cellulose has been used for pollutant removal in industrial wastewater by modifying with polyaniline (PANI). Bagheri et al. [27] published a study on the modification of carboxymethyl cellulose (CMC) with AA. The process requires an aqueous solution of CMC, AA, APS as initiator, and MAB as crosslinking agent for the PAA grafted over the CMC. The polymer obtained was mixed with a 1.0 M aniline solution in hydrochloric acid to obtain a PANI hydrogel. The final product was then washed, dried, and characterized using FTIR and TGA. The moisture absorption capacity, the electrical properties, and the antibacterial capacity using *P. aeruginosa, S. aureus,* and *B. subtilis* were measured, showing acceptable results in all cases. Finally, biodegradation studies were carried out under aerobic conditions in the soil.

They observed a mass loss of 47% in 110 days and a practically complete loss after 7 months of testing.

5 Grafted Chitosan with Acrylic Polymers and Nanocomposites

Chitosan is another natural polymer used as a template for graft modification with acrylic monomers (see Fig. 5). These materials have been used for water absorption, flocculation, controlled release of drugs and fertilizers, chelating metals, and other pollutants in industrial or municipal effluents. It is an aminopolysaccharide, structurally composed of β-D-glucosamine and *N*-acetyl-D-glucosamine, randomly distributed along its polymeric chain. Some of the most up-to-date reports on this polymer are presented below.

Melo et al. [28] developed a nanocomposite based on a chitosan hydrogel grafted with AA and cellulose nanowhiskers. The synthesis of the grafted copolymer was performed by solubilizing the chitosan in a slightly acidic aqueous solution with APS as the initiator. After that, AA and small concentrations of MBA are added as a crosslinking agent under an inert atmosphere. Once the crosslinked graft copolymer was obtained, it was washed, dried, and purified. The material obtained was formulated with different concentrations of cellulose nanowhiskers and used for methylene blue (MB) adsorption in an aqueous solution. The removal capacity of this nanocomposite reached up to 1968 mg of MB per gram of copolymer. Although its adsorption exhibits Langmuir I-type behavior, i.e., monolayer chemisorption, this material can be regenerated and reused up to 5 times continuously with a minimal loss of MB removal efficiency.

A similar study was done by Lin et al. [29]. In this case, they used a solution of chitosan with maleic anhydride and dimethylformamide (DMF), which was heated at 115 °C for 8 h under a nitrogen atmosphere to obtain maleochitosan in the first stage. This material was dissolved in DMF; KPS was subsequently added and vigorously stirred under an inert atmosphere. After that, an aqueous solution of AA was added, and the system was polymerized. The graft copolymer is characterized by spectroscopic, microscopic, and thermal techniques and evaluated as an adsorbent of Cu^{2+} in aqueous solutions under different temperature conditions. The greatest adsorption of the ions on the surface of this copolymer was found at 40 °C. A Langmuir-type adsorption behavior was observed, which indicates a typical monolayer of a chemisorption process. The maximum concentration of Cu^{2+} adsorbed was 203 mg per gram of copolymer, which was higher than reported in other studies.

In addition to the study carried out by Lin et al. [29], Tian et al. [30] prepared a neutral solution of AA, to which chitosan, sodium lignosulphonate (SLS), and MBA were added. APS was then added as the polymerization initiator, and the reaction was subjected to ultrasound waves at 65 °C for 3 h, to obtain a copolymer used for the adsorption of Cu^{2+} and Co^{2+} metal ions. They found an evident dependence of

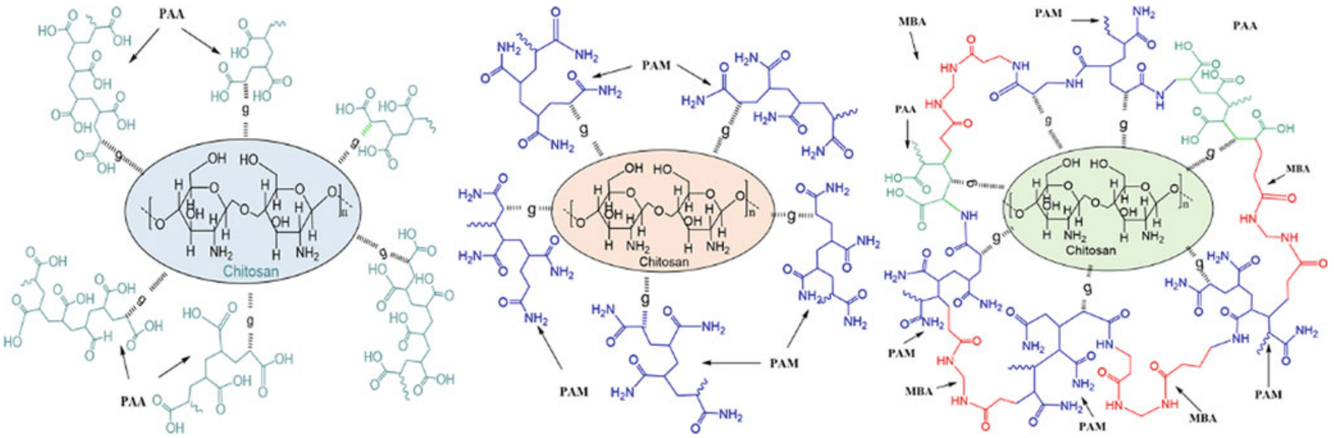

Fig. 5 Schematic representation of graft-modified chitosan with acrylic polymers. Modification with AA (left), with AM (center), and with AA and AM, cross-linked with MBA

pH in the adsorption of these ions, obtaining their optimal conditions at a pH of 6.0. The adsorption kinetics approximates second-order behavior and fits the Freundlich model.

A nanocomposite prepared with graphene oxide (GO) and chitosan modified with AA was reported by Medina et al. [31]. The nanocomposites were used to remove lead in solutions with different pH values. The best results were obtained at a pH of 5.0 with 5 wt% of GO, reaching an adsorption capacity of up to 138 mg of lead per gram of material. The adsorption kinetics obtained is of the Langmuir type, indicating a chemisorption.

Modified chitosan has also been used for controlled drug release. Bashir et al. [32] reported the preparation of a modified chitosan nanocomposite with succinic acid (AS), copolymerized with PAA and theophylline, a well-known drug for the treatment of respiratory diseases. For the functionalization of chitosan, a slightly acid solution of chitosan was prepared. After that, a solution of AS in acetone was added and reacted for 48 h. Finally, the modified chitosan is precipitated, washed, and dried. An aqueous solution was prepared with the material obtained, AA, and APS added as initiator. MBS and tetramethylethylenediamine (TEMED) were used as crosslinking agents. The polymerization reaction conditions were 60 °C for 10 min. Once the crosslinked copolymer was obtained, it was washed, dried, and characterized. The nanocomposite is obtained using aqueous solutions of theophylline, where the copolymer is swollen to obtain the encapsulated drug. The water absorption tests performed showed that at a neutral pH, swelling is favored, while the opposite occurs at a highly acidic pH. The biodegradation tests of the synthesized materials were carried out in vitro in a pancreatin solution, specifically in a Simulated Intestinal Fluid (SIF) pH 7.4. A weight loss of 71% is observed after three weeks, observing that 50% of it occurred during the first week. This fact was accused to the degradation of the polysaccharide bonds of the copolymer, which favors the release of the encapsulated drug.

Fang et al. [33] developed a superabsorbent copolymer based on chitosan modified with amine groups. In the first stage, a solution of chloroethylamine was used to obtain aminated chitosan. Subsequently, this material was dissolved in deionized water, APS was added as initiator and stirred vigorously. After that, a solution of AA and another of MBA were added, and the temperature was raised to initiate the radical polymerization under constant stirring and an argon atmosphere. At the end of the reaction, the product was neutralized, washed, and dried. The material was characterized by spectrophotometric and thermal techniques, and its water adsorption capacity was measured. In this case, unlike the results reported by Luo et al. [25] for cellulose, they found that the maximum adsorption of water is found at a neutral pH and reaches levels above 500 g/g of the copolymer. However, they do not present results of the biodegradability of the synthesized material.

6 Polylactic Acid Grafted with Acrylic Polymers and Nanocomposites

Lactic acid is a monomer that can be obtained from natural or through biotechnological or synthetic processes and allows, through a polycondensation process, the synthesis of polylactic acid (PLLA). This polymer can also be obtained from lactides by ring-opening polymerization. It is a biocompatible and biodegradable polymer used as a template for the preparation of acrylic copolymers for different types of applications, especially in nanocomposites for the controlled release of drugs. A representation of this polymer modified with acrylic monomers is shown in Fig. 6.

Xu et al. [34] synthesized a copolymer based on polyethylene glycol (PEG) and L-lactide to obtain a PLLA-PEG-PLLA block copolymer. They firstly prepared a solution with both substances. Then, a catalyst was added and heated at 130 °C for 12 h. The system was cooled after the reaction time, and the copolymer was precipitated and purified. In a second step, the copolymer is dissolved in dichloromethane and triethylamine, and a solution of methacrylic anhydride in dichloromethane is added dropwise under stirring for 48 h at 30 °C. Finally, the MA-PLLA-PEG-PLLA-MA copolymer is precipitated, washed, and characterized by FTIR and NMR. After that, an aqueous solution was prepared with AA, *N*-isopropylacrylamide (NIPAM), and the copolymer with a photo-initiator under a nitrogen atmosphere to obtain a hydrogel.

The prepared hydrogels were separately immersed in two phosphate-buffered saline PBS ($pH = 7.4$) solutions. The first one contained doxorubicin (DOX), a known anticancer agent, and the other one with tetracycline (TET), a bactericidal antibiotic. The release of these drugs was tested in a pH range from neutral to similar conditions of gastric juices. The authors found that DOX is better released at a pH close to neutrality, which effectively eliminates HeLa cells, while TET is released at a more acidic pH. Parallel studies demonstrated the effectiveness of these hydrogels in eliminating *E. coli* bacteria. In this case, biodegradation studies of these materials were performed in-vitro. Materials were immersed in PBS under intense stirring, and weight loss readings were taken as a time function. The total degradation of these materials occurs due to hydrolysis of the ester group.

Regarding the release of DOX, the group of Ahmadi et al. [35] presented a study in which they firstly prepared PAA telomeres with –OH termination, using a AA solution and mercaptoethanol (ME) in THF and AIBN as initiator. In this case, ME works as a chain transfer agent to obtain a PAA-OH at the end of this process. In the second stage, ring-opening polymerization of glycolide and L-lactide was carried out in the presence of PAA-OH at 120 °C under Ar atmosphere using a tin catalyst. At the end of the reaction, the copolymer obtained was dissolved in chloroform and precipitated in diethyl ether. The product was washed, dried, and spectroscopically characterized by FTIR and NMR.

The preparation of the nanocomposites with DOX and hydroxytyrosol (HT), a powerful antioxidant compound, was carried out by dissolving the prepared copolymers in dimethylsulfoxide (DMSO), adding the drugs in small concentrations. Drug

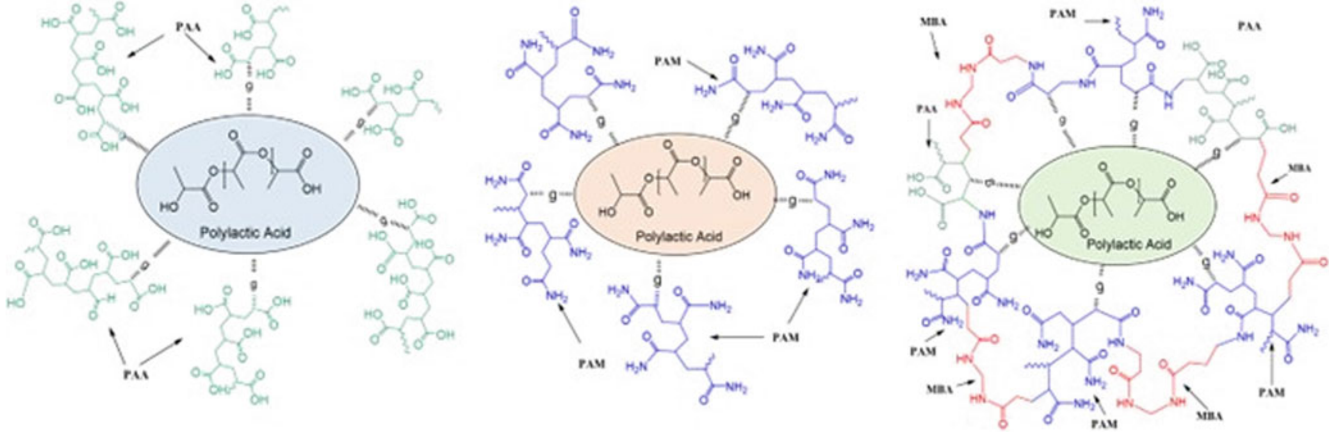

Fig. 6 Schematic representation of PLLA graft-modified with acrylic polymers. Modification with AA (left), with AM (center), and with AA and AM, cross-linked with MBA

release tests were successfully verified by UV–vis spectroscopy in PBS at different pH conditions. In vitro assays were also performed as in the study presented by Xu et al. [34]. In vivo studies showed that as the biodegradation of the copolymers occurs, the drug is gradually released, which can convert these materials into potential vehicles for anticarcinogenic substances.

PLLA is one of the most conventional materials for 3D printing. Considering that some drugs require structural supports to be placed in a certain position in the body, Gagliardi et al. [36] carried out a study on the molecular printing of biodegradable nanoparticles based on poly(lactide-co-glycolide) matrix (PLGA). In this study, they develop 3D structures of PLGA combined with chlorinated PLLA to obtain a completely biodegradable material formulated with biotin and used to print parts through additive manufacturing. The final objective of this study is to manufacture parts with specific geometries that, when biodegraded, leave the drug deposited in specific positions in the body.

7 Alginates Grafted with Acrylic Polymers and Nanocomposites

Another natural polysaccharide grafted with acrylic monomers is alginate (SAG), as shown in Fig. 7. This natural polymer can be extracted from the cell wall of brown seaweed. One of the most recent studies is the one presented by Samanta et al. [37]. They firstly prepared a solution of SAG. After that, a mixture of MA-co-2 acrylamino-2-methyl-1-propane sulfonic acid, TiO_2, and APS were added in the presence of MBA as cross-linking agent. The nanocomposite prepared was used as a superabsorbent material for water and for the removal of two inks, methylene blue MB and carmosine-a (CR-A). The tests were carried out at different temperatures and pH, finding two maximum peaks of ink adsorption at 2.0 and 8.0. The maximum adsorption temperatures were 60 °C for MB and 4 0 °C for CR-A, and the maximum concentrations of adsorbed dyes were 403 mg/g and 106 mg/g for MB and CR-A, respectively.

In 2019 Cheaburu-Yilmaz et al. [38] reported a study on the controlled release of Theophylline (THP), a well-known drug used as a bronchodilator, using AM-modified alginic acid as a vehicle by mixing aqueous solutions of the substances in different proportions using KPS as an initiator. Once the grafted polymers were obtained, they were washed and dried. Subsequently, the polymer samples were swollen in aqueous solutions of THP, which were then dried by lyophilization. The drug release assays were performed in vitro using a solution that simulates the gastrointestinal conditions, i.e., pH 2.2 and 37.5 °C. In vivo tests were also performed in laboratory rats. The results indicated that the prepared materials were biocompatible and non-toxic and with good capacity as carriers of this drug, which was released in up to 302 min.

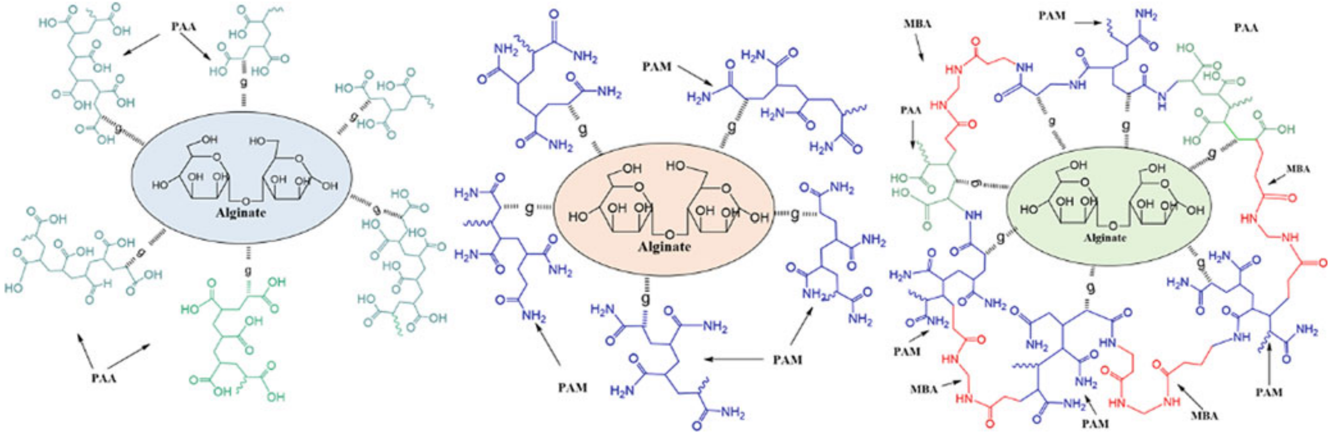

Fig. 7 Schematic representation of graft-modified alginate with acrylic polymers. Modifications with AA (left), with AM (center), and with AA and AM, cross-linked with MBA

Nanocomposites based on SAG grafted with acrylic monomers and montmorillonite (MMT) were developed for drug-controlled release for cancer treatment. Reddy et al. [39] prepared aqueous solutions of SAG, AA, and AM mixed under continuous agitation. APS is added to this mixture as initiator and a small concentration of MBA as cross-linking agent. In this case, different concentrations of MMT were added to the solutions to prepare the nanocomposites. Once the materials were obtained, they were washed and dried. Finally, the drug is encapsulated by swelling the nanocomposites in aqueous solutions of paclitaxel (PT), an anticancer drug.

PT controlled release assays were performed in vitro using a DS8000 dissolution tester LABINDIA, observing that up to 80% can be released within the first 72 h. This release rate was identified as a function of the cross-linking degree. To evaluate the extraction of the drug released by the nanocomposites, cytotoxicity of the extracts was evaluated on HaCaT cells, using a methodology MTT, which allows evaluating the cellular metabolic activity. The study concludes that the prepared nanocomposites can be used as vehicles for the controlled release of this type of drug.

Regarding the biodegradation studies of modified alginates, it is reported the biodegradation of alginates modified with ethyl acrylate (EA). Samples of these materials were tested in the presence of fungi, specifically fungus *Alternaria* spp., in a culture medium of dextrose and potato agar, finding that this species can grow up to 50% of the surface on the tested samples. Therefore, according to the international standard ISO 846, it can be concluded that the prepared materials are effectively biodegradable [40].

8 Other Reports of Acrylic Nanocomposites and Their Applications

Due to the limitations of exposing in previous sections a general overview of many other interesting reports on the research of nanocomposites based on acrylic polymers and their applications, this section collects in Table 1 other reported works of the modifications with acrylic monomers of natural rubbers, biobased, biocompatible and biodegradable polymers and the nanoparticles used in their formulations, as well as the applications given to these materials. This with the objective to complement the information provided in sections prior to this one in this chapter.

9 Conclusions

According to the different reports in the literature related to green-based acrylic polymers and their nanocomposites, it can be concluded that this type of matrices are actually copolymers based on biodegradable polymers that are grafted through

Table 1 Green-based acrylic polymers derived from biodegradable polymers

Polymer	Particle/substance	Application	References
Guar gum	GO	Drug release	[41]
Guar gum	TiO_2; Ag	Dye removal	[42, 43]
Guar gum	GO	Biomedical applications	[44]
Xanthan gum	CNT, RhB, GO, SiO_2	Dye removal	[45–48]
Xanthan gum	Ag	Agriculture/release	[49]
Xanthan gum	Li-ion	Batteries	[50]
Xanthan gum	C	Dust suppressant	[51]
Xanthan gum	Semi-coke	Agriculture/release	[52]
Tara gum	Ag	Antibacterial activity	[53]
Arabic gum	Li-ion	Batteries	[54]
Arabic gum	MB	Dye removal	[55]
Manihot gum	Cellulose	Agriculture/release	[56]
Tragacanth gum	TiO_2	Dye removal	[57]
Gellan gum	Al^{3+}	Metal removal	[58]
Ghatti gum	PPy	Metal removal	[59]
Starch	CNT	Metal removal	[60]
Starch	Au	Biomedical applications	[61]
Starch	Cellulose	Metal removal	[62]
Starch	Char, bentonite, clinoptilotite, hallosite	Agriculture/release	[63–66]
Starch	Ag	Drug release	[67]
Starch	C	Food packaging	[68]
Starch	Fe_3O_4	Dye removal	[69]
Starch	MB, CR, cellulose	Dye removal	[70–73]
Starch	Char	Agriculture/release	[74]
Starch	Cellulose	Biomedical applications	[75]
Starch/Chitosan	Acid Blue 113	Dye removal	[76]
Starch/Chitosan	Cu^{2+}, Ni^{2+}, and Co^{2+}	Metal removal	[77]
Starch/Gelatin	Ag	Antibacterial activity	[78]
Cellulose	Cu^{2+}; Cd^{2+}, Pb^{2+}, U^{6+}, Na^{+1}, Ca^{2+}, Al^{3+}	Metal removal	[62, 75, 79, 80]
Cellulose	B12, gentamicin	Drug release	[81, 82]
Cellulose	PPy, GO	Conductive materials	[83, 84]
Cellulose	GO, MB, Cu^{2+}, sefranin-O	Dye removal	[85, 86]
Cellulose	K, P, semi-coke, urea, GO, bentonite, soil particles	Agriculture/release	[87–92]
Cellulose	Li-ion	Batteries	[93]

(continued)

Table 1 (continued)

Polymer	Particle/substance	Application	References
Chitosan	Cellulose, bio-char, CNT	Metal removal	[94–96]
Chitosan	Bio-char	Soil treatment	[97]
Chitosan	Fe_3O_4, ibuprofen, diclofenac	Drug release	[98–100]
Chitosan	ZnO, Al	Biomedical applications	[101, 102]
Chitosan	Halloysite	Structural materials	[101]
Chitosan	Bentonite, TiO_2, GO, Fe_3O_4, TiO_2/MMT	Dye removal	[103–107]
Chitosan	MMT	Water treatment	[108]
Chitosan	MMT	Dye removal	[109]
Chitosan	Li-ion, Fe_3O_4	Conductive materials	[110, 111]
Chitosan	MMT	Agriculture/release	[100]
Chitosan/cellulose	Ca^{2+}, Mg^{2+}, Na^{+1}, MMT	Metal removal	[112, 113]
Polylactic acid	ZnO, TiO_2, MgO	Antibacterial activity	[111]
Alginate	Graphite	Dye removal	[114]
Alginate	CNT	Metal removal	[115]
Alginate	Ag	Biomedical applications	[116]
Alginate	MMT	Agriculture/release	[117]
Alginate	Bentonite	Dye removal	[118]
Alginate	ZnO	Dye removal	[119]
Alginate	Ag	Conductive materials	[120]
Alginate	GO	Metal removal	[121]
Alginate	GO	Dye removal	[122]
Alginate	Cu^{2+},	Dye removal	[123]
Alginate	Halloysite	Agriculture/release	[124]
Alginate	Sepiolite	Dye removal	[125]
Alginate	$FeCl_2$, $FeCl_3$	Drug release	[126]

Active substances/particles and applications

radical chain polymerization, with AA or AM derived polymers and in some cases both at the same time.

Although there are some exceptions, in most cases, copolymerization is carried out in aqueous solutions of selected biodegradable polymers, to which solutions of AA and AM monomers are added and polymerized in-situ by free radicals. The most used initiators are AIBN, APS, and KPS.

Numerous publications consider the use of a crosslinking agent, normally MAB, during polymerization to obtain three-dimensional molecular networks that can act as hydrogels and release active substances. These materials have been proposed for applications such as eco-friendly moisture retainers or for controlled release of nutrients. The same materials are used as carriers of medicinal principles and for the preparation of nanocomposites with drugs for specific applications such as cancer treatment.

Biodegradability tests are normally carried out in three main methods: through composting, the soil burial method, and in some cases by enzymatic biodegradation. For the particular case of graft copolymers for drug carriers, biodegradation is performed by simulating pancreatic systems at highly acidic pH conditions or using standardized buffer solutions.

In summary, it results interesting how acrylic monomers, conventionally used to make non-biodegradable polymers, are effectively used in combination with biodegradable polymers to form eco-friendly copolymers. These copolymers are not only biodegradable, but even bio-compatible, taking advantage of the great affinity that polar acrylic sections have as superabsorbent materials, to develop promising applications in diverse fields, like metal and ink removers, as well as drug carriers.

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Nanocomposites Comprise of Collagen and Acrylate-Derived Polymers for Biomedical Applications

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Abstract Collagen is a natural polymer with the capacity to generate three-dimensionally complex (3D) matrices made up of fibrils arising from the nanoscale. These matrices have excellent biological properties for the modulation of biomedical strategies, since they constitute a natural scaffolding where mammalian cells can carry out their vital functions, so that they have been used widely in fields such as regenerative medicine, controlled release of drugs, in vitro cell culture models, cell phenotype modulation and tissue engineering. The modulation of the mechanics, swelling and degradation of these nanometric collagen matrices requires functionalization techniques with synthetic polymers that promote such adaptation, generating nanocomposites with improved properties for biomedical applications. Acrylate-derived polymers such as polyacrylamide (PAM), polyacrylic acid (PAA), polymethyl methacrylate (PMMA), polyhydroxyethyl methacrylate (PHEMA), polybutyl acrylate (PBA), and poly2-ethylhexyl acrylate (P2EHA) have been used extensively for functionalization with collagen, designing nanocomposites with different architectures, mechanical properties, adherence, swelling and biofunctionalities. The use of these synthetic derivatives must be tailored inside the fibrillar collagen matrices to avoid the loss of their native biological properties. This chapter aims to present the fundamentals related to the synthesis processes, characterization of properties and application of nanocomposites based on collagen and acrylate-derived polymers in biomedical fields.

Keywords Nanocomposite · Collagen · Acrylate-derived · Polymer functionalization · Biomedicine

1 Introduction

In nature, collagen is one of the most abundant proteins and natural polymer, it has a fibrillar structure, and it is found predominantly in conjunctive and connective tissues,

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that is, joints, bones, and skin, referring to the human body [1]. About 28 types of collagens have been identified. However, type I collagen is the one commonly found in bones, tissues, organs, ligaments, teeth, etc. Type II collagen is present predominantly in cartilage [2]. The importance of natural polymer such collagen, is that can be obtained from natural sources and the biproducts often can be used in other applications. For example, natural polymer can be extracted from food wastes such feather, fishbones, animal hair, bones, cartilage, fish scales, crustacean shells, mammals among others [3]. The abundant sources to obtain collagen is one of the reasons for its use in several application.

For just over three decades, the scientific community has been interested in the use of type I collagen (mostly) for biomedical applications, such as tissue and cell engineering, bone regeneration, drug delivery, among others. Pure collagen can form three-dimensional porous sponges, which have been used in regenerative therapy in endodontics, wound dressings, in 3D cultures of various cell types, bone-cartilage substitutes, tampons, adhesives, cornea substitute, among others [4, 5]. Collagen sponges have also been used by different research groups as vehicles for the release of antibiotics [6, 7]. Current uses of collagen sponges for clinical applications and their ease of being loaded with drugs or biomolecules make them attractive materials for use as drug delivery carriers [4].

Unlike the components of the extracellular matrix (ECM), type I collagen does not have good ability to trap biomolecules, which results in an abrupt release of the agents that have been added to it, which can cause inflammation and other detrimental effects [8]. Even so, type I collagen remains highly versatile because its fibrillar architecture and physical properties can be controlled with the help of different factors such as pH, temperature, collagen concentration, and ionic strength. Collagen hydrogels have been used across virtually all types of tissue engineering research, cosmetic applications due to ease of injectability, drug delivery, intervertebral repair, and wound healing [1, 9–11].

Now, since the good properties of collagen for various applications have been discussed (mainly due to its biocompatibility), it is also well known that its use may have certain disadvantages, for example, limited thermal stability and inadequate mechanical properties (modulus, strength). This means that collagen-based materials can easily be degraded by high temperatures, ionic strength, pH or by enzymatic action [12]. For this reason, often the collagen is associated in various biomedical formulations with a series of synthetic biopolymers in order to improve its mechanical properties, thermal stability, or biodegradation rate, on the one hand, and to increase the acceptance level of the developed device by the body [13].

As mentioned above, collagen has poor mechanical properties and in order to enhance them, scientific community has been working in the development of nanocomposites whit different types of collagen-based nanocomposites. For example, in the field of bone regeneration collagen has been mixed with different materials to improve mechanical properties and biocompatibility, such as hydroxyapatite, β-tricalcium phosphate and calcium silicate. Collagen composites and carbon-based materials have also been formulated, such as carbon nanotubes (CNTs) and graphene oxide (GO), as well as natural and synthetic polymers [14].

Different natural polymers have been used to form matrices with collagen, to mention a few, cellulose, alginate, and lignin, among others. Similarly, synthetic polymers have also been used to form composites with collagen, some of them are polyethylene glycol (PEG), poly(N-isopropylacrylamide) (PNIPAM), polyvinyl alcohol (PVA), polyacrylic acid (PAA), etc. [15].

Within the synthetic biopolymers that have gained great importance in recent decades are acrylates. Acrylates (or polyacrylates) are part of the family of vinyl polymers made from acrylate monomers and their derivatives, among which methacrylate is one of the most common. Acrylates themselves are the esters, conjugate bases, and salts of acrylic acid (AA) with its derivatives. They are composed of the acrylate monomer, which is usually comprised of esters which contain vinyl groups, meaning that in the structure two carbons are joined by a double bond [16]. This makes acrylates bifunctional in nature: the vinyl group is susceptible to polymerization, therefore. The carbonyl group has a myriad of functionalities using alcohols or amines [17].

Acrylate derivatives have very varied applications due to their versatility and resistance, hardness, transparency, good resistance to temperature, elasticity, they also have good wettability and resistance to ozone and their glass transition temperature (T_g), normally found by below room temperature, among other characteristics. These types of materials are used for many applications, including the manufacture of cosmetics, adhesives, diapers, orthopedic devices, paints, coatings, contact lenses, and bone cements [16].

Acrylate-derived polymers such as polyacrylamide (PAM), polyacrylic acid (PAA), polymethyl methacrylate (PMMA), polyhydroxyethyl methacrylate (PHEMA), polybutyl acrylate (PBA), and poly2-ethylhexyl acrylate (P2EHA) have been used extensively for functionalization with collagen, designing nanocomposites with different architectures, mechanical properties, adherence, swelling and biofunctionalities. The use of these synthetic derivatives must be tailored inside the fibrillar collagen matrices to avoid the loss of their native biological properties. This chapter aims to present the fundamentals related to the synthesis processes, characterization of properties and application of nanocomposites based on collagen and acrylate-derived polymers in biomedical fields.

2 Collagen/Acrylate-Derived Nanocomposites

2.1 Polyacrylamide (PAM)

Polyacrylamides are synthetic linear polymers which are soluble in water. This kind of polymer emerge of the combination of acrylamide and acrylic acid [18]. Their principal characteristics include stability, nontoxic, nonimmunogenic, no resorbable and with cohesive, viscoelastic, hydrophilic, and biocompatible properties [19, 20]. The first reported use of synthetic polyacrylamide (derived from the acrylamide monomer) was as a support for the matrix of gels used for electrophoresis, in the year 1959 [21]. Since then, materials with PAM have gained great importance and are promising for various biomedical applications, among which can be mentioned ophthalmic operations, drug treatment, injectable implant, in cell-based studies, and wound dressing [20, 22, 23].

In this section, we describe several research works where nanocomposites of collagen polyacrylamide (PAM) have been studied. In 2015, Baek et al., proposed, the in situ assembly of an interpenetrating polymeric network (IPN) gel comprise of polyacrylamide and collagen aiming a cell-adherent hydrogel system. The hypothesis of this work was that the in situ assembly process could favor the Michael reaction between the amine groups of collagen and vinyl of acrylamide. In this way, the collagen fibers would be chemically connected to the PAM, without interfering with the crosslinking reaction of the PAM itself. To verify the above, this research group carried out studies such as the effect of collagen concentration and the molar radio between polyacrylamide and the crosslinker, determining the elastic modulus, swelling radio and the degree of crosslinking in the composite gel. What was described above was carried out to find out if the material would be able to regulate the behavior of cell adhesion. As a result, of the previously described tests, they found that the combination of the variants allowed them to find a material that presented wide values of elastic modulus, minimizing the changes in the degree of swelling. The compression elastic modulus of the hydrogel (collagen/PAM) changed from 10 to 100 kPa, when the concentrations of bis acrylamide (crosslinker) were varied. On the contrary, if the polyacrylamide concentration of the pure PAM gel was increased, lower elastic moduli and variations in the degree of swelling of the gel were obtained. The resulting material (interpenetrated collagen/PAM gel) can present great advantages to be used as a scaffold for in vitro cell culture [24].

Another example where collagen/PAM nanocomposites have been used is in bone regeneration and repair. The architecture of the material used for these applications is crucial, since it must not only provide the necessary support for cell integration, but must also allow substitution, differentiation, and migration to form functional tissues [25]. When an implantation is made, the biomaterials must offer the damaged site (bone tissue) enough support so that it can regenerate, for which it is sought that the scaffolds have very high compressive forces initially. Nanomaterials can offer this feature compared to conventional materials. For example, collagen-PAM shaped gels with an elastic modulus of 25–40 kPa favors osteo-genic differentiation of human mesenchymal stem cells (MSCs) [25, 26].

On the other hand, in 2010 Yamamoto et al. fabricated a hydrogel composed of collagen/polyacrylamide (PAM) with the immobilization of the type I collagen gradient within the polyacrylamide hydrogel. This biomaterial was achieved by coupling the amino groups of collagens and the carboxylic groups generated in the PAM gel by treatment with sodium hydroxide. In vitro studies showed that the number of fibroblast cells (L929) that adhered to the hydrogel directly depended on the amount of collagen immobilized in the composite. Their results indicate that cell adhesion can be optimized by varying the concentration of collagen. The controlled manufacture of this material makes it viable as a scaffold for the coupling of biomolecules to be used in various desired biomedical applications [27, 28].

As mentioned, collagen type I is the most widely used for biomedical applications, nevertheless, there are several applications for the diverse collagen types existing. For example, PAM gels have been coated with collagen type IV to model defects in the basement membrane, where some peptides of collagen type IV are responsible of cell adhesion, spreading and motility [4, 29].

It is well known that the skin is the largest organ in the human body, protecting us from microbial invasion and damage and keeping body fluids stable [30]. However, epithelial damage can arise from accidents, diabetes, surgeries, and burns, among other causes, which is why collagen/PAM-based nanocomposites have also been proposed for wound healing. With this background, Bai et al. proposed the synthesis of a hydrogel to heal wounds that had good adherence, high resistance (hardness) and good biocompatibility. For this goal, he used a crosslinker based on dopamine and oxidized sodium alginate (COA), which was used in the synthesis of the PAM/collagen hydrogel. The composite material, PAM-Col-COA, showed mechanical properties, good water absorption, adhesion to epithelial tissue and good biological activity. In addition, performed in vivo wound healing experiments, the results showed that the synthesized hydrogel accelerated the healing process, which makes it a promising nanomaterial for biomedical applications [31].

2.2 *Polyacrylic Acid (PAA)*

Polyacrylic acid (PAA) is a compound that arises from the polymerization of acrylic acid monomer. Its main characteristics are high molecular weight and solubility in water. Because it contains carboxylic acid groups in its structure, PAA is often appropriate for developing pH-dependent delivery systems. In addition, it has the necessary characteristics to be a good material for biomedical applications, such as biocompatibility, biodegradable nature, non-toxic behavior, etc. However, at the same time it does not possess sufficient mechanical strength [32].

As mentioned above, PAA is an excellent material that can be used for biomedical applications. Hydrogels that are composed of acrylic acid (AA) or polymerized methacrylic acid (MMA), are reported as materials that have high water absorption due to the hydrophilicity of their carboxylic groups, in addition to their pH and thermal-sensitive properties [33, 34]. For this reason, they are materials of great interest in the field of drug delivery. For example, the oral administration of insulin through the use of hydrogels is of great interest, to avoid pain in those patients who inject the medication recurrently.

For this reason, Noppakundilograt et al. proposed the synthesis of hydrolyzed colla-gen-grafted-poly[(acrylic acid)-co-(methacrylic acid)] hydrogels, which were prepared by solution polymerization using N,N^0-methylenebisacrylamide (N-MBA) and N,N,N′,N′-Tetramethyl ethylenediamine (TEMED) as crosslinking agents and co-initiator, respectively, while ammonium persulfate (APS) was used as initiator.

The resulting hydrogel was a material that proved to be both pH and temperature sensitive, which are desired characteristics in this type of material since it is sought that insulin can reach the intestines for its absorption, that is, that it can resist acid stomach conditions [35].

As mentioned, collagen is a protein that is present in connective tissues and in the extracellular matrix and has been used in the field of biomaterials, food engineering and regenerative medicine due to its mechanical and biological properties. However, its limited stability and mechanical strength make it unsatisfactory for in vitro and in vivo applications [36, 37]. With the recent advances in protein sciences and chemistry, the chemical modification of proteins has become very important.

For this reason, Zhang et al. achieved fibrillogenesis of acrylic acid (AA)-grafted collagen by induction with natural collagen. Co-assembly was confirmed by a turbidity study. Fibrillogenesis, which is the process of formation of fine collagen fibers, was achieved with the AAc-g-Col material without the self-assembly property of natural collagen. In this work, they assume that the co-assembly mechanism of the material is as follows: AAc-g-Col would participate in the self-assembly of Col to give the hybrid fibrils until the it was exhausted, which process might be attributed to hydrophobic forces, hydrogen bonding and electrostatic interaction. The formation of Col/AAc-g-Col hybrid fibrils was determined by the TEM technique. Moreover, coassembly of Col/AAc-g-Col resulted in a softer hydrogel with similar fibrillary structures and biocompatibility compared to Col hydrogels. This development provides an alternative to perform fibrillogenesis with a “natural collagen-induced method”, which can help the design of biocompatible materials with similarities in the structures of natural tissues [38].

In the same sense, and in order to create materials with adjustable properties for regenerative medicine, Duceac et al. proposed the synthesis of hydrogels based on chitosan, collagen and polyacrylic acid (PAA). The preparation of the citraconyl-chitosan/citraconylcollagen/poly(acrylic acid) scaffold was carried out by mixing citraconyl-chitosan (CS) and critraconyl-collagen solutions in different proportions. AA and APS/TEMED complex (constant ratios) were added dropwise under solution pressure. The systems had a 3D scaffold model and were kept at 70 °C for two hours, and then washed for 7 days to reach a constant pH and finally, proceed to lyophilization. Characterization studies (IR and SEM) confirm that the chemical structure and the proposed formation of porous materials with variable morphology depend on the composition of the material. In addition, the swelling behavior of the synthesized materials revealed higher water absorption when the hydrogels had a higher CS concentration, but a higher degree of swelling due to the presence of collagen. The materials synthesized in this research work had the ability to resist enzymatic attack and remained stable for more than 7 days. Information was obtained from cytotoxicity studies that hydrogels present good cell-material interactions. With the results described, this type of materials based on chitosan, collagen and PAA, may be promising for use in matrices for regenerative medicine [39].

2.3 Polymethyl Methacrylate (PMMA)

Polymethyl methacrylate (PMMA) is a polymer obtained from the reaction of methyl methacrylate (MMA) monomer with the ester groups of polymethacrylic acid. It is regularly obtained by the radical polymerization of MMA, although it can also be obtained by anionic and coordination polymerizations. Among the characteristics of PMMA is that it is a transparent thermoplastic polymer and has properties of chemical, impact, and water resistance, for which it has been used as an option to replace materials in various applications such as optical materials, automobiles, electronics, and biomedicine, among others [40].

Within the field of biomedicine, PMMA has been given various applications, such as cement and bone substitute, lenses, and drug delivery systems. In addition, the properties such as low modulus of elasticity, adequate porosity and thermal and electrical inactivity are very similar to human dentin, this polymer has been used for the development of dental implants as well as to replace tooth roots. PMMA has also been used to permanently remove wrinkles and scars from the skin [41].

In 2017, Ng et al. proposed the use of PMMA collagen microspheres to treat scars caused by acne, since it is a problem that affects many individuals worldwide and generates psychological effects in people who suffer from it. They propose it as a hypothesis based on the review of various clinical cases and antecedents of the use of PMMA for the elimination of scars [42].

As mentioned above, Karnik et al., sought to demonstrate through a clinical study the effectiveness of treating patients with nasolabial scars with a nanocomposite of collagen spheres with PMMA, this through a blind study. To carry out the study, 147 subjects were studied, of which some received the treatment of collagen spheres with PMMA and the rest injections of saline solution. The follow-up for the patients was for 6 months and the study showed that the PMMA collagen treatment has good efficacy in treating patients with atrophic acne scars and that it is also safe for the subjects. However, the authors suggest a larger study (longer follow-up) to confirm the results [43].

Regarding the generation of bone tissue (osteogenesis), Vedhanayagam et al. propose the use of a collagen structure with PMMA reinforced with nanocomposites (NC) of PdO–TiO_2 (in different molar radii). The purpose of the study is study was to improve the physical and chemical properties, as well as the biocompatibility of the materials. The results obtained from the research indicate that the incorporation of PdO–TiO_2 nanoparticles 1:1 with a molar radius (size range 5–11 nm) in the PMMA and collagen scaffolds, provide the material with better thermal stability and greater strength. mechanical stability (Young's modulus) compared to the collagen matrix, increasing thermal stability from 71.64 to 83.45 °C, while Young's modulus had a significant increase from 11.7 to 105.57 kPa. In addition, the in vitro tests showed that the PMMA-Collagen-NC scaffolds were not toxic to human MG-63 cells (human osteosarcoma), in addition to increasing the activity of alkaline phosphatase (ALP), this enzyme indicates the presence of osteoblast cells, which regulate bone formation [44].

Bone cement based on bioinert PMMA is the most used for the fixation of orthopedic implants in hip replacement surgeries. However, it is common that a good fixation does not normally occur when the patient suffers from osteoporosis, since adequate osseointegration is not achieved. To improve this situation, Zheng et al. proposed the use of mineralized collagen (MC), incorporating it into PMMA (PMMA-MC). As relevant results of their work, it can be mentioned that the wettability and dynamic and mechanical performance of the composite material in vitro were improved compared to pure PMMA. They compared the bulk material with the composite material in osteoporotic bone marrow stromal cells, the results indicated that the addition of MC to PMMA could significantly regulate the expressions of osteoblastic genes. In vivo osseointegration was tested in rat models with osteoporosis (bone defects) and it was observed that after 8–12 weeks of implantation the PMMA-MC group had significantly more bone formation around the implant and greater adhesion than PMMA cement [45].

A common condition in older adults is degeneration of the lumbar discs, a minimally invasive surgery called percutaneous cement discoplasty (PCD) is currently used. So, likewise, in the case of the hip, PMMA-MC was also tested for this condition. Different characteristics were analyzed, such as injectability, mechanical properties and hydrophobicity. The results of this research showed no changes in the type of application and setting of PMMA compared to PMMA-MC, and it was manageable for minimally invasive surgery. The composite material was shown to be hydrophilic, and its elastic modulus was adapted to complement the mechanical performance of bone under dynamic stress. In vivo tests were performed in a goat model that presented induced disc degradation, and it was examined with both materials. After 3 months of installation, the PMMA-MC showed a higher circumferential contact index (36.4%) than the PMMA. With the cell staining it was possible to observe that the PMMA-MC material was in direct contact with the bone, unlike the PMMA that was found covered by fibrous tissue. In order to elucidate the cause of the previous behavior, in vitro tests were carried out with macrophages, from this test it was found that adding MC to the material hinders the fusion and protrudes from the macrophages. Interestingly, fibroblast-stimulating growth factors, such as insulin-like growth factor, basic fibroblast growth factor, and tumor necrosis factor-b, were also significantly reduced. For these reasons, it follows that the PMMA-MC material is viable for use in the treatment of lumbar disc degeneration [46].

2.4 Polyhydroxyethyl Methacrylate (PHEMA)

Polyhydroxyethyl methacrylate (PHEMA) is a stable, optically transparent hydrophilic polymer that is one of the most widely applied hydrogel biomaterials [47]. PHEMA-based hydrogels can be manipulated to give tissue-like characteristics, such as water content and mechanical properties, while also possessing excellent cytocompatibility. Among the applications that have been given, are the manufacture of contact lenses, bandages and as a material for the release of drugs [48]. It is synthesized using free radical precipitation polymerization of 2-hydroxyethyl methacrylate [49, 50].

The development of composite materials of PHEMA and collagen for their application in the generation of bone tissue has been reported, as well as the advantages that this type of composite can present. Among the advantages that these materials can present is that they do not form fibrous tissue around the implanted material, which indicates that they are biocompatible compounds and that they promote the generation of bone tissue [51].

Based on the aforementioned and in order to find a biomaterial with potential biomedical use in implants, Rezaei et al. proposed the modification of poly(dimethyl siloxane) (PDMS) films with PHEMA by the method of interpenetrating polymeric networks (IPN). In addition, they added type I collagen on the modified films using methyl sulfonyl chloride. To compare the properties of PDMS films against those modified with PHEMA and collagen, different characterizations such as FTIR-ATR, XPS and SEM, among others, were performed. The results obtained from FTIR and XPS showed that the collagen was successfully grafted on the surface of the films. The wettability of the PDMS films with and without grafted collagen was enhanced. The system with grafted collagen showed greater cell adhesion and growth (L929 fibroblasts) than the system without collagen. With the results, a strategy was found to use biomacromolecules that could improve cell interactions [52].

Among the common human illness, corneal diseases rank second as causes of blindness, affecting more than 12 million people globally [53]. Corneal transplantation is a usual treatment method. However, the availability of donor tissue fails to meet the demand in many countries [54]. This is the reason why several studies have been carried out to find biomaterials that can replace the cornea.

As has been described, ocular problems related to corneal damage are worrying, which is why for some decades great efforts have been made to find an alternative to conventional corneal transplantation, that is, to find biocompatible materials with good mechanical properties that can function as a corneal implant. A material that has been tested for this type of application is polyethylene glycol (PEG). However, its mechanical properties are not considered optimal for some applications. For this reason, Park et al., proposed the synthesis of interpenetrated network hydrogels (IPN) based on PEG and PHEMA, to improve the properties of the hydrogel. The synthesis of the IPN hydrogels was confirmed by the weight difference between the PEG hydrogel and those containing PHEMA, as well as being corroborated by ATR-FTIR. Hydrogels with high PHEMA content were optically transparent and highly crosslinked, with low water content and mechanical properties improved by approximately sevenfold (tensile strength). When the amounts of PHEMA used were lower (less than 90 wt%), the hydrogels obtained were opaque due to phase separation between the PHEMA and the water. Once these hydrogels were characterized, proceeded to combat the poor cell adhesion of the hydrogel-type materials by covalently anchoring collagen to the surface of the IPN hydrogels using carbamate linkages to hydroxyl groups in PHEMA. IPN hydrogels with collagen were shown to be non-cytotoxic and the presence of collagen in the material produced a significant improvement in cell adhesion. The results obtained by this research group indicate that the IPN hydrogels obtained in this work are promising biomaterials that can be used as artificial corneas and a variety of other load-bearing tissue engineering applications [55].

In order to find a biomaterial with possible application in corneal repair, the fabrication of a porous matrix of PHEMA by the paraffin template method was proposed, which was used to adhere collagen fibers to develop an inter-connective porous collagen/PHEMA (Col-PHEMA) composite hydrogel. Fourier transform infrared spectroscopy (FTIR) showed that new hydrogen bonds were formed between the collagen fibers and the PHEMA hydrogels. In addition, the composites showed high hydrophilicity, good mechanical properties, and excellent capacity to absorb water. The Col-PHEMA hydrogel-type composites also showed good biocompatibility, and, in addition, the collagen fibers were able to promote the proliferation of fibroblasts. These characteristics obtained from the synthesized composite material make it a biomaterial with high possibilities of being used in biomedical applications, in this case, could be a promising candidate for artificial cornea skirt [56].

2.5 Polybutyl Acrylate (PBA)

Butyl acrylate (BA), presents the molecular formula $(C_7H_{12}O_2)_n$. BA can be synthesized in different reactions. Thus, 1-butyl alcohol, acetylene, carbon monoxide, hydrochloric acid and nickel carbonyl can react to yield butyl acrylate. Other chemical route in the synthesis of BA is based on the reaction of methyl acrylate with butanol or acrylic acid [16].

Some of its derivatives can improve some physical properties (e.g. impact strength) of other plastics, which is why they are often used as impact modifiers [57]. Homo- and copolymers of acrylate monomers are used in a wide variety of applications, e.g., in adhesives, coatings, and biomedical materials [58, 59]. PBA is a polymer with low glass transition temperature and high UV resistance [60].

Materials science is currently searching for products that combine the adaptability of synthetic polymers with the structure and functionality of biopolymers. Due to this need, Luque et al. proposed the synthesis of a hybrid nanocomposite based on acrylic/collagen, for its possible use as bioadhesives. The synthesis was performed by emulsifier-free emulsion polymerization to obtain water-based hybrid nanocomposites based on acrylic monomers and fully hydrolyzed as collagen (HC) with film-forming capacity. The high solubility of collagen is the reason why emulsion polymerization is the most suitable way to stop the formation of hybrid nanoparticles. The acrylic phase of the hybrid latexes was composed of acrylic acid (AA) and butyl acrylate (BA), and the HC contents were varied from 15 to 50% weight based on monomer (wbm) while the AA/BA ratio was kept constant at 70/30. As relevant results of the research are that they achieved the synthesis of a new collagen/acrylic hybrid hydrogel, the selection of the emulsion polymerization method was an adequate technique for obtaining nanomaterials. The latexes obtained showed an excellent ability to form films, giving rise to materials with different mechanical properties and adhesion depending on the ratios used in the formula. Neutralization of the latexes caused the adhesion properties of the manufactured films to change markedly. That is, the neutralized films showed different behaviors compared to those that were not neutralized, exhibiting greater deformability and better adhesion properties. The materials obtained by this research group showed promising properties to be used as bioadhesives in which the moisture content could be a switch to control adherence [61].

In addition to the search for nanomaterials that have good mechanical properties and biocompatibility, there is a need to take advantage of resources that are considered waste. In this sense, waste from the fishing industry such as skin, bones, and tails are sources of collagen [62, 63].

Materials based on fish collagen also have a wide variety of applications such as collagen obtained from mammals, in addition to being structurally very similar to human collagen, thus providing a high level of biocompatibility [62, 64, 65].

For this reason, Uromicheva et al. [67] proposed the synthesis of PBA copolymers grafted with collagen obtained from fish in the presence of different initiators (azobisizobutyronitrile (AIBN) and the initiating triethyl borane (TEB)—oxygen system). The collagen used was isolated from cod skin with acid acetic solutions according to the method reported in the patent by Semenychev et al. [66]. The BA was washed with 5% NaOH solutions and then with distilled water until reaching neutral pH and then dried with anhydrous sodium sulfate for subsequent purification by vacuum distillation. AIBN was recrystallized from ethanol to remove impurities from the initiator. The obtained crystals were dried under vacuum at a temperature of 25 °C.

For the synthesis of the BA copolymer with collagen, the following procedure was carried out: collagen isolated in solution and butyl acrylate in a 1:1 weight ratio, the mixture was bubbled with argon for 15 min at room temperature. Once this time had elapsed, the initiator was added to the reaction mixture and the temperature was raised to 50 °C immediately. The synthesis time for the formation of the copolymer was 1 h in an inert atmosphere. Finally, the aqueous phase containing the copolymer was recovered.

The infrared spectra showed that the resulting material shows a combination of bands of both pure collagen and BA, thus confirming the formation of the nanocomposite. This is a material with promising applications as a material resistant to fungi and as a bactericide [67].

2.6 Poly2-Ethylhexyl Acrylate (P2EHA)

Poly2-ethylhexyl acrylate (P2EHA) is a hydrophobic polymer. The long ester side group strongly influences properties such as viscosity and glass transition temperature, T_g, which is far below room temperature. A high number of applications of P2EHA have been reported in the literature [68]. The main ones are waterbornecoatings, pressure sensitive adhesives and nanocomposites thanks to its low T_g [69, 70].

In addition to the applications mentioned, P2EHA has gained importance in the field of biomedicine and cosmetics, thanks to the fact that it can increase the hydrophilicity of materials, which makes it promising for cosmetic products and drug release, among others. This is why Sadeghi et al., in their research work, propose the synthesis of a Collagen-g-Poly(Sodium Acrylate-co-2-Hydroxyethylacrylate) [71].

For the preparation of the hydrogel, 2 g of hydrolyzed collagen (HC) were weighed and dissolved in 50 mL of distilled water, the resulting solution was filtered to remove insoluble phosphate. The filtrate (containing collagen) was kept under stirring for 10 min at a controlled temperature of 80 °C. Over time acrylic acid (1–4 g), 2-hydroethyl acrylate (4–1 g) and methylene bisacrylamide (0.05–0.20 g) were added to the reactor at the same time. The reactor temperature was kept constant at 80 °C and under stirring at 300 rpm. After the reaction time, the resulting compound was poured into ethanol and dehydrated for 12 h. Finally, the product was washed with ethanol and filtered to proceed to drying at 50 °C for 12 h.

The composites were subjected to various tests for their characterization, such as swelling and degradation kinetics, infrared spectroscopy, SEM and thermal analysis. Thermogravimetric analysis and FTIR spectra show that graft copolymerization does take place. The swelling capacity of hydrogels is affected by the amount of crosslinking agent (MBA) used in the formulation and likewise the ratio of monomers used. The behavior is that the swelling decreases when the concentration of MBA increases and also step up when the ratio of AA/HEA increases. The swelling capacity of collagen-g-(PAA-co-PHEA) hydrogels in solutions at different pHs (1–13), as well as the swelling-deswelling behavior of the materials exhibited pH-sensitivity and reversible pH-responsiveness properties. These characteristics make the compounds excellent candidates for the design of new systems for the controlled release of drugs [71].

3 Conclusions

The field of biomedicine is wide, and the efforts required to cover all areas are of substantial importance. It is evident that for decades several research groups have been putting great effort into the development of biomaterials and composite biomaterials that can cover the needs that involve the improvement of human health. Collagen, being one of the most abundant proteins in mammals, fishes, and crustaceans (among other sources), has been shown to be a protein that can be used to manufacture mimetic materials for human tissues and bones. However, the deficiency of some properties such as mechanical and thermal in collagen, has led to the development of nanocomposites with other materials such as those derived from carbon, ceramics, natural and synthetic polymers. As has been observed in this chapter, collagen nanocomposites with acrylate derivatives are highly promising materials in the field of biomedicine such as tissue repair, wound healing, corneal implants, drug delivery, among others, due to their that help to improve or fine-tune the characteristics of the nanocomposites, offering sufficient stability to the collagen, without losing the absorption capacity, low cytotoxicity, and biocompatibility, which is desired for biomedical applications.

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PVA Blends and Nanocomposites, Properties and Applications: A Review

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Abstract Due to the environmental concern caused by polymers from non-renewable sources and that consequently their decomposition time is excessively slow, the demand for polymers from renewable sources or biopolymers for various uses has recently increased. One of the disadvantages of biopolymers is their thermal and mechanical properties, therefore research on biopolymers is currently being carried out on physical or chemical modifications or even metallic nanoparticles have been added to obtain reinforced polymers (nanocomposites) with the intention of improving mechanical, thermal properties, antimicrobial, water vapor/oxygen barrier properties that have shown improvements in materials for various applications. Polyvinyl alcohol (PVA) is a polymer widely studied for its ease of forming films, hydrophilicity, good processability, good biocompatibility and interesting chemical and physical properties, which when combined with another polymer can form very resistant films. In addition, they have been shown to be polymers of low toxicity and cytotoxicity. This chapter discusses the studies carried out on blends of PVA with different polymers, nanocomposites of PVA with different nanoparticles, their properties and their applications.

Keywords PVA · Blends · Nanocomposites · Properties

1 Introduction

PVA is a widely studied polymer due to its biocompatibility, good processability, hydrophilicity and its interesting chemical and physical properties, in addition, it has low toxicity and cytotoxicity. It has also been reported that by adding nanoparticles to a PVA matrix, a nanocomposite material with improved mechanical, thermal or

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antimicrobial properties would be obtained. This polymer exhibits very interesting properties, but when it is in contact with polar solutions for long periods of time, it tends to solubilize, presenting an obstacle for various applications such as biomedical ones where the polymer is required to be in contact in physiological environments. Due to this problem, the crosslinking of PVA has also been studied, which involves the formation of ionic or covalent bonds between the chains of the same polymer or to a crosslinking agent, resulting in the formation of a three-dimensional network. To avoid the drawbacks of its solubility, it is also common for this polymer to be mixed with other polymers or nanoparticles are added to it to solve the solubility problem.

This polymer has a wide field of application, being the field of medicine and pharmacy the most promising. Some properties that these biomaterials must possess are flexibility, elasticity and hydrophilic behavior, without becoming soluble in water, that is, they must be able to absorb water without losing their structure.

Another important characteristic is that it is water-soluble, since in its structure there are functional groups that favor the formation of three-dimensional networks, commonly called hydrogels, which swell in water or physiological fluids. PVA is one of the most widely used biopolymers in the preparation of hydrogels, due to its hydrophilic behavior, biodegradability, biocompatibility, good processability in film formation, non-toxicity and its easy mixing with other polymers, whether from renewable or non-renewable sources, which makes it an excellent matrix to form hydrogels and nanoparticles can even be added to improve properties. These hydrogels can be produced by chemical or physical cross-linking generating interpenetrated networks. Due to the properties of hydrogels in physiological environments, are excellent candidates for biomedical applications.

The fact that this polymer is soluble in water is one of the main characteristics for the formation of films. However, this characteristic could be a disadvantage because if it is soluble in water, it is also likely that it will solubilize on contact with fluids (thinking of a biomedical application such as wound dressings). For this reason, the appropriate combination of other polymers and even the use of crosslinking agents could reduce the solubility in water and even modify or increase the mechanical properties of the polymer, to obtain useful films for medium or long-term implants.

Because PVA has –OH groups and hydrogen bonds, it can interact with organic and inorganic materials or with other water soluble polymers, forming hydrogen bonds with each other. These types of interactions influence the blending of polymers in many ways, including solubility and mechanical properties. In this sense, the use of crosslinking agents is important to obtain materials with some ideal properties.

Since the solubility in water, it is necessary to mix PVA with another polymer or promote chemical crosslinking to ensure a more stable and less soluble structure. In this case, carboxylic acids are a non-toxic and low-cost alternative to cross-link PVA. Citric acid, is a non-toxic metabolic product that can react with polymers containing hydroxyl groups under relatively mild conditions. Furthermore, it is cost-effective and has numerous applications in items such as food additives, anticoagulants, antivirals, and cleaning agents.

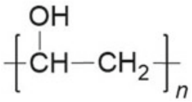
Glutaraldehyde has been used as a crosslinking agent which reacts with PVA producing an intermolecular crosslinking network, its addition allows the easy formation of a transparent film at room temperature. These types of mixtures have currently been gaining interest in the pharmaceutical and biomedical areas due to the fact that materials are obtained for applications such as tissue engineering, regenerative medicine, as well as for drug delivery systems. Other applications are in the textile area, in the pharmaceutical industry, being used as an excipient, as an adhesive and as a film former for food packaging. This chapter will discuss the synthesis, properties and applications of PVA-based nanocomposites and blends.

2 Structure of the PVA

Polyvinyl alcohol (PVA) is a linear polymer that has a formula $[CH_2CH(OH)]_n$ as shown in Fig. 1, which is usually prepared from a subsequent polymerization reaction of a homopolymer obtained from a protected monomer, such as vinyl acetate [1].

Considered as a hydrophilic synthetic polymer, which can chemically bind to the surface of materials and retains water on the surface, and is a non-toxic and biodegradable polymer [2].

However, PVA can be classified into two groups (a) partially hydrolyzed and (b) fully hydrolyzed Fig. 2. The molecular weights obtained for PVA products may vary (20,000–400,000), depending on the length of the initial vinyl acetate polymer and the level of hydrolysis [3].



$$\left[\overset{OH}{\overset{|}{C}H} - CH_2 \right]_n$$

Fig. 1 Polyvinyl alcohol

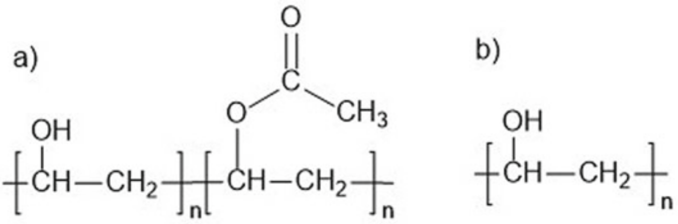


Fig. 2 Structural formula for PVA: **a** partially hydrolyzed; **b** fully hydrolyzed

3 Synthesis and Obtaining of the PVA

PVA is a thermoplastic biopolymer which is synthesized from the hydrolysis of its precursor [2]. In 1924 it was synthesized by Hermann and Haehnel by saponification of poly(vinyl ester) in a solution of sodium hydroxide [4].

Unfortunately, there is a problem with this synthesis since vinyl alcohol is a chemical that is very unstable. Which easily tautomerizes into acetaldehyde, which is a very stable resonance structure of vinyl alcohol [5] Fig. 3. Therefore, given the unstable nature of the structural monomer (vinyl alcohol), PVA cannot be synthesized by polymerization of its structural monomer [4].

Therefore, because of this, other methods are necessary for the elaboration of the PVA. The most used method for its commercial production is vinyl acetate hydrolysis (see Fig. 4), where vinyl acetate is used as a monomer as raw material, and controlled partial alkaline hydrolysis of vinyl acetate is performed where the ester group of vinyl acetate is partially replaced with a hydroxyl group in an aqueous sodium hydroxide environment. The duration of the saponification reaction determines the degree of hydrolysis of PVA [4].

There are three other routes to obtain the PVA however, they are unusual being these:

Fig. 3 Tautomerization of vinyl alcohol

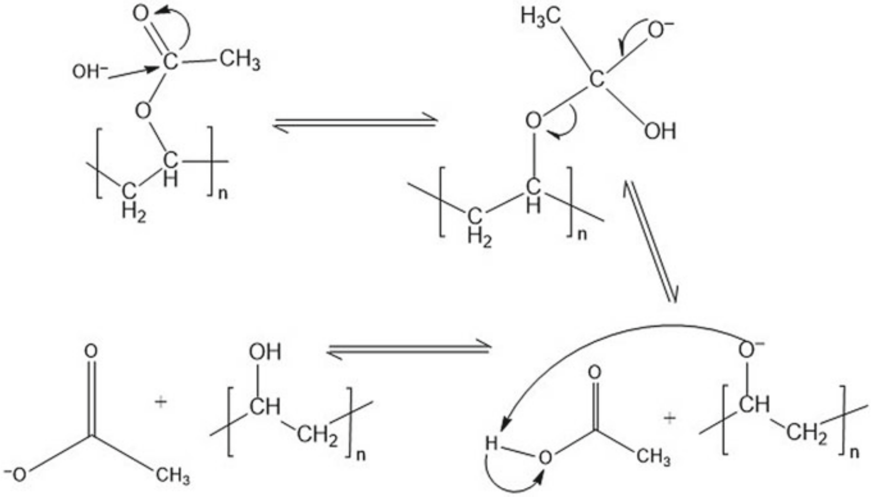


Fig. 4 PVA hydrolysis method

Route 1: Polymerization of vinyl tert-butyl ether was carried out at −78 °C with boron trifluoride diethyl etherate in toluene.

Route 2: Polymerization of vinyl tert-butyl ether was carried out at −78 °C with methylene chloride.

Route 3: It is a route normally used at the laboratory level from poly (vinyl trimethylsilyl ether), which is polymerized with ferric chloride in nitroethane at −78 °C.

It is necessary to consider the different methods of synthesis since the tacticity of the PVA will depend on them; for example, when the synthesis is carried out employing the hydrolysis of polyvinyl acetate, atactic PVA will be obtained; nonetheless, when the polymerization of vinyl tert-butyl ether in toluene is carried out isotactic PVA is obtained as a result. On the other hand, slightly syndiotactic PVA is the result of polymerization of vinyl tert-butyl ether in methylene chloride, and a highly syndiotactic form is the result of polymerization of polyvinyl trimethylsilyl ether in nitroethane [6].

4 Properties of PVA

The physical properties of PVA (density, crystallinity, solubility, degree of polymerization, etc.) will depend on the percentage of hydrolysis, molecular weight, and humidity [3, 4].

It is considered one of the polymers with superior tensile strength, flexibility, toughness, and gas barrier characteristics, [2, 3] as well as markedly superior characteristics such as oxygen barrier which is important for packaging applications of food, [2] however, to avoid gas permeability it must be protected from moisture. The properties are shown in Table 1.

This material is a semi-crystalline polymer that involves an amorphous and a crystalline phase, it is biodegradable, hydrophilic and non-toxic with good thermal and chemical stability to be considered as an excellent film former [7, 8].

Table 1 Properties of PVA

Properties	Range	References
Density	1.19 g/cm^3	[1]
Glass transition temperature (T_g)	53 °C	[1]
Melting temperature (T_m)	131 °C	[1]
Refractive index	1.47 at 630 nm	[1]
Molecular weight	20,000 to 400,000 g/mol	[4]
Solubility	Soluble in water. Insoluble in aliphatic and aromatic hydrocarbons, oils, ketones, and esters	[4]

The tensile properties of PVA will depend on the molecular weight and the degree of hydrolysis, so in 2015 Gaaz et al. mentioned that the PVA complete hydrolysis has a tensile strength of 1.6 GPa, and a Young modulus of 48 GPa with a breaking elongation of 6.5% [3]. On the other hand, Vorrada et al. in the same year presented values of 25.4 MPa in tensile strength and Young module of 27.6 MPa with partial hydrolysis [9].

Additionally, the oxygen barrier property is expressed from the oxygen permeability coefficient, which is defined as the amount of oxygen transferred through a packaging material per unit area and time. Considering that PVA is soluble in water, it has a high permeability to water but a very low oxygen permeability due to its crystal structure and the strong interaction between molecules [10]. Therefore Krumova et al. in 2000 demonstrated that the barrier properties of PVA could be improved when crossed with 3–5% boric acid due to the creation of a new hydrogen bond between the –OH groups of PVA and boric acid [11].

In the case of surface tension, it appears that, at a lower concentration, the macromolecules of the PVA will be on the surface acting as a surface tension reducing surfactant [6].

5 PVA Blends

Currently, mixtures of different materials are becoming more common due to the need for materials with more specific applications, one of which is directly related to the food industry, more specifically to food packaging. Although the food industry has used polymeric materials for decades, the problem has always been their high environmental impact, a problem that society has been taking more seriously over the years.

Although PVA has biodegradable properties, unfortunately, these biodegradability rates are very low, which justifies a reason to be mixed with other materials [12]. The biocompatibility of PVA in conjunction with other polymers such as those derived from chitosan can be used in the development of scaffolds [13].

Chitosan (Fig. 5) is one of the most abundant natural polymers; it is obtained by deacetylation of chitin which is mainly found in the exoskeletons of many insects and crustaceans. Chitosan has good antimicrobial properties, and low toxicity without forgetting the fact that it is a renewable material [14]. The good properties of PVA and its excellent ability to form films in combination with the antimicrobial properties of chitosan make PVA/chitosan blends excellent for packaging and medical applications [15].

Starch is composed of two polymers, amylose and amylopectin; their proportions can vary depending on the organism in which starch is present. This material can be found mainly in different foods such as corn, rice, and potatoes, among others [16–18]. Starch/PVA mixtures can improve the material's mechanical properties without losing its biodegradable properties, especially in the presence of plasticizers, nanocomposites, surfactants, and crosslinking agents [19].

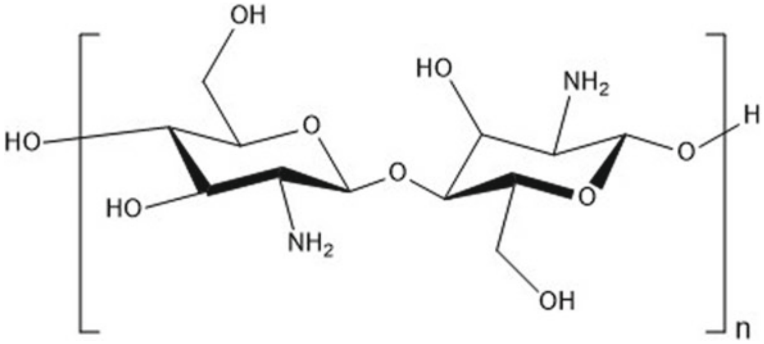


Fig. 5 Chitosan structure

There are also several reported studies of mixtures of different polymers of synthetic origin with PVA, such as polylactic acid (PLA), polyvinylpyrrolidone (PVP), and polypropylene (PP) [20–22]. PVA/PLA blends, for example, are often used in medical applications such as artificial skin, surgical sutures, and tissue engineering, where they can take advantage of their biocompatibility and biodegradability properties [23]. At the same time, PVA/PVP mixtures in the form of films have been studied for the development of biomaterials with application in the pharmaceutical area, formation of nanospheres and nanorods, and release of nitric oxide [24]. It has also been reported that polymer blend fibers such as PVA/PP have been used in the development of various textile products, this is due to their mechanical and biodegradable properties [25].

Other mixtures of natural polymers whose study is still under development are natural rubbers (NR); these are commonly obtained from latex which originates in the bark of trees and has properties such as high elasticity, elongation at break, and resilience. Researchers such as Kalkornsurapranee et al. reported in 2017 the mixture of natural rubber (NR) and PVA using glutaraldehyde as a curing agent with the objective of improving the mechanical properties of the final material [26]. Additionally, various researchers have studied mixtures of PVA and gelatin. Gelatin is a translucent and colorless material used mainly as an ingredient in cooking; its production comes mainly from the hydrolysis of collagen, which comes from the skin and bones of animals [27]. Kim et al. in 2018 developed Gelatin/PVA scaffolds manufactured by 3D printing process for hard tissue regeneration [28]. While Alonso et al. in 2018 fabricated ceramic-reinforced gelatin/PVA hydrogels for application in 3D printed prosthetics [29].

Lignin is one of the most abundant biomacromolecules in the world; it is relatively inexpensive and widely available because it is a renewable material. Commercially, lignin is obtained as a by-product of “wood-free” papermaking. Lignin is mainly burned as a source of energy; it is an essential part present in the cell walls of plants. It maintains the space between the cell walls among other components such as cellulose, hemicelluloses, and pectin that provide mechanical resistance to the cell wall [30]. Lignin has been used in multiple applications like polymer synthesis and composites materials over the years. However, in recent decades, authors such as Korbag et al. studied the mechanical and biodegradable properties of PVA/lignin blend films, reporting that blending PVA with lignin improves the tensile strength and the modulus of elasticity and causes a slight decrease in elongation at break, as well as good biodegradability and an increase in the degradation rate of the mixtures, indicating that the PVA/lignin blended films can be potentially suitable as an ecological packaging material [31].

6 Properties of PVA Blends

A wide variety of materials that can be mixed with PVA with the purpose of obtaining new properties that materials do not normally have individually. Thanks to this, a better cost-effectiveness ratio can be guaranteed in some of the existing applications for PVA, such as medicine and the food industry.

Researchers in recent years have been looking for a way to develop mixtures of materials for packaging in the food industry based (totally or partially) on polymers with biodegradable properties from renewable resources, which is presented as a good alternative to reduce the environmental impact [32]. Regardless, PVA is not limited to mixing only with one material; in fact, PVA blends and multiple additional materials have been studied with the aim of obtaining multiple properties in a single material [33].

Tanwar et al. in 2021 manufactured a film with antioxidant properties using PVA and corn starch (ST) and incorporated for the first time coconut shell extract (CSE) and sepiolite clay (SP) with the aim of evaluating the microstructure and the physical properties of the material. The authors reported that CSE provided excellent antioxidant properties to PVA-Starch films. The number of phenols in the films made from PVA/ST/SP/CSE improved with the increasing amount of CSE. The addition of coconut shell extracts affected the physical properties of the fabricated biocomposite films. The addition of CSE and sepiolite significantly improved the mechanical properties (elongation at break) of the films [34].

Although PVA is a synthetic polymer, it is characterized by low toxicity, biodegradability, water solubility and is excellent for forming films when blended with other materials. Souza et al. in 2021 developed films for food packaging based on chitosan (CS), zein (ZN), and PVA using hot extrusion and molding methods. Demonstrating that the films with a greater amount of CS and ZN presented greater roughness, presence of globular clusters, and heterogeneity that may have influenced the values of water permeability and better barrier property due to the tortuosity that decreases as the PVA content increases [35]. Hydrogel films with potential biomedical properties have also been reported, Hubner et al. in 2021 obtained hydrogel films by mixing PVA/gelatin with potential for wound dressing, indicating that the hydrogel is 65% intumescent, 146% elastic, and has a mechanical resistance of 3.40 MPa [36].

It has also been reported that blending PVA with PLA improves PLA's ability to degrade in water, which would reduce its environmental impact when the latter is disposed. Huang et al. in 2020 developed a series of seawater degradable PVA/PLA blends by blending PLA with water-soluble PVA, conducting a degradation test in natural seawater for 180 days, indicating that weight loss and changes in molecular weight show that the weight loss rate of PVA/PLA blends in seawater increases significantly, and the degradation rate of the PLA phase also improves compared to pure PLA, concluding that the PVA can be used as an effective hydrolysis accelerator in a PLA matrix, helping to significantly reduce PLA molecular weight and increase PLA degradation rate [37]. Also, authors such as Kanca et al. have carried out studies on the tribological properties of PVA/PVP hydrogel blends against articular cartilage in the area of medicine, indicating that the PVA/PVP blend has a low coefficient of friction (COF) (between 0.12 ± 0.01 and 0.14 ± 0.02) concluding that because PVA/PVP hydrogels produce a low COF against articular cartilage and do not damage the contact surface of articular cartilage, they are attractive as cartilage-mimicking materials [38].

7 PVA-Based Nanocomposites

Polymer-based nanocomposites have received attention from researchers in recent years due to the high demand for materials that are lighter, cheaper, or have better properties and performance [39]. The use of materials with nanometric sizes such as nanoparticles, nanofillers, nanotubes, nanofibers, nanoclays, and nanocrystals in conjunction with biodegradable polymers such as PVA presents good opportunities to improve their properties with a good cost-effectiveness ratio.

Graphene oxide (GO) has been used as a nanofiller to create a PVA-based nanocomposite for applications such as optoelectronics, to its use as a material with antibacterial capacity in the pharmaceutical area. Gulshan et al. in 2021 studied PVA-based nanocomposite and GO nanofiller using a casting method for their preparation, indicating that PVA/GO nanocomposite with tunable optical properties can be used in optoelectronic devices [40]. Furthermore, Hajeeassa et al. in 2018 used graphene mixed with carbon nanotubes to form a PVA-based nanocomposite through the solution mixing method with the assistance of ultrasonic radiation, with the aim of creating a material with potential antibacterial activity such as *Escherichia coli*, indicating that the nanocomposite has 100% inhibition compared to pure PVA [41].

Due to the high demand for eco-friendly materials, researchers have also been developing devices to obtain renewable energy easily. Khan et al. in 2021 developed a methodology for the development of organic photovoltaic solar cells using PVA and ZnO nanoparticles for the formation of the nanocomposite in the form of films, all with the objective of improving the performance of solar cells [42]. Alternatively, Kaler et al. in 2018 studied the development of thin films of TiO_2/PVA nanocomposites which were prepared by the solvent casting method to be used as anti-reflective and UV masking coatings for their application in solar cells [43].

Over the years, cellulose nanocrystals have been used in multiple applications due to their ease of obtaining; in fact, they are one of the most abundant natural polymers in the world without mentioning that they come from renewable sources. Its applications range from the manufacture of electronic device screens to the development of filters and membranes. Tortensen et al. in 2019 studied the use of cellulose nanocrystals as an additive for PVA nanocomposite membranes for the separation of CO_2/N_2 mixed gases, indicating that cellulose nanocrystals have a higher performance compared to membranes developed with carbon nanotubes [44].

Carbon nanotubes (CNT) have been used for years in various polymeric and inorganic materials due to their surprising mechanical and thermal properties. For example, Yanmaz et al. in 2021, developed a nanocomposite based on sodium dodecyl sulfate (SDS) and modified single-walled carbon nanotubes (SWCNT) in a PVA matrix with the purpose of evaluating the thermal properties of the nanocomposite, indicating that the SDS added to the polymeric matrix and the modification of SWCNT raised the glass transition temperature of PVA, to increase the adhesion of the material. Concluding that these types of nanocomposites can be used for new kinds of coatings, adhesives, and related areas [45].

8 Properties of PVA-Based Nanocomposites

Multiple improvements have been reported on PVA-based nanocomposites; in contrast to pure PVA; the thermal, optical, and mechanical properties are able to be improved based on the type of material used. Some properties, such as the mechanical ones, depend on the type of nanofiller, quantity, incorporation method, and its dispersion in the polymeric matrix. Graphene and graphene oxide, for example, have been used as nanofillers to increase thermal properties such as T_g, T_m, and initial decomposition temperature (T_{di}) as reported by Park et al. in 2019 [46]. Other authors, such as Mousa et al., took a more economical and eco-friendly approach using bamboo charcoal (BCs) as nanofiller; BC has a good affinity with PVA and thus is able to obtain excellent properties of PVA/BC nanocomposites. PVA/BC nanocomposite films were fabricated fully environmentally friendly using a simple solution casting method to achieve the high dispersibility of BCs. With the inclusion of only 3% by weight of BC, the tensile modulus and tensile strength of the PVA/BC nanocomposite films were improved by 70.2 and 71.6%, respectively, compared to the pristine PVA films [47]. On the contrary, Parthasarathy et al. in 2020 evaluated the optical, thermal, and mechanical properties of PVA nanocomposites with F_2O_3 nanofiller incorporated in the material, indicating an increase in the thermal stability of the material compared to pure PVA, Young's modulus (Y) and the tensile strength obtained an increase from 1.4 to 11.1 GPa and from 85.2 to 117.4 MPa after the nanofillers were dispersed in the material [48].

Different materials have also been used with the objective of improving the electrical properties of PVA nanocomposites, generally with applications related to optoelectronics. Devikala et al. in 2019 carried out the study of the electrical properties of the PVA/Alumina nanocomposite (PVA/Al_2O_3), concluding that the electrical properties of the nanocomposite increased as the concentration and temperature of the alumina increased [49]. Soliman et al. in 2020 developed films of a nanocomposite based on PVA and barium titanate ($BaTiO_3$) with the aim of evaluating the structural and optical properties of the material, indicating that the energy of the optical band gap decreased as the concentration of $BaTiO_3$ in the PVA matrix increases. The direct band gap decreased from 5.9 eV to 5.65 eV, and the indirect band gap decreased from 4.9 eV to 4.2 eV, also indicating that the optical conductivity increases with increasing concentration of $BaTiO_3$ in the matrix, concluding that PVA-$BaTiO_3$ films can be used in high industrial technology such as organic circuit boards and in electronic applications [50].

Various researchers have also been studying in recent years the possibility of manufacturing PVA-based nanocomposites with antimicrobial properties in order to provide a more ecological approach to this type of material. Sarwar et al. in 2018 developed PVA/nanocellulose/silver nanocomposite films for antimicrobial food packaging, indicating that the nanocomposite films exhibited strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [51]. Other researchers have also reported the development of PVA-based nanocomposites with antifungal activity. Chougale et al. in 2020 designed a PVA/TiO_2-based nanocomposite used against fungal strains, indicating that the nanocomposite exhibits a large zone of inhibition compared to the standard drug streptomycin [52].

9 Applications

Some of the main applications of PVA, mixtures, and PVA-based nanocomposites are found in the packaging sector, considered the most important global industry in the development of countries. Packaging is very important to improve the added value of food since it maintains its safety and quality by controlling its exposure to factors such as oxygen, light, humidity, pathogenic bacteria, and various contaminants. In this way, it seeks to maintain and ensure the quality of the products for a long time. The study of biodegradable materials for this purpose has increased in recent years due to the social pressure that exists regarding environmental problems due to the uncontrolled use of plastics. In this sense, Wu et al. in 2017 developed films for food packaging based on Starch/Polyvinyl Alcohol/Citric Acid, demonstrating strong antimicrobial activity against Listeria monocytogenes and Escherichia coli; they also found that these films prevented the formation of condensed water on their surface when subjected to freshness tests in fresh fruits. They finally concluded that these mixtures can be used as an active food packaging system [53]. The simple mixture of starch and PVA presents low mechanical properties however, Ali et al. in 2021 incorporated coir fiber particles as reinforcement using the solution casting method; they observed that the tensile strength increased with the incorporation of fiber particles, so they inferred that these films can be used in packaging applications due to its better tensile strength [54]. The films developed by Ravindran et al. in 2020 are extremely interesting because in their study, they mixed PVA/PEG and silver nanoparticles obtained by green methodologies using the broth of *Capparis* *zeylanica* leaves, the thin films they obtained showed excellent characteristics in several aspects to be used in food packaging and biomedical uses [55].

On the other hand, studies have been made of membranes with a mixture of chitosan/PVA/ZnO processed using the electrospinning technique, which has shown very encouraging results in healing diabetic wounds [56]. Accelerating the wound healing process is being studied in the development of new materials for wound dressings. In the study by Solak et al. in 2021 demonstrated antibacterial activity in the biomaterial developed from polyvinyl alcohol (PVA) and sodium alginate (NaAlg) to prepare microspheres, which were obtained by the crosslinking method in emulsion with calcium chloride ($CaCl_2$), they concluded that this biomaterial could be effective in treating infections [57]. Furthermore, PVA and PVP patches plasticized with glycerol and propylene glycol have been developed for application in cardiac tissue engineering, this study was carried out by Pushp et al. in 2021, where the patches were evaluated for mechanical and physicochemical properties. The viability, ability to support in vitro attachment, and in vitro beating behavior of neonatal mouse cardiomyocytes and proliferation were also evaluated. The patches were evaluated in vivo by performing the study under the subcutaneous skin pocket and in rat heart models, revealing that they are biodegradable, biocompatible, and safe for cardiac tissue engineering applications [58]. Another type of wound dressings to prevent wound infections are those made with poly(ionic liquid)/PVA, which exhibited effective antibacterial ability against molds, bacteria, and fungus. In addition, histological tests showed that after 15 days of the healing process, the hydrogel can promote the reconstruction of the epidermis, resulting in great potential as an antibacterial dressing for wound healing [59].

The World Health Organization has identified the water pollution crisis as the main problem. For this reason, it is important to treat water to eliminate contaminants such as heavy metals, chemical materials, suspended gases, biological contaminants, among others [60]. In recent years, nanocomposite films have been developed for water purification due to their advantages, such as permeability, hydrophobicity, and others. Ahmed et al. in 2019 investigated PVA nanocomposite films incorporating functionalized multi-walled carbon nanotubes at different concentrations to evaluate them in environmental applications and analyze their ability to capture pesticides, heavy metals, bacteria, and fungi from wastewater. They demonstrated that these films present efficiency in the elimination of heavy metal and pesticide capture, which is enhanced as the concentration of functionalized multi-walled carbon nanotubes increases, they also present antimicrobial activity. These results demonstrate that these films have potential in wastewater treatment applications [61].

10 Conclusions

PVA films are often attractive in various areas of industry due to improvements in their mechanical, chemical and thermal properties. These improvements arise due to the investigations that have been carried out regarding the mixture of different polymers, fillers or crosslinking agents with PVA to promote improvement in its properties. A favorable advantage of these cross-linked films is their ability to swell on contact with an aqueous solution, making them interesting in various areas of industry with great economic potential. The potential applications of these cross-linked films vary from biomedical applications such as regenerative biomaterials, drug release, scaffold generation, in the textile and pharmaceutical area and in the food area as films for packaging vegetables and fruits. The addition of nanoparticles such as graphene, graphene oxide, TiO_2, ZnO and even cellulose nanocrystals, among others to PVA, is opening new paths for different applications in areas such as optoelectronics, obtaining organic photovoltaic solar cells and even in membranes for the separation of gas mixtures. Also, PVA can be used in new types of coatings, adhesives, and related areas.

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Diversifying Polyhydroxyalkanoates: Synthesis, Properties, Processing and Applications

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Abstract Polyhydroxyalkanoates (PHAs) are polyesters synthetized by microorganisms or a chemical synthetic route with inherent chemical and physical properties comparable to conventional non-biodegradable polymers but a less environmental impact. Furthermore, the new generations of PHAs have found engineering and specialties applications in biotechnological sector, biomedical for tissue engineering, drug delivery, etc. Similarly, synthesis, processing and recycle of PHAs involves processes that helps to change until a circular and green economy. Nevertheless, the low cost-effectiveness associated with fermentation and downstream processing for recovery and purification of PHAs after biopolymerization are one of the issues that remains. Additionally, PHAs offer several mechanical behaviors from hard to elastic due to partial crystallinity, wide values in glass transition temperature, variety of structures of repeating units, as well as several additives and fillers to design tailor-made properties. Moreover, PHAs are usually blended with other biodegradable polymers searching synergistic interactions (e.g., in mechanical, biodegradability, barrier properties, etc.) through miscibility modification and microdomains interactions for the diversification of their applications. Eventually, single use products of PHAs for packing could improve the managing plastics waste through reach short times of biodegradation, a carbon neutrality and the use of some residues and contaminants sources as raw materials for PHAs synthesis.

Keywords Polyhydroxyalkanoates synthesis · Synergistic interactions · Biodegradability

1 Introduction

In the last years, the production rate of plastics has reached above 360 million tons per year [1] even during the economy contraction by COVID-19 pandemic. Considering the substantial quantities of plastic waste generated, only 9% is recycled, 19% is

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incinerated, 50% reaches landfills and 22% remains as uncollected litter or mismanaged [2]. Additionally, approximately of 0.02% of plastic waste leaked into aquatic environments and 0.005% flowed into oceans [2]. While a build international willingness and participation to curb plastic pollution through summits, agendas, social and individual strategies [3, 4] and governmental regulations [5, 6] the plastic waste exceeds the efforts to mitigate plastic pollution [7, 8].

Green polymers are produced using green chemistry, and IUPAC defined the latter as the invention, design and application of chemical products and processes to reduce or to eliminate the use or generation of substances hazardous to humans, animals, plants, and the environment [9]. Polyhydroxyalkanoates (PHAs) are one of the most attractive types of green polymers due to biodegradability [10], compostability [11], biocompatibility [12, 13], hydrophobicity [14], wide availability in mechanical behavior [15], barrier properties (for single-use products) [16], etc. [17]., in medical, environmental, energy and other areas [18].

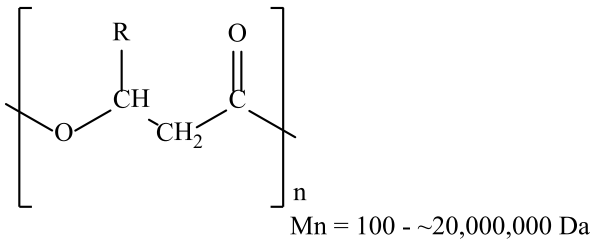
The PHAs are polyesters synthetized by a wide variety of microorganisms (generally by bacteria) or via pure chemical synthesis with inherent chemical and physical properties so useful as the single-use and non-biodegradable polymers showing a less environmental impact compared with conventional polymers [17]. Furthermore, the new generations of PHAs have found specialties and engineering applications as in biotechnological sector, for example, in biomedical for tissue engineering, drug delivery, cosmetic surgery, etc. [19].

For the most part, the PHAs are synthetized by microorganisms producing lipid inclusions for energy storage in granular form inside the microorganisms' structure. The advantage of PHAs considering other biopolymers is based on mechanical and thermal response because are natural polyesters of 3-, 4-, 5-, 6- and m-hydroxyalkanoic acids which produces generally thermoplastic polymers. In the backbone of polymer chain there is a –O–C– chemical bond that it makes sensitive (due to polarity of the covalent bond) to chain scission via hydrolysis using a biotic and/or abiotic degradation, see Fig. 1.

Compared to other biopolymers, polyhydroxyalkanoates have more than 150 different repeating units [22, 23], with a general structure that is shown in Fig. 1. If the group $R = \mathrm{CH_3}$ the polymer is named polyhydroxybutyrate of poly (3-hydroxyalkanoate) (P3HB). Nowadays, there are available several chemical functionalizations of the side groups of PHA searching for specific activities and interactions to diversify their applications. The molecular weight of PHAs is from hundreds to several million Daltons according to type of microorganisms, metabolic route, the type of reactor operation and carbon sources fed for the PHAs obtained with microbes and stability of polymerization mechanism, as well as conditions and type of reactor for a pure chemical synthesis.

According to the size of repeating units the PHAs are classified into three main groups:

- Short chain length (*scl*-PHA): if the PHAs have 3–5 carbon atoms into repeating unit, leading to a mechanical behavior rigid and brittle, not recommended for biomedical and packing film applications.



Polymer name by number of carbon atoms if R =

R	Polymer name/abbreviation	R	Polymer name/abbreviation
hydrogen	poly (3-hydroxypropionate)/P3HP	octyl	poly (3-hydroxyundecanoate)/P3HU
methyl	poly (3-hydroxybutyrate)/P3HB	nonyl	poly (3-hydroxydodecanoate)/P3HDD
ethyl	poly (3-hydroxyvalerate)/P3HV	decyl	poly (3-hydroxytridecanoate)/P3HTriD
propyl	poly (3-hydroxyhexanoate)/P3HHx	undecyl	poly (3-hydroxytetradecanoate)/P3HTD
pentyl	poly (3-hydroxyoctanoate)/P3HO	dodecyl	poly (3-hydroxypentadecanoate)/P3HPD
hexyl	poly (3-hydroxynonaoate)/P3HN	tridecyl	poly (3-hydroxyhexadecanoate)/P3HHD
heptyl	poly(3-hydroxydecanoate)/P3HD	tetradecyl	poly (3-hydroxyheptadecanoate)/P3HHpD

Fig. 1 General structure of polyhydroxyalkanoates [20, 21]

- Medium chain length (*mcl*-PHA): for PHAs having 6–14 carbon atoms, shows an elastomeric response to mechanical tests but a lower mechanical stress that limits the applications.
- Long chain length (*lgl*-PHA): the repeating unit with more than 15 carbon atoms with elastomeric properties [15].

The mechanical properties and green characteristics depends or their pathways synthesis [24], chemical structure [25] and the hierarchy structure at nano and micro level. Because of chemical and structure difference of repeating units in PHAs, the physical, chemical, physicochemical properties as well as biodegradation differs from each other.

The PHAs have several disadvantages of microbial biopolymers in comparison to synthetic polymers, one of them are the high costs involved in the fermentation process, the carbon source, the efficiency of PHAs yield, the productivity of the process and down-stream processing [26, 27]. The final cost of PHA is directly related to PHA accumulation capacity of microorganism and productivity of the process. Furthermore, the economy of the process is still governed by the final application of the end products [28]. Nevertheless, these are eco-friendlier materials for production of single-use products, and these have several applications in medical fields. In addition to the advantages mentioned, the life cycle analysis has shown that PHAs are more sustainable that conventional synthetic polymers [17]. The big challenges are focus on segregate the pure monomers from complex mixtures and the biotechnologies of genetic engineering of microorganisms that allows the develop of new strains with higher accumulation capacities.

2 Synthesis of Polyhydroxyalkanoates

The synthesis of PHAs has been focused in two main routes: (1) biosynthesis to obtain semiprecision/speciality/engineering polymers [29, 30] and (2) precision polymers obtained by chemical routes for advanced applications searching stereoregularity [31], tacticity [32], sequence [33] and specificity [15] as well as incorporation of new side groups or functional groups [34]. Nevertheless, both ideas contribute to develop of mechanisms for diversification of structures' polyhydroxyalkanoates and with this an increase of applications with a variety of chemical, physical and physicochemical properties.

2.1 Natural and Synthetic Synthesis of Polyhydroxyalkanoates

Biological and bioinspired polymerizations make use of the key feature in biology, the sequence control during biopolymerization. Here, the high complexity, and advanced properties of proteins of proteinaceous (e.g., enzymes) or polynucleotide (as in ribosomes) in nature involved in these polymerization mechanisms is simplify through metabolic pathways to obtention of PHAs.

The PHAs can be obtained by microorganisms are from ten thousands to several million Dalton controlling the PHA synthase structures and activities [35] and metabolic pathways that is not easily by a chemical synthesis approaches [36]. Besides, is easy to understand the lower cost in production at industry level because of diversification of raw materials. On the other hand, a higher control in the structure, sequence of repeating units, stereoregularity and chain's architecture of the polymer can be expected using ring-opening polymerizations (ROP), among other polymerization types.

2.1.1 Natural Synthesis of Polyhydroxyalkanoates

Natural Microorganisms

The P3HB was the first PHA and was found by Maurice Lemoige in 1926 as intracellular granules in bacterium *Bacillus megaterium* [37]. Since that time, the P3HB has been the most studied and well characterized to be used as reserve material in bacteria above 80% of the cell dry weight (CDW) [38]. A biological polymerization is commonly described through metabolized mechanisms normally catalyzed via enzymatic process. However, natural biosynthesis of PHAs regularly does not allows a high control over the polymer chain [30] and isotactic polymers, as well as random copolymers are the usual PHAs produced.

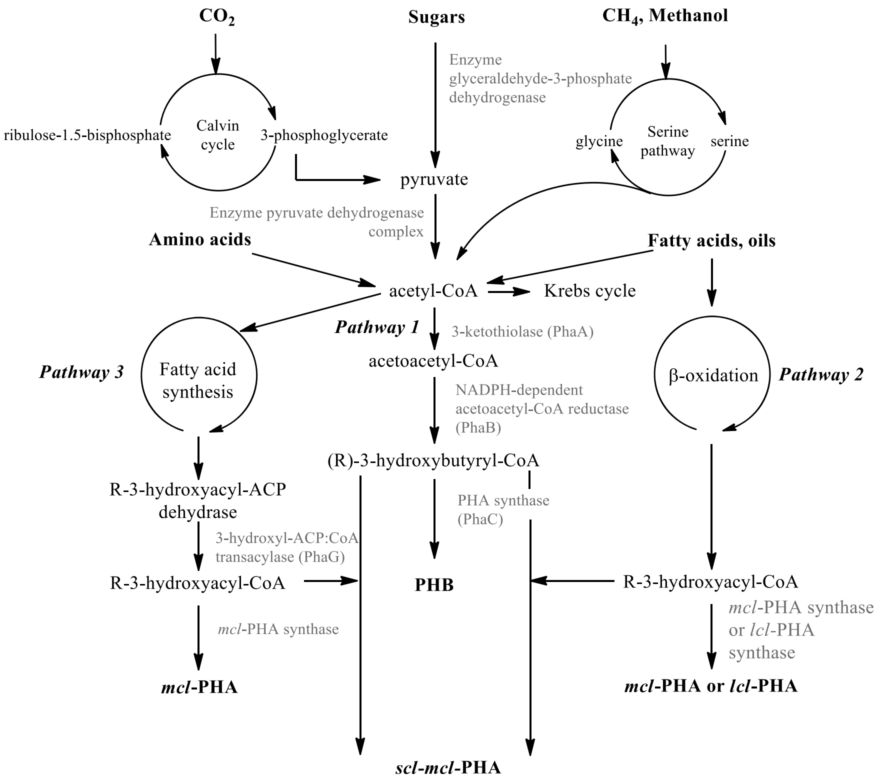


Fig. 2 Mainly metabolic pathways to synthetize polyhydroxyalkanoates [42]

A key factor for the structure of PHAs synthetized inside of microorganisms is the carbon sources fed. Thus, the molecules supplied as raw materials to the enzyme PHA synthase define the ended structure of PHAs. Considering the requirement of nutrients, nutrient stress (i.e., the deficiency or excess of nutrients along the time), and their growth pattern, PHA accumulating bacteria have been classified into two main groups: (G1) those that needs limited nutrients such as phosphorous, nitrogen, oxygen, sulfur and magnesium to store PHAs and are not able biosynthesize PHAs during their growth periods [39, 40] (e.g., *A. eutrophus*, *Protomonas extorques*, *Pseudomonas oleovorans*, and *Pseudomonas*) and (G2) the bacteria not affected by nutrient limitation, and it can accumulate PHAs during its growth phase [41] (e.g., *Alcaligenes latus*, *Promotomonas extorquens*, and recombinant *Escherichia coli*) [17].

Heretofore, there are three primary metabolic pathways (see in Fig. 2) reported to synthetize PHAs:

Pathway 1: To obtain *scl*-PHAs between 3 and 5 carbon atoms, generally starts with the production of 3HB monomers by the Krebs cycle, involving the acetyl-CoA in presence of 3-ketothiolase enzyme (or PhaA) from sugars, fatty acids, oils or amino acids that are converted to acetoacetyl-CoA reductase to hydroxybutyryl-CoA and polymerized by *scl*-PHA synthase to produce P3HB [17, 43, 44].

Pathway 2: Initiate with the fatty acids as raw material by way of the β-oxidation in presence of 3-hydroxyacyl-CoA enzyme and *mcl*-PHA synthase [45] or *lcl*-PHA synthase [46], which preference of size of the repeating unit is due to proteins in the β-oxidation pathway for each one microorganism. Here, the fatty acids are transformed into enoyl-CoA and then by R-3-hydroxyacyl-CoA hydrates to R-3-hydroxyacyl-CoA to produce *mcl*-PHA polymers through *mcl*-PHA synthase. Moreover, a few PHA synthases in natural (e.g., *Thermus thermophilus* HB8, see Table 1) and engineered microorganisms (e.g., *Transgenic arabidopsis thaliana* [36], see Table 2) can produce copolymers of *scl-mcl*-PHAs, simultaneously utilizing pathways I and II.

Pathway 3: The in situ fatty acid synthesis or the use of substrates as glucose, sucrose and fructose pathway starts with R-3-hydroxyacyl-(acyl carrier protein) ACP dehydratase in presence of 3-hydroxyl-ACP-transacylase (PhaG enzyme) to produce R-3-hydroxyacyl-CoA and with *mcl*-PHA synthase finally obtain *mcl*-PHAs [17, 84].

Considering the activities of PHA synthase and depolymerase can affects the size of molecular-weight, M_n, the effect can be minimized in presence of chain transfer agents (as in traditional polymerizations) being helpful for the control using poly(ethylene glycol) (PEG), methanol, ethanol and isopropanol in the culture or via mutations in the N-terminus of PHA synthase [85]. Nevertheless, small changes during biopolymerization normally leads to variations in final molecular weight between a batch and another even at the same conditions.

Extremophile Microorganisms

Extremophiles, which concept is different from extremotolerant, are highly adapted and metabolically active under uncommon environmental. Their use for the synthesis of PHA increases the biological conditions to production of biopolymers at high temperatures, extreme pH conditions, salinity, radiation, desiccation, man-made toxic environments (such as toxic metals, surfactants, etc.) and other unfavorable environmental conditions to overcome, for example, in landfills, ocean, vinasses and toxic waste.

Extremophilic archaea could be an economically viable option in conventional aerobic processes [86]. However, their pathways and PHA accumulation capacities are less-know, even so, specific adaptive mechanisms towards extreme environments by extremophiles and specific role of PHAs are grow up [87]. Additionally, genetic engineering and process engineering approaches are required for high-rate PHA production using extremophilic archaea [86].

Haloarchaea and halophilic bacteria are considered a promising cell factories for PHA synthesis due to its several unique characteristics as high salinity requirement to avoid microbial contamination, high intracellular osmotic pressure allowing easy cell lysis for PHA recovery, and the use of a wide variety of low-cost substrates [64]. Within these, *Hfx. mediterranei* can produce PHAs from various organic substrates up to 70% of dry weight biomass [64].

Table 1 Synthesis of polyhydroxyalkanoates by microorganisms and extremophilic microorganisms

PHA	Carbon source	Microorganism	Cell dry weight % (w/w)
Natural synthesis of PHAs			
P3HB	CO_2	*Cupriavidus necator*H16	88.9 [47]
	Glucose	*Novosphingobium nitrogenifigens Y88*	81.0 [48]
	Fructuose, glucose	*Azohydromonas lata*	76.5–79.4 [49]
	Malt waste	*Azohydromonasaustralica*	70.0 [50]
	4-hydroxyhexanoic, CO_2	*Alcaligeneseutrophus*TF93	67.2 [51]
P3HV	Pure glycerol	*Bacillus* sp. ISTVK1	85.2 [52]
	Glucose	*Bacillus* sp. ISTC1	47.0 [53]
	$NaHCO_3$ and glucose	*Serratia* sp. ISTD04	45.5 [54]
scl-PHA	Sugarcane liquor	*P. fluorescence* A2a5	70.0 [55]
	Commercial glycerol	*Cupriavidusnecator* DSM 545	62.0 [56]
	Activated sludge	Acetate	59.0 [57]
mcl-PHA	Unsaponifed olive oil	*Aeromonascaviae*	96.0 [58]
	Nonanoic acid	*Pseudomonas putida* KT2440	26.8–75.4 [59]
	Fatty acids	*P. putida* Bet001	49.7–68.9 [60]
scl-*mcl*-PHA	Whey	*Thermus thermophilus* HB8	35.6 [61]
P3HB3HV	Vinasse	*Haloferax mediterranei*	50.0–73.0 [62]
	Hydrolyzed whey and valerate	*Hydrogenophagapseudoflava*	40.0 [63]
Natural synthesis of PHAs by extremophile microorganisms			
P3HV3HV	Pre-treated vinasse	*Hfx. mediterranei*	70.0 [64]
PHB	Cellobiose	*H. halphila*	90.8 [64]
P3HB3HV	Maltose	*H. campisalis*	45.0–81.0 [64]
P3HB	Glucose	*Rubrobacter xylanophilus*	51.5 [65]
P3HB	Glycerol	*Chelatococcus daeguensis*	73.0 [66]
P3HB	Glycerol	*Zobellella denitrificans* MW1	66.9 [67]
PHA	Plant	*Bacillus licheniformis*	62.0 [68]
PHA	Palm oil mill effluent	*B. licheniformis M2–12*	88.7 [69]

The actinobacteria *Rubrobacter xylanophilus* has been exposed to multi-extremophilic growth conditions shown highly radiation-resistant, halotolerant, thermotolerant or even thermophilic with accumulation of PHAs [65], see Table 1. A wide spectrum of thermophilic microorganisms had been reported for the production of PHAs as *Chelatococcus daeguensis* TAD1, *Zobellella denitrificans* MW1, *Bacillus*

Table 2 Genetic engineered and metabolically engineered microorganisms involving in polyhydroxyalkanoates synthesis

PHA	Microorganism (bacterial strain)	Key genes	Modification	Pathway
Poly(lactic acid) (added as a reference)	Metabolically engineered *Escherichia coli*	pct$_{cp}$	Plasmid, knockout (OK)	Synthetic/non-natural [70]
Homopolymers				
Polyyhydroxyvalerate (PHV)	*Pseudomonas putida* KT2442	PhaPCJ	Plasmid, KO	Synthetic/non-natural [71]
Poly(3-hydroxypropianate) (P3HP)	Recombinant *Escherichia coli*	Gpd1, gpp2, dhaB123, pduP	Plasmid	Synthetic/non-natural [72, 73]
Poly(hydroxybutyrate) (P4HB)	Recombinant *Escherichia coli*	PhaP, sucD, 4hbD	Plasmid, KO	Succinate degradation [74]
Random copolymers				
Poly(3-hydroxybutyrate-co-3-hydroxypropionate) (P3HB3HP)	Recombinant *Escherichia coli*	gpd1, gpp2, dhaB123, pduP	Plasmid	Synthetic/nonnatural [75]
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	Recombinant *Escherichia coli*	cimA, pct, bktB	Plasmid, KO	Citramalate pathway, threonine synthesis pathway [76]
Poly(3-hydroxybutyrate-co-hydroxyhexanoate) (PHBHHx)	Recombinant *Cupriavidus necator*	PhaC, PhaJ, bktB, PhaB1	Plasmid	PHA synthesis, β-oxdation cycle, BktB-dependent condensation pathway [77]
Block copolymers				
Poly(3-hydroxybutyrate)-b-Poly(3-hydroxypropionate) (P3HB-b-P3HP)	Metabolically engineered *Escherichia coli*	dhaB, pduP, PhaA, PhaB	Plasmid	Synthetic/non-normal [78]
Poly(3-hydroxybutyrate)-b-Poly(hydroxyvalerate) (P3HB-b-P3HBV)	Metabolically engineered *Cupriavidus necator*	PhaCAB	None/Wild type	PHA synthesis [79]

(continued)

Table 2 (continued)

PHA	Microorganism (bacterial strain)	Key genes	Modification	Pathway
Poly(hydroxybutyrate)-b-Poly(4-hydroxybutyrate) (P3HB-b-P4HB)	Mutant *Pseudomonas putida* KTHH06	PhbC, orfZ	Plasmid KO	Synthetic/non-natural [80]
Functional PHAs				
Poly(3-hydroxy-5-phenylvalerate) P(3HPhV-co-3HDD)	Recombinant *Pseudomonas entomophila*	PhaJ, PhaC	KO	Weakened β-oxidation cycle [81]
Poly(β-hydroxyalkanoate) (having aromatics groups) PHPhAs	Mutant of *Pseudomonas putida U*			[82]
Poly(hydroxydodecanoate-co—hydroxy-9-decenoate) P(3HDD-co-3H9D)	Recombinant *Pseudomonas entomophila*			[83]

licheniformis and *B. licheniformis* M2–12 with a higher content of dry weight over 60% of biomass [88].

The metabolic pathways do not exist in all extremophiles and the specific production of PHAs in extremophiles are not well-understood yet. Nevertheless, a necessity to develop genetic engineering approaches might improve their capacity for their commercialization and application in real scenarios.

2.1.2 Synthetic Biosynthesis of Polyhydroxyalkanoates

The production of PHAs can also be obtained via biosynthetic pathways, via genetic engineering, metabolic engineering, or synthetic biology approaches. In addition, genetically engineered polymers are designed by sequence-specific polymers by genetic engineering [89] as the case of proteins and polynucleotides used in genetic tools, technologies, processes, and other methods. With the development of genome editing and molecular biology approaches and modifying the PHA synthase enzyme, tailor PHAs can be synthetized with some degree of control in sequence of repeating units (random or block copolymers) and composition of repeating unit, changing the biodegradability, biocompatibility, as well as the thermal/mechanical and other properties [36].

In natural biosynthesis of PHAs the molecules fed to microorganism are structurally related to structure of repeating units of PHAs. However, the enzyme PhaC synthase is a key to create new metabolic pathways to use new molecules as raw materials as sugar that are low-cost [36, 90, 91]. PhaC is the most important element to determine the PHA composition because in bacteria depends not only on monomer supply, but also on specificity of PHA synthases [92]. Other advantage on the use of genetic modified microorganisms is for the search of a high production of PHA by microorganism or design tailor-made and robust microbes to produce PHA even for waste.

The case reported of the fatty acid in β-oxidation pathway and the 3-hydroxyacyl-ACP:CoA transacylase (PhaG) in *Pseudomonas putida*, increases the fatty acid flux and obtaining a higher accumulation of *mcl*-PHA syntheses [93]. The genetic engineering of genes to modify the PhaC encoding a lees-specific PHA synthase in pathway 2 allows the synthesis of PHAs as homopolymers, random or block copolymers, and functional polymers [94, 95], see Table 3. Some PHA synthases have been reported to polymerize *scl* and *mcl* repeating units as enzymes with the feature of low-specify PHA synthases which can be modified by molecular evolution or by chimera formation [36].

Taking into consideration one of the tools of genetic engineering, the system clustered regularly interspaced short palindromic repeats (CRISPR) associated to protein 9 (Cas9) has been used to edit eukaryotic genomes [98]. CRISPR Cas9 has been applied to control PHA biosynthesis pathway flux and to adjust PHA composition changing the composition of PHA synthase [99].

Table 3 Mechanical and thermal properties of P3HB and their copolymers [96, 97]

Material	Maximum stress (MPa)	Maximum deformation (%)	Young's modulus (GPa)	Glass transition temperature (°C)	Melting point (°C)
P3HB	45	4	3.8	9	175
P(3HB-co-3HV) (89.0/11.0 w/w %)	38	5	3.7	2	157
P(3HB-co-3HV) (80.0/20.0 w/w %)	26	27	1.9	−5	114
P(3HB-co-3HV) (72.0/28.0 w/w %)	21	700	1.5	−8	102
P(3HB-co-3HV) (66.0/34.0 w/w %)	18	970	1.2	−9	97
P(3HB-co-4HB) (86.0/14.0 w/w %)	8	391	1.3	−12	68
P(3HB-co-4HB) (64.5/35.5 w/w %)	18	510	1.1	−4	78
P(3HB-co-4HB) (24.8/75.2 w/w %)	–	–	–	−10	59
P(3HB-co-3HHx) (91.0/9.0 w/w %)	7	167	1.2-	−2	63
P(3HB-co-3HHx) (83.6/16.4 w/w %)	12		320.2	−1	57
P(3HB-co-3HHx) (62.0/38.0 w/w %)	–	–	–	0	71

Clustered regularly interspaced short palindromic repeats interference (CRISPRi) has been employed to control the PhaC transcription and thus PhaC activity to maximize the P3HB contents [100]. A higher PhaC activity leads to higher accumulation with a less molecular weight and a wider molecular weight dispersity. PHB controlled in the intervals of 2.0 for 75.0% of CDW [85].

Finally, the extraction methods of PHAs from inside of microorganisms at the end of bioprocess can be classify in: solvent extraction, floatation, supercritical fluid extraction or aqueous two-phase extraction [17]. The PHA extraction is one of the costliest procedures in PHA production, it requires the separation of PHA-containing cells and the cell lysis to release intracellular PHA granules. Such as other synthesis process, the chemical and physical separation process needs more develop searching efficiently to reduces and grow up to industrial scales.

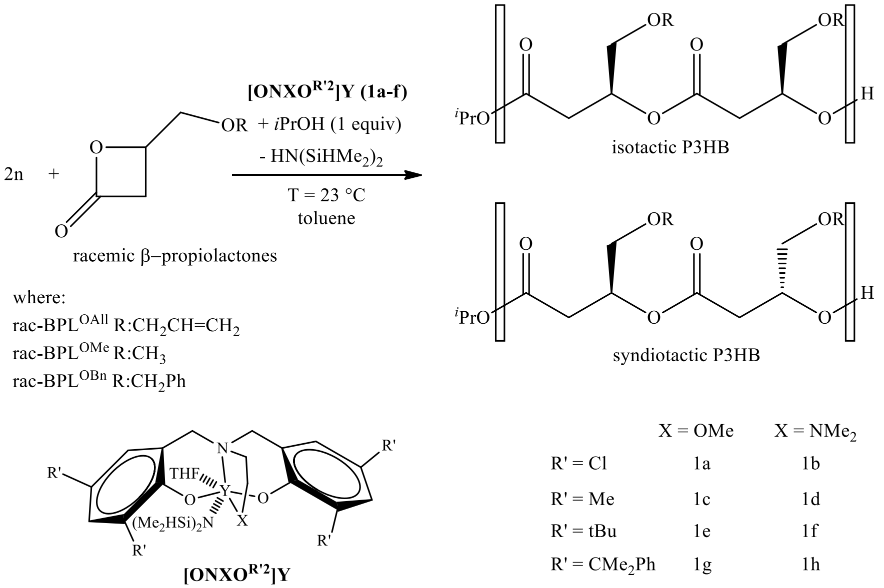
A high production of PHAs is obtained by fed batch or continuous fermentation. In fed-batch culture of bacteria belonging to G1, a two-step cultivation method is often employed. A desired concentration of biomass without nutrient limitation in the first stage after and in the second stage a limiting concentration of nutrients. In second stage, the residual cell concentration remains almost constant, and the cell concentration increases only because of the intracellular accumulation of PHA. Other bacteria belonging to G1 produce PHA more efficiently when a nutrient is limited but not completely depleted. For a bioreactor working in fed-batch of G2, the nutrient feeding strategy to produce yield of PHAs must be applied along with the selection of microorganism based on cell's ability to use an inexpensive carbon source, growth rate, polymer synthesis rate, and the maximum accumulation of PHAs [40].

2.2 Chemical Synthesis of Polyhydroxyalkanoates

PHAs produced by microorganisms have limitations in advances applications by the isotactic and not sequence controlled of repeating units along polymer chain. However, PHAs produced by bacteria commonly are purely isotactic polymers containing a chiral site in each repeating unit leading to thermal and mechanical properties as a function of length of the side group (R-group in Fig. 1), making them more useful for a wide range of applications [31]. Unlike of natural synthesis of PHA, the chemical mechanisms have the aim of incorporation of atypical side groups as aromatic pendant groups to increase the glass transition temperature ($T_{g)}$ or long branches as side groups to increase the flexibility of the chain and with that modify the crystallinity [34]. Additionally, the molecular weight and molecular weight dispersity are reasonably more controllable using chemical synthesis in comparison with microbes because even using the same microorganism and the same conditions and raw materials during the microorganism growth and reproduction are several changes at molecular level. Other advantage to synthesis using metabolic pathways, a pure chemical polymerization might provide a better scalability and faster reaction kinetics [31].

The PHAs has been obtained via ring-opening polymerization of cyclic esters since 1960s, adding up PHA with different chemical structures and stereoregularities. Some bespoke polyesters can be obtained through an organocatalyzed mechanism via ring-opening polymerization of cyclic esters as stereoselective synthesis of isotactic and syndiotactic P3HB by racemic 4-alkoxymethylene-β-propiolactones (BPL^{OR}s) [101] as shown in Fig. 3. Using catalytic activity of alkoxy-functional poly(3-hydroxyalkanoate) and the simple modification of o, p-R' substituents on $[ONXO^{R'2}]^{2-}$ plataform of yttrium catalyst for the switching syndioselective to isoselective polymerization of β-lactones.

Ligny R. et al., has demonstrated that o, p-dichloro-substituted ligand deliver highly isotactic polyesters ($Pi > 0.90$) from three racemic BPL^{OR} monomers (where R: allyl, benzyl and methyl groups). Yttriumisopropoxide catalysts/initiators were generated during the reaction from the activity of 1 equiv. of iPrOH from precursors 1a-g differing by the nature of the R' phenolate substituents. The final number molecular weight was until to 18,300 Da and a molecular weight dispersity about 1.1 [101] similar to other ring-opening polymerizations utilizing other catalyzers [34].



	X = OMe	X = NMe$_2$
R' = Cl	1a	1b
R' = Me	1c	1d
R' = tBu	1e	1f
R' = CMe$_2$Ph	1g	1h

Fig. 3 Ring-opening polymerization from racemic β-lactones to produce isotactic and syndiotactic poly(3-hydroxybutyrate) [101]

3 Physical, Chemical and Physicochemical Properties and Characterization

PHAs shows properties analogue to conventional polymers such as hydrophobicity, resistance to UV, high degree of polymerization. They are usually thermoplastic polymers, exhibiting a wide variety of mechanical properties from hard to elastomeric response with the advantage of biodegradability and biocompatibility.

3.1 Physical Properties

Glass transition temperatures of PHAs is about 0 °C, triggering the flexibility of the polymer chain and a melting point from 50 to 179 °C [96], see Table 3. For other hand, thermal degradability starting at 260 °C for copolymer with 3HV, 4HB and 3HHx repeating units until 300 °C for P3HB [96].

The crystalline structure of P3HB determined by X-ray indicated an orthorhombic unit cell with dimensions a = 0.576 nm, b = 1.320 nm, and c = 0.596 nm. Typically, P3HB forms lath-shape crystals with dimensions of around 0.3–2 μm for the short and 5–10 μm for the long axes. The ability of PHA to crystallize is determined by the inner properties and the structure of repeating units that for P3HB a 70% or higher of crystallinity degree it is found. For random copolymers with 3HB monomer is from

49 to 60% for *mcl* and 2% to 50% for *scl*. For copolymers with 3HB/3HV, 3HB/3HP and 3HB/3HHx the degrees of crystallinities measured by X-ray shown a slightly increased (50–70%) for the first because of isodimorphism, i.e., co-crystallization of the two repeating units of the homopolymer crystal lattices of P3HB and the second monomer. This leads to a reduction near to 50/50% for the rest of copolymers due to defects in the P3HB crystal lattice by the repeating units of 3HB [102].

3.1.1 Mechanical Properties

The wide variety of mechanical properties from hard to elastic is a function on composition, crystallinity degree and structure of repeating unit [40]. The maximum stress at break point is achieve at 45 MPa for P3HB, which has the shorter repeating unit and decrease as is expected for longer repeating units, see Fig. 1. A higher toughness and an elastic behavior are expected for *mcl*-PHA in comparison to *scl*-PHA because of long branches or long repeating units made of –C–C– bonds trigger a flexible polymer chain. Furthermore, a low crystallinity degree by side groups for chain folded and a glass transition temperature below of 0 °C (around −80 °C for monomers with more than 6 carbon atoms) for *mcl*-PHA and *lgl*-PHAs usually shows an elastomeric response.

The PHAs of high molecular weight (> 40 kDa) obtaining via fermentative processes exhibits mechanical properties which can be grouped into three subcategories: *scl*-PHA, which short chain length and repeating units until five carbon atoms with thin crystals and the higher melting point. Young modulus, tensile strength, impact strength and, UV resistance and oxygen permeability, due to this, the P3HB has a similar behavior to isotactic polypropylene, useful as packaging material that can increases with the copolymerization with soft repeating units or blending with elastic and partial o completely miscible polymer to avoid the brittleness that appears after several days. The *mcl*-PHA are amorphous macromolecules with decreasing glass transition temperature with increasing side groups length [103]. For *lcl*-PHA an higher crystallinity degree is expected with longer side groups and similar flexibility on the chain to *mcl*-PHA.

The length and mechanical flexibility of the side chain and its functional group modify considerably the properties like melting point, glass transition temperature, crystallinity, and the biodegradability. The T_g of the amorphous domain can be related to the maximum deformation in PHA along with the crystallinity degree according with the temperature of use of polymer material. However, a higher crystallinity degree leads to poor mechanical properties and PHAs need to be tailored to achieve a better performance and tunable mechanical properties blending with different types of PHAs, other synthetic biodegradable polymers or a chemical modification of PHA.

Some PHA processing methods as the incorporation of additives as plasticizers can lead to modulate the mechanical behavior changing the glass transition temperature in the final product. The PHA as commodity material can be added: poly (ethylene glycol), oligomeric lactic acid, glycerol diglycidyl ether or soybean oil as plasticizers.

3.2 Chemical Properties

Some post-polymerization modification to expand the structural variety of PHAs are focus on the chemical modification of unsaturated bonds. Here, the desired functional groups are attached to the side groups of the polymer chains to crosslinking [104].

The PHA modification add new properties as an enhanced hydrophilicity and thermoresponsibility in PHA-graft-PNIPAm [105], temperature controllable protein adsorption in PHA-graft-PDMAEMA [106], higher thermal degradation temperature and electrical conductivity for PHA-graft-graphene [107], intense photoluminescence under UV laser excitation with PHA modified with rare-earth [108], formation of hydrogels as in PHA modified with PDT [109], and superhydrophobicity in physical surface modification [110].

The carboxylation is the addition of carboxylic (–COOH) functional group to the substrate. These groups are an active binding site for biologically active moieties like hydrophilic as the phase transfer and dissociating agent [15, 111]. The halogenation enhances the properties, functions, and applications of polymers for the addition and substitution reactions. For other hand, hydroxylation makes easier acid or base catalyzed reactions for PHA modification in the presence of low molecular weight mono or diol compounds by the process of hydroxylation [112]. In addition of previous chemical modifications, the epoxidation increases the reactivity attaching epoxy groups that response as anionic and polar groups. These kinds of groups can be used for crosslinked polyhydroxyalkanoates for self-healing applications.

3.3 Properties for Single-Use Products of Polyhydroxyalkanoates

The use of PHAs for bottles, disposables, coatings with cardboard boxes, films, animal feed supplements and biofuels as hydroxyalkanoates methyl esters are some of their single-use applications. The general properties for food packaging material are:

(a) Antimicrobial function.
(b) Mechanical properties.
(c) Optical properties.
(d) Thermal properties.
(e) Eco-friendly.
(f) Barrier properties: gas barrier, aroma barrier, vapor barrier.
(g) UV-resistance.

The polyhydroxyalkanoates shown excellent barrier properties to gases [113]. The permeability of water vapor values of P3HB and its copolymer with 3HV in films are comparable to opponents such as PET and poly (vinyl chloride) [114, 115]. Evaluation of water vapor barrier properties of the packaging material is one of the most importance as physical or chemical deterioration of the packed food and important to maintain and extend the shelf-life of packaged food [116].

The oxygen permeability value of a packaging material is a decisive key to preserve fresh foods (e.g., fruits, vegetables, meats, etc.). The oxidative deterioration affects its color, flavor, and microbial stability for lacteous products. If the oxygen transmission rate is below of 2 nmol $\text{ms}^{-1}\text{GPa}^{-1}$; the material is often labeled as "Barrier polymers" [116]. The CO_2 is another gas of interest to conserve fresh vegetables and food sensible to oxidation. For these barrier properties, a low CO_2 permeability of P3HB exhibits a diffusion coefficient value comparable to poly(vinylidene chloride) [114]. However, the water and gas transport properties of commercial PHAs films shows a higher water and gas permeability values for solvent cast films compared to compressed counterparts [117].

The nature acidic or basic of foods may cause hydrolytic reactions to the packaging material. Unfortunately, the chemical resistance to several values of pH in PHAs are known to undergo acid-catalyzed hydrolytic degradation [114]. This kind of degradation change drastically the mechanical properties of films' polymer. An easy solution can be found in the composite materials or blending to modify the susceptibility to chemical degradation.

Finally, the migration of subproducts as monomers or additives added for the processing plays an important role for food safety. The P3HB using for food packaging, commercial grade BIOCYCLE, obtained through injection process shows a performance compared to polypropylene [118].

The PHA exhibit greater UV-resistance than compounds such as propylene and the addition of UV stabilizer led to improvement in the retention of mechanical properties final products.

4 Processing, Blends and Composites of Polyhydroxyalkanoates

The processing of PHAs usually is leading by extrusion or injecting process with extrusion temperature profiles (140–160 °C) about 100 °C below the degradation temperatures (240–270 °C) [119]. For this reason, the residence time distribution in melting processing must be careful in extruders and injector to minimizing the thermal degradation. The rheological properties depend on the chemical structure and molecular weight because of melting regime the viscosity always increases with the size of molecular weight of the polymers. Some additives for manufacturing for conventional polyesters can be used for PHAs but green additives for are not completely extended for PHA.

Electrospinning is also used to fabricate nanofibers with the selection of an appropriate and non-hazardous solvent o solvent system that determines the rheological properties. The electrospin nanofibers have several applications such filtration sysrems, chemical and optical sensors, tissue engineering, drug release due to their high surface are to volume ratio, small pore size and high porosity.

Blending is a simple and effective approach to produce new polymeric materials with improved properties. Polymer blending has attracted the attention because of polymers with extraordinary properties can be obtained via chemical synthesis are more expensive than existing polymers and blending operations. To achieve this aim, a high or partial miscibility must be searched to ensure at least a micro or nano segregation of phases to achieve a synergistic interaction between domains. On the contrary, a compatibilizer needs to be added to increase the interaction between polymeric phases.

The properties of blends using PHAs with starch, poly (lactic acid), poly (ε-caprolactone) and cellulose can be improved and adding the new properties as homopolymers if a phase segregation at micro or nano level is ensure. The blending of PHA with natural raw materials usually help to reduce the cost and increase the biodegradability for single-use product in packaging products. The cellulose derivatives are of great interest as blending components with PHA because of their compatibility with and the enhance of rate degradation. The PHA blending with starch allows the compostability of materials due to inherent biodegradability and abundance. The PHA blending with lignin makes the blend valuable for the high functional groups presents in lignin. Besides, the thermal analysis indicated that lignin can improve the total thermal stability of P3HB [15].

The blending of PHA with other biodegradable polymers as poly (lactic acid) is environmentally friendly. The P3HB/PLA blend is one of the most studied blends, due to mechanical properties shows an intermediate response following the blend law between the individual components. PCL/PHAs shows miscibility with a PCL content between 0 and 30 w/w% with high flexibility and good biodegradability to be applied in packaging products [120].

Blends of PHBV and polyurethane (TPU) improves mechanical and barrier properties, at highest concentration of TPU and increased water vapor permeability[121].

4.1 Nanocomposites and Fillers

The application of nanoparticles and nanofillers as composite is attractive because of the nanofillers not only enhance the polymer crystallization, the gas-barrier, thermomechanical and physicochemical properties [122]. The mainly nanofillers are: sylylated kaolinite, carbon nanotubes, graphene, organophilic montmorillonite, nanoclay and cellulose nanocrystal [122], which properties added require a minimum concentration to incorporate the partial characteristics of material, e.g., electrical o thermal conductivity with the addition of carbon nanotubes or graphene.

An option to reduce significantly the oxygen transmission rate was obtained with composite of P3HB/cellulose nanocrystal (CNC) by the positive effect on crystallinity of CNC [97].

5 Biotechnological Applications of Polyhydroxyalkanoates

5.1 Drug Delivery Carriers

The PHAs are biodegradable and biocompatible polymers [13] reason for which are used as nano and micro particles to release drugs. The PHAs have been applied in several and in humans to treat the gingivitis [123, 124]. The PHB have also been used in transdermal tissue as vehicle to increase the transdermal permeability of tamsulosin drug [125]. The P3HB and its copolymers in nanoparticles have been used for the release of molecular drugs able to cross intracellular membranes in applications as anticancer drugs, immunomodulatory agents, antibiotics, and hormones [126].

Different processing routes for fabrication of PHAs for drug delivery can be chosen as: spherical or worm-like particles obtained by emulsion or supercritical fluids that normally has been studied until an optimal rate and dose regimen in order to minimize side effects and toxicity.

For in vivo therapy poly (3-hydroxybutyrate-co-4- hydroxybutyrate) are used as antibiotic in Sulperazone® and Duocid® to the controlled release for chronic osteomyelitis [127]. To release an hormone for osteoporosis therapy the P3HB3HHx is used [128]. For cancer of colon, the release of 5-fluorouracil is leading with blend spheres of P3HB3HV and cellulose acetate phthalate [129].

5.2 Scaffolds and Medical Devices

The aforementioned properties for drug delivery and low inflammatory response makes attractive their use in tissue regeneration for human body ad bio-implants [130]. For this, PHAs are used as matrices in vitro for proliferation of human cells as endothelium cell, liver cells, and fibroblasts as they show adhesive properties to PHAs [131]. Endothelium cells, isolated hepatocytes and fibroblast show similar adhesion to P3HB and P3HV when are used as matrices for regeneration or cellular growth [131]. Microspheres of PHBV have been used in brain tissue engineering to support primary neurons [132].

Porous materials can be design via 3D printing, gas forming, phase separation/evaporation as scaffolds in tissue engineering for bone tissue regeneration, tissue engineering blood vessels and cardiac valves. The application of PHA, especially PHA, and biodegradable scaffolds are used to replace defective valves in human heart [133]. Fibers can be obtained through extrusion or electrospinning.

In vivo implants as the poly (3-hydroxyoctanoate)/poly (3-hydroxybutyrate) blends have been used as highly tensile wires for sutures [134]. Poly(3-hydroxybutyrate-co-3- hydroxyhexanoate) has been applied in peripheral nerve tissue engineering for rat model experiments for nerve regeneration [135]. Blends of poly (3-hydroxyoctanoate)/poly (3-hydroxybutyrate) for preparation of blood vessels stents [136].

6 Degradation and Biodegradability of Polyhydroxyalkanoates

The biodegradability of PHAs is well-known due to PHA hydrolases and depolymerases enzymes produced by microorganisms which assist the degradation of PHA. The key factors involving the structure of PHAs in biodegradability are chemical composition, molecular weight, and crystallinity degree. For other hand, the environmental conditions as temperature, pH, moisture, and the oxygen content are the most important factor in biodegradation.

The PHAs are degraded to H_2O and CO_2 under aerobic conditions and to methane under anaerobic conditions by microorganisms in soil, sea, lake water and sewage. The PHAs are solid polymers with a high molecular weight and before to be transported through the cell wall usually the microorganisms excrete extracellular PHA depolymerases to hydrolyze PHA into water-soluble oligomers and monomers to be used as nutrients [40].

The abiotic degradation of PHA and their blends is through hydrolytic mechanism by the chain scission in the –O–C– chemical bond that is accelerated to 60 °C. To increase the degradation rate, carboxylic groups, amine groups or any polar groups promote the water penetration into the polymer making attractive for degradation in water as in oceans, lakes, rivers, etc.

The PHA are high resistant to chemical decomposition by strong agents such as NaOH but easily degraded by strong acids and soluble in chloroform and other chlorinated hydrocarbons.

The enzymatic activities depend on the composition of the polymer chain and the environmental conditions during degradation [42], see Table 4. The biodegradation of PHAs polymers in anaerobic sewage is from months to years in saline water and ultraviolet light increases the fate of degradation [137]. Additionally, inside of mammalian systems, the hydrolysis and degradation are very slow [138].

Finally, the PHA and their copolymers are readily degraded in a several environmental conditions, with faster degradation rates in anaerobic sewage and slowest in sea water. Nevertheless, the locations for biotic (biodegradation) and abiotic

Table 4 Microorganisms in degrading polyhydroxyalkanoates in several environments [40]

Environment	Microorganism
Soil	*Aspergillus fumigatus*
	Acidovorax faecalis
	Comamonas sp
Activated sludge	*Acidovorax faecalis*
	Pseudomonas fluorescens
Sea water	*Comamomas testosteroni*
Lake water	*Pseudomonas stutzeri*
Anaerobic sludge	*Ilyobacter delafieldii*

degradation always requires be tested due to diverse microorganisms and the local conditions.

7 Conclusions

Polyhydroxyalkanoates can be synthetized by microorganisms as a reserve for energy and carbon (under stressful conditions where there is an excess of carbon and an absence of an essential nutrient) or a pure chemical route to achieve a higher control on the structure and composition of polymer chain. Biobased plastics will certainly be an effective way to reduce the carbon footprint when gradually replace the conventional plastics.

The reduction of the production cost of PHAs can introduce a new platform for their applications in biomedical fields. With the advances in genetic engineering and synthetic biology, the PHAs will be produced with a comparative price compared with petroleum-based polymers.

Cost-efficient extraction method is also considered a crucial factor, which determines the economy of these green polymers. However, The PHA nanocomposites produced with nanosized fillers, have reached the level at which they can compete with the properties of conventional plastics, making attractive in the packaging industry. Searching create green polymers, additives, fillers and reinforcements need to be developed to improve the physical and mechanical properties for the diversification of applications.

Although biodegradation studies of PHA in soils, aquatic, and atypical environments (as in human body) need a wider geographical reach, a great part of evidence shows a full degradation for long periods of time decreasing the contamination impact. The PHA are becoming attractive in the future to replace at least the conventional plastics and for advanced applications as the medical fields, for example, in drug release and tissue engineering making use of some polymer tools.

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Polyhydroxybutyrate-Base Nanocomposites and Blends. Applications

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Abstract Given the growing need to take care of the environment and to create more environmentally-friendly alternative materials for the manufacturing of various products, research and development in biodegradable and bioplastic polymers has emerged. Among the most widely studied biopolymers, polyhydroxyalkanoates (PHAs) have attracted increased attention due to their thermoplastic, biodegradable, biocompatible and renewable nature. Polyhydroxybutyrate (PHB) is the most frequently used PHA, their properties are related to those of various synthetic plastics including polypropylene. However, PHB has some disadvantages such as high cost, slow crystallization rate, high degree of crystallinity that can be overcoming by the obtention of nanocomposites. The development of nanocomposites promises to expand the use of biodegradable materials, broadening their applications including properties as the antimicrobial performance with the use of nanotechnology. This chapter it's a review of PHB-base nanocomposites, including cellulose nanocrystals and silver nanoparticles, and the applications of the obtained materials.

Keywords PHB · Nanocomposites · Biodegradable

1 Introduction

Frequently, many synthetic materials were made to fulfill human needs. These synthetic materials have potential impact on the environment and life causing hazardous effects over land, water, and air [1]. The environmental and sustainability challenges associated with the use of synthetic polymeric materials can be substantially addressed by using bio-based polymers and other biomaterials, which come from renewable sources [2].

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Among many biodegradable and biocompatible biopolymers, polyhydroxyalkanoates (PHAs), generated by microorganisms, have highly attracted attention in various fields due to their unique physicochemical properties [3]. Some of the efforts that has been made in environmental and engineering fields by employing PHAs, is the nanoarchitecture that has become a newly emerging trend among researchers. PHA is a group of polymers obtained from various bacteria such as *Cupriavidus necator* and *Bacillus megaterium* [4]. PHA's could be homopolymers such as polyhydroxybutyrate (PHB) or co-polymers of PHB with valeric acid, polyhydroxybutyrate-*co*-valerate copolymers (PHBV), in which the ratio of valeric acid determines the mechanical properties of the final product [5]. Also, physicochemical characteristics of PHAs are different and they depend on the bacterial species, growth conditions, and the number of carbon atoms in a monomer unit [4]. Table 1, shows various types of bacterium strains which can produce PHB.

The PHB polymer was discovered in 1926 by Maurice Lemoigne, who carried out its isolation and characterization [12]. There are various biosynthetic pathways to produce PHB with isotactic structure, some of the pathways leads a product with 100% of R configuration, although it could also get syndiotactic PHB through synthetic routes by combining monomers of *R* and *S* configuration [13]. PBH obtained by biosynthesis is a degradable semicrystalline stereo regular polymer with a *Xc* value usually between 60 and 70% depending on the structural configuration developed during the biosynthesis.

PHA's are environmental-friendly due to they can be degrades naturally and are produced either by some bacterial species and haloarchaea or by chemical synthetic processes via ring-opening polymerization of β-lactones [14]. They are nontoxic polyesters that can be bond to diverse monomers to create materials with different

Table 1 List of some bacterium strains involved in PHB production

Bacteria	Applications	References
Alcaligenes eutrophus	Applications including commodity plastics in bulk, fishing lines, woven materials, and potential biomedical usages	[6]
Chromatium vinosum	Applications including commodity plastics in bulk, fishing lines, woven materials, and potential biomedical usages	[7]
Thiocystis violacea	Mostly used in medical and drug delivery applications	[8]
Brevundimonas vesicularis	Applied in bioplastics packaging, pharmacology and medicine	[9]
Recombinant *E. coli* bearing *C. necator*	Applied in packaging, pharmacology and medicine	[10]
Paracoccus denitrifcans	Used in biofuels, medicine, drug delivery, packaging, stretchable films	[11]

mechanical properties. Despite having many advantages, such as its natural biodegradation, the applications of PHA's are restricted due to weak mechanical and thermal properties and slow degradation rate. To overcome these limitations, has been resorted to the use of nanotechnology. To improve the properties of biopolymer, nanofillers have been applied as reinforcing agents. As an example, high melting temperature and high crystallinity of PHB make it fragile and brittle and thermally unstable which causes a fall in molecular weight during the processing. Then, materials as graphene, carbon nanotubes, clay and silica nanoparticles [3], as well as polysaccharide nanocrystals, including cellulose, starch and chitin which are biocompatible and non-toxic.

Besides the obtention of PHB-nanocomposites as it was said above, the physical properties and biodegradability of PHAs can be regulated by blending with synthetic or natural polymers [15], about blends of PHB with synthetic polymers there is a very little information. One of the most studied biodegradable polymers is poly(lactic acid) (PLA), it is a biocompatible crystalline polymer that can be produced from renewable resources hence its blends with PHB are of great interest [16]. Some studies on PLA/PHB blends showed that the miscibility between these two polymers depended on the molecular weight of the PHB [17] and that the blends properties also depend on composition, chemical or physical cross-links and processing conditions [18]. PHB/PLA blend can provide improved stiffness and reduce brittleness to PHB and increase crystallinity and mechanical properties of PLA [19].

This chapter it's a review of PHB-base nanocomposites and PHB blends, including cellulose nanocrystals and silver nanoparticles among other nano reinforcements, and the applications of the obtained materials.

2 PHB Nanocomposites

2.1 Nanoparticles

Rey and coworkers developed a material consisting in gold nanoparticles encapsulated by PHA. They first coated gold nanoparticles with the enzyme to synthesized PHA on the nanoparticle surface, then produced PHB by further addition of 3-Hydroxybutyryl-CoA monomer to obtain the gold nanoparticles encapsulated by PHB. The nanocomposite obtained was found to be a potential agent which can be simultaneously applied in both diagnosis and therapeutic cases, coated PHB granules have enhanced imaging capabilities and are capable of heating upon near-infrared laser irradiation [20]. Another research with gold and silver nanoparticles is the surface PHB modification made by Slepička and coworkers. They activated the PHB by plasma modification and a subsequent process of either immersion into a solution of nanoparticles or direct metallization and found a strongly hydrophilic PHB by the PHB surface modification which can led to the preparation of an antibacterial coating that inhibits significantly the growth of *S. epidermis* and *E. coli* [21].

In the subject of microorganisms, the increased multidrug resistant (MDR) bacteria are considered one of the most challenging problems of the present century, therefore, the antibacterial activity of some nanoparticles, including silver and Co_3O_4 in the obtention on PHB composites, has been studying. Safaei et al. have identified the optimum conditions for synthesis of PHB-Co_3O_4 bionanocomposite with the highest antibacterial activity via in situ synthesis. They made nine experiments with different amounts of PHB biopolymer and Co_3O_4 nanoparticles and different stirring times. The results obtained showed that nanocomposite have improved structural properties, thermal stability and antibacterial activity. PHB-Co_3O_4 bionanocomposite can be used in various fields of pharmacy, medicine and dentistry due to its desirable antibacterial properties [22].

Talking about the PHB's applications, one of the main problems with PHB for food packaging applications is the lack of gas barrier. In order to overcome this demerit, several fillers like silver nanoparticles and nanoclays were added to form PHB based polymeric composites/nanocomposites. Zare et al. added zinc-silver oxide nanoparticles (NCs) into poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)-chitosan (PHBV-CS) to fabricate the novel degradable biopolymer (BP) nanocomposite, they found that the incorporation of uniformly dispersed NCs into biopolymers matrix improved the thermal stability and tensile strength due to barrier properties and the strong interfacial adhesion of NCs with BP in the system [23].

2.2 Nanohydroxyapatite and Nanocrystals

Recently, a new application of PHB/hydroxyapatite (HAp) in the biomedical field have been proposed, the nanocomposite of PHB/HAp helps to repair and regenerated the bone structure. Nevertheless, there are still a very limited studies about the effect of HAp on rheological and thermal behavior of PHB. The results of Sadat-Shojais et al. showed that both rheological and thermal properties can be tailored by incorporating appropriate amounts of HAp nanoparticles, for example, increasing the PHB melting temperature and therefore the thermal stability, but perhaps, the most important finding was the strong effect of the as-synthesized HAp on the cell proliferation and differentiation; a significant increase in metabolic activity and hence cell growth of both cell types were observed for the HAp-filled composites compared to the neat PHB [24]. Taking about bone treatments, in recent decades interdisciplinary techniques based on tissue engineering have been proposed, among these methods, electrospinning has received much attention, and among the biodegradable polymers that have been electrospun for bone tissue engineering applications is PHB. The work of Mehdi Sadat-Shojai was focused on the fabrication of electrospun PHB fibers containing a high concentration of well dispersed HAp nanoparticles along with their ability to interact with bone-like cells. To know more about the dispersion of HAp inside the PHB matrix without the use of any additional additives, SEM, EDX and TGA studies were made. The results showed that HAp nanoparticles could be encapsulated and distributed inside the PHB fibers. Then in vitro cell assays showed that the preosteoblasts could be attached to the nanocomposite and there was no significant difference in cell viability between the samples of nanocomposites and the control. Accordingly, the PHB/HAp nanohybrids reported by Sadat-Shojai, may provide a new opportunity to create biocompatible fibrous constructs for bone regeneration applications [25].

As was mentioned above, the crystallinity of pure PHB could be a disadvantage for some applications; chitin nanocrystals, with some modification, has been incorporated to PHBV through chlorination, improving PHBV characteristics by suppressing crystallization and increasing the melting temperature [26], which can open new application opportunities.

2.3 Graphene Nanocomposites

As can be read in previous sections of this review, PHB has moderate mechanical thermal and barrier properties that limit its use, for example in food packaging applications. In order to overcome these drawbacks, have been developing PHB nanocomposites incorporating graphene nanoplates (Gr-NPs), and testing their morphology, mechanical, thermal, barrier, cytotoxicity and biodegradable properties. The analyses revealed that Gr -NPs were uniformly distributed in the PHB matrix at an optimum concentration of 0.7% (w/v). PHB/Gr-NPs with 0.7 wt% Gr-NPs displayed improved melting point, thermal stability, tensile strength, barrier properties and negligible cytotoxic effect. improved the shelf life of certain food products and showed high biodegradability by soil microorganisms. Thus, the PHB/Gr-NPs nanocomposites prepared by Arul Manikandan et al. could be used for packaging applications [27]. The PHB/graphene nanocomposites also have application in other fields different from food packaging. Anishiya Cehella Daisy et al. have explored the fabrication of a novel scaffold consisting in curcumin and *Gymmema sylvestre* incorporated graphene oxide PHB-sodium alginate (GO-PHB-SA-CUR&GS composite as an extracellular matrix to treat diabetic wounds for better tissue regeneration application. They found that GO-PHB-SA-CUR&GS composite showed good biocompatible characteristics in terms of increased migration of wounded cells and could serve as a promising candidate as a new wound dressing material that is effective and inexpensive. Thus, the GO-PHB-SA-CUR&GS composite could significantly accelerate the healing of normal and diabetic wounds [28].

2.4 Reinforced PHB Blends

About PHB blends, scientific studies about, for example PHB/PLA blends, showed that the reinforcement of PHB by blending with PLA improves barrier properties and ductility, increases crystallinity and the mechanical properties and PLA can provide improved stiffness and reduced brittleness to PHB [29, 30]. As was said above, properties of PLA/PHB mixtures depend on the composition, chemical or physical crosslinking and processing conditions, but in general, PLA/PHB blends exhibit better barrier properties than PLA, but still relatively low strain at break values. It is well known that the incorporation of a plasticizer could decrease the glass transition temperature (*Tg*) of a polymer. In this case, the plasticizer incorporation could help to overcome the low strength at break of PLA/PHB blend. The use of a plasticizer in the PLA/PHB blend leaded a material with lower *Tg* and better processability as well as higher flexibility [16]. For example, elongation at break values reported for PLA/PHB (75:25 w/w) blended with different plasticizers are in the range of 6%–15% for poly (ethylene glycol) (PEG) [31]. In the patent EP2710076B1 were demonstrated that the use of a suitable type of plasticizer added into a PLA/PHB blend, could improve the mechanical properties and flexibility of the blend. Also, the plasticizer had the effect of the suppression of the thermo-mechanical degradation during the blend processing [32]. Another way to improve the compatibility of the PLA/PHB blends is to use a compatibilizer. These compatibilizers may include glycidyl methacrylate (GMA) [33] or poly (vinyl acetate) (PVAc) [34].

PHB-PLA blends were made with natural fibers such as cotton [35], kenaf and lyocell fibers [36] and limonene [19], all the study results showed that physical and mechanical properties of the PHB/PLA blends generally were shown to improve with cellulosic filler reinforcement [30]. Although PLA/PHB blend has various applications, some materials should be added to the blends to endow the desired properties, this is the case of nanofillers, that may be added to improve a desire property.

As could be seen in previous sections of this review, the addition of various nanofillers to biopolymer matrices also can provide enhancement of the structural and material properties of biopolymers. In blends of PHB/PLA and nanocellulose, nanocellulose can provide high oxygen barrier properties and improved mechanical properties. The work of Deniz Aydemir and Douglas Gardner showed that the addition of cellulose nanofibrils (CNF) increased the mechanical properties of the PHB/PLA blends, but the improving effect of PLA on the mechanical properties was higher than that of cellulose nanofibrils at 1% CNF wt%, however while the loading rate of CNF was raised from 1 to 4 wt%, the mechanical properties of the blends didn't show an improvement, which was statistically insignificant, attributable to filler aggregations, so the results indicate that the concentration in which the nanofiller is added is very important [30]. Anther work about PHB/PLA reinforced blends with cellulose is the work of Arrieta et al., they fund that the disintegration process in composting conditions of PLA was delayed by the addition of PHB, while the addition in the blend of cellulose nanocrystals (CNC) speeded it up. PLA-PHB-CNCs formulations showed enhanced mechanical performance, improved water resistance, reduced oxygen and UV-light transmission, as well as appropriate disintegration in compost suggesting possible applications as packaging materials [19, 37]. Other research about the application of PHB/PLA reinforced blends is the work of Dhar et al. they prepared green and eco-friendly films with improved recyclability, biodegradability and non-toxicity polylactic acid (PLA)/cellulose nanocrystal (CNC) composites, for potential applications as food packages [38].

Table 2 PHB nanocomposites and its applications

Material	Nanofiller	Application	References
PHB	Gold and silver nanoparticles	Antibacterial coating	[21]
PHB	Co_3O_4 nanoparticles	Pharmacy, medicine and dentistry applications	[22]
PHB-*co*-hydroxyvalerate-chitosan	Zinc-silver oxide nanoparticles	Food packaging	[23]
PHB	Hydroxyapatite nanoparticles	Bone repairs and regeneration	[24, 25]
PHB	Nanoclay	packaging applications	[40]
PHB	Calcium–Iron Layered Double Hydroxide (Ca–Fe LDH)	sustained drug delivery	[41]
PHB	Graphene nanoplates	Food packaging	[27]
PHB-sodium alginate	Graphene oxide	Medical application	[28]
PHB/PLA	Nanocellulose	Packaging materials	[37]

On the other hand, PHB not only has been blended with biodegradable polymers like PLA, but also whit non-biodegradable polymers as Polypropylene (PP) that can help to overcome the thermal instability and fragility of pure PHB, as in the case of Pachekoski et al. the results indicated that the PHB/PP blends had better mechanical properties than pure PHB, as well as improved immiscibility and higher degradation in alkaline soil, the blend also showed a tendency for lower crystallinity and stiffness of the polymer matrix, proportional to the amount of polypropylene in the blends, rendering them less stiff and fragile [39].

The Table 2 resumes the PHB and its blends with different materials using various nanofillers, as well as its possible applications.

3 Theories of Crystallization Analysis

One of the promising and environmentally-friendly alternatives to replace synthetic plastics from petroleum are bio-based polymers such as polyhydroxyalkanoates (PHA). One of the most studied PHAs is polyhydroxybutyrate (PHB). PHB is a rigid and brittle material due to its high stereo regularity, high degree of crystallinity, and formation of very large and overlapped spherulites with a high tendency to crack. In addition, PHB has a very narrow range of temperature processing due to is susceptibility of thermal degradation at temperatures close to the melting point (See Table 3).

Up to now, there have been many efforts by the scientific community to address the PHB drawbacks including the improvement of mechanical properties maintaining the biodegradability as well as guarantee its quality, large scale production and low cost. In this sense, the melt polymer blends between PHB and other polymers, represents a simple and economical process to obtain a polymer with intermediate properties between the original polymers, with the intention of improving the mechanical properties and processability of the PHB. For this aim, it is important to understand the origin of the PHB's stiffness and brittleness.

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The stiffness and fragility of PHB has been explained in the literature by the progressive tension of the amorphous molecular chains [46]. To explain the progressive tension of the amorphous chains, there are two hypotheses: The first hypothesis is based on a secondary crystallization process, which occurs slowly over time [47], while the PHB sample is at rest. When PHB is processing, it is molten, then, during the cooling process, occurs a crystallization event. Over time, a secondary crystallization occurs while the material is resting or storage at room temperature. This hypothesis predicts that the crystallization of amorphous chains occurs in the interlamellar region, joining the adjacent crystalline lamellae and freezing the remaining amorphous chains of the PHB, resulting in increased stiffness and brittleness. That is, cold crystallization (secondary crystallization) comes after the primary crystallization; this event produces small crystals which are growing among the crystals

Table 3 PHB physic-mechanical properties reported in the literature [42–45]

Property	Unit	Mean value
Density	g/cm^3	1.23
Crystallinity	%	40 to 90
T_g	°C	-15 to -9
T_m	°C	160 to 180
Processability temperature	°C	180 to 190
Young modulus	MPa	1000 to 3500
Tensile strength	MPa	4 to 40
Elongation at break	%	1 to 15

formed in the first event of crystallization as the resting time overs. Simultaneous to the cold crystallization, there is a freezing of the amorphous chains out of the primary crystals.

The secund hypothesis is based on the coexistence of two amorphous-phases, one rigid and one mobile, one of the amorphous-phases it is in the interlamellar region (rigid phase) and the other amorphous-phase it is out of the interlamellar region (mobile phase) [48]. In this hypothesis, the molecular interaction between the crystal lamellas and the amorphous rigid phase, restricting the molecular mobility of the amorphous chains and increasing the stiffness of the material as the room temperature resting time increases, the above words could be the explanation of the progressive embrittlement sometimes observed in semicrystalline materials during physical aging.

As the resting and storage time, at room temperature, increases, it is clear that the stiffness and fragility increasing. This progressive increase in stiffness and fragility, could be due to the fact that one of the two theories of progressive tension of amorphous chains occurs inside the PHB, may be both or perhaps another not yet revealed.

In order to theoretically understand the progressive stiffens and brittleness increasing of PHB in resting or in storage at room temperature, a previous report has been analyzed. The work of Zitzumbo-Guzmán et al. reported the analysis of PP/EPDM blends with carbon nanofillers, where it was demonstrated that the polymers chains of the mixture presented molecular mobility during the blend rest and that as the rest time increases, more stable molecular structures inside the nanocomposite was formed [49]. The results suggested that the molecular structures formed in the blend interior, were due to two molecular reorder processes, one fast process and other one much lower. The first one was attributed to the entanglement of the polymer chains that form stressed and unstable molecular structures, while the second one was referred to the molecular mobility that takes place inside the stressed entangled polymer. In such a way that the nanocomposite internal structure is reaching molecular stability as the resting time increases. Besides in the PP/EPDM blend has been demonstrated that the more stable molecular structures required a greater effort to unentangled that the less stable ones.

Similar to the reported in the two processes of molecular rearrangement of a resting polymer, it could understand that the process of rapid molecular mobility inside PHB samples would not be the one related to the primary crystallization process that takes place inside the material to form crystalline lamellae, but rather, the fast molecular mobility process is the one that the amorphous molecular chains have, trying to accommodate each other but that do not form crystals. This molecular mobility of amorphous chains could take place immediately after primary crystallization has occurred and crystalline lamellae have formed in the material. That is, the primary crystallization process that forms the crystalline lamellae inside the PHB is different from the rapid process of molecular mobility that amorphous molecular chains have in the resting material. While in the first case there is crystal formation, in the second one there could only be stressed molecular structures that were unable to rearrange themselves to form crystals.

On the other hand, the slow molecular mobility process in PHB resting samples could be related to the movement of amorphous molecular chains that could be found agglomerated in stressed molecular structures instead of had formed crystals during the cooling process of the melt PHB. These stressed molecular structures could be located in or out of the interlamellar region. To better understand, the stressed molecular structures are formed by amorphous molecular chains and are located around the formed crystalline lamellae. In such a way that, as the resting time at room temperature in PHB increases, these stressed molecular structures changed due to the molecular movement of the amorphous molecular chains which it is formed with. In other words, stressed structures gradually relax and with a resting time large enough tend to stabilize.

This slow molecular movement could be the main responsible for the progressive tension that takes place inside the PHB at room temperature resting time, because it could explain the formation of secondary crystals and the rigidity of the amorphous molecular chains between the crystalline lamellae. However, slow molecular motion depends on the formation of stressed molecular structures caused by rapid molecular motion of amorphous molecular chains.

The hypothesis of the cold crystallization establish that small crystals are forming between the crystallite lamellas as the resting time increases in PHB. Besides, according with the two processes of molecular mobility present in storage or resting polymers, in the polymer interior there are conformational changes leading to more stable molecular structures. The structural changes occurring in the material interior, could explain either both hypotheses related with the progressive tension in the PHB amorphous chains. On one side, the structural changes in the material interior could be the small crystals mentioned by the cold crystallization hypothesis. If it were the case, it could be considered that these small crystals could have irregular non-stable shape, or at least less stable than the primary crystals forming the crystalline lamellae. On the other hand, the structural changes occurring in the material interior, could be the rigid amorphous phase mentioned in the coexistence of one rigid amorphous phase and one mobile phase hypothesis.

It is worth mentioning that during the PHB resting state, internal structural changes due to amorphous molecular chain reordering could be originated. Molecular chain reordering is due to the energy conserved in the amorphous molecular chains took from the melting process where they come from. In such a way that as the material resting time at room temperature, increases, the amorphous molecular chains tend to demand more energy quantity to move on one side to another, reordering in crystallites or frozen structures more stable than the one they had before the rearrangement, but without forming crystals. The formation of irregular crystallites or frozen molecular structures restricts the molecular mobility of the amorphous chains that could not cluster or to be reordered.

If the molecular rearrangement may originate small irregular crystals, this could indicate that the PHB chains has strong intermolecular interactions. In such a way that, as the small crystals are forming, the interlamellar space volume and, probably, the separation distance between the adjacent lamellas, could be decreasing, leading a strong and progressive increasing in the interlamellar molecular interactions, enormously restricting the movement of the molecular amorphous chains in the crystalline lamellas that have not been able to reaccommodate.

If the possibility of strong intermolecular interactions in PHB is accepted, then the crystalline lamellas will tend to form crystallites or an internal structure with progressive stabilization, even if they are or are not between the crystalline lamellas. Regard to the cold crystallization hypothesis, we do not find a convince reason that explains why it consider that only the amorphous chains between the crystalline lamellas can crystalized while the others did not and just can froze. On the contraire, amorphous molecular chains in the interlamellar region could experiment harder molecular movements than the ones located outside of the interlamellar region, hindering the possible formation of crystallites, due to the strong interactions that they are expose.

On the other hand, the crystallization theory stablishes that the crystals are forming molecular folding of polymer chains. In such a way that after the formation of the crystalline lamellae, the molecular mobility of the chains of the amorphous phase decreases considerably, and more those that are occluded between the crystalline lamellae than those that are outside them, due to the molecular interactions [50]. It could be added that the part of the amorphous molecular chains occluded in the interlamellar region forming a primary crystal, do not come off to form other crystals, due to the primary crystal stability, where the internal structural bond is so strong that even acid conditions cannot make it apart [51].

Now, after the PHB es subjected to a melting and cooling process, a quickly rearrangement occurs leading crystalline lamellae and then a slow molecular rearrangement, the slow molecular rearrangement occurs in the molecular chains of the amorphous phase. However, according to the molecular rearrangement of a resting material, the crystallites late formation es more complicated due to amorphous molecular chains needed a greater energy demand to move and fold, that the energy required by the amorphous chains before folding in primary crystals and form crystalline lamellas. The energy demand that the occluded amorphous chains need to move, could hinder the crystallites forming process due to the mobility restrictions of the molecular chains in the amorphous phase. These restrictions are due to two reasons: first and probably the main reason, is the presence of crystalline lamellae already formed and, second, the amorphous chains need to overcome the strong intermolecular interaction between themselves to move and fold with each other.

3.1 Partial Conclusions

As detailed in the above paragraphs, there are two hypothesis that explains the amorphous molecular chains progressive tension, both could be partially convincing [50]. As stated above, first there is the possibility of a cold crystallization process during the material resting time, although this crystallization could not occur between the crystalline lamellas, but outside them, due to the amorphous molecular chains located outside the interlamellar region have fewer restrictions on molecular movement because their molecular interactions with the crystalline lamellae could be weaker than those experienced by the amorphous chains that are confined in the interlamellar region, and therefore, they require less energy to move and fold with each other. Second, there is the possibility that the rigid amorphous phase, which is located in the interlamellar region, forms a frozen molecular structure. That is to say, during the resting time of the PHB there are polymeric chains movements, where the chains of the rigid amorphous phase form internal molecular structures between the crystalline lamellae, but which frozen before becoming crystals, due to the strong molecular interactions to which are subjected. However, the molecular chains of the mobile amorphous phase that are outside of the interlamellar region, present less restriction to movement with respect to the rigid amorphous molecular chains located in the interlamellar region. For this reason, they could form irregular and unstable crystallites before freezing completely, since the strong molecular interactions between the amorphous chains themselves and the resting time of the PHB, prevent or significantly hinder the free movement of its amorphous polymeric chains.

4 General Conclusions

Overall, the reviewed literature highlights PHB is a very promising polymer for a wide range of applications. In spite of its intrinsic brittleness, a lot of progress reported here has been made recently through the formulation of PHB with nanofillers and blends with other biodegradable polymers leading to greatly improved mechanical profiles, keeping its biodegradability as well as suitable processability via extrusion or injection molding, among others. These advances will improve its capacity to get markets such as food packaging or automotive industry. The recent advances are ongoing regarding the improvement of the applications of PHB that could be ranging from surgical sutures, tissue engineering and agricultural foils. The characteristics mentioned along the chapter, makes that PHB may be a good candidate to substitute PP and PE, but also PET in certain applications. Nevertheless, some limitations still persist such as its elevated cost, and further improvements are still needed to allow a brother range of properties and thus of applications, including scientific effort to reduce the productions const and increase the industrial sustainability.

Therefore, there are a lot of opportunities to explore different nanofillers and its effect on PHB especially in fast growing industries that requires biodegradable materials to reduce its environmental impact and fully replace conventional fossil oil-base polymers.

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Poly(Butylene Succinate). Functional Nanocomposite Materials and Applications

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Abstract Poly(butylene succinate) (PBS) is one of the biodegradable polymers that has attracted major attention to replace the non-biodegradable and pollutant petroleum-based polymers. This chapter presents current research on the preparation, properties and application of PBS. Easy processing and good balance in thermo-mechanical properties make this polymer suitable for applications in sectors such as food packaging, agriculture, and medicine, among others. In recent years, much research work has been done in developing biodegradable polymer nanocomposites and PBS has been considered one the most promising platforms, not only because it can compete with some of the major commercial polymers (polyethylene and polypropylene) but also because it offers the possibility of being processed by different free-solvent techniques like extrusion, injection molding, thermoforming, foaming, etc. Lately, PBS has been loaded with different carbon allotropes to make it an electro- and thermo-conductor. Biodegradable PBS-based nanocomposites with reinforced and functional properties could find application in modern technologies such as those required in electronics, robotics, biosensors, etc. High performance biodegradable nanocomposites, including those prepared with PBS, will certainly contribute to alleviate actual and future environmental problems.

Keywords Poly(butylene succinate) · Succinic acid · Biodegradable polymers · Nanocomposites

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1 Introduction

The big global environmental issues are prompting the polymer industry to change towards the bio-based and biodegradable polymers [1–3], biodegradable biocomposites [4–6], and biopolymer-based compounds [7, 8]. These eco-friendly polymers have found application in the major sectors utilizing plastics such as food packaging [9–12], and also in high value-added areas such as medicine [13, 14] and electronics [15–17]. This reorientation will not only decrease our dependence on the fossil sources but also will reduce the contamination problems associated with the final disposal of the long-life traditional plastics.

Different routes for obtaining chemicals and building blocks (monomers) from natural sources have been explored, being perhaps those obtained from the hydrolysis of lignocellulose that have aroused the greatest interest [18–20]. These are ethanol [21, 22], lactic acid [23, 24], itaconic acid [25, 26], diols such as 1,3-propanediol and 1,4-butanediol [27–29], adipic acid [30], azelaic acid, succinic acid, and ε-caprolactone, among others [31]. From these building blocks, bio-based and biodegradable polymers like poly(lactide acid) (PLA), polycaprolactone (PCL), and poly(butylene succinate) can be obtained. Partially bio-based and non-biodegradable polymers such as polyurethanes (PU), polyamides (PA), polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET) can also be obtained [31].

In this chapter, we will address the synthesis, properties and applications of PBS, which is one of the most promising bio-based and biodegradable polymers for commercial use. It can compete with PE and PP in low performance areas such as packaging, but also with specialized polymers (PLA) in fields such as biomedicine. At present, there are several industrial efforts to market PBS available through biotechnological fermentation processes, which are seen as the most eco-friendly technology platform to obtain plastic materials.

2 Poly(Butylene Succinate): Synthesis and Properties

2.1 Synthesis of Bio-Based Succinic Acid

The succinic acid is a dicarboxylic acid of four carbon atoms also known as 1,4-butadienoic acid (Fig. 1). Actually, a great volume of succinic acid is produced through catalytic hydrogenation of maleic anhydride from petroleum sources. However, in the last two decades biotechnological processes have been developed to produce it from the fermentation of sugars using different microorganisms [32–34].

In most research work, the biotechnologically obtained succinic acid is considered as the chemical platform for obtaining bio-based polymers [35], although the real chemical platform are sugars like glucose, saccharose, xylose, and others. The principal raw materials for the synthesis of biotechnological succinic acid are sugars

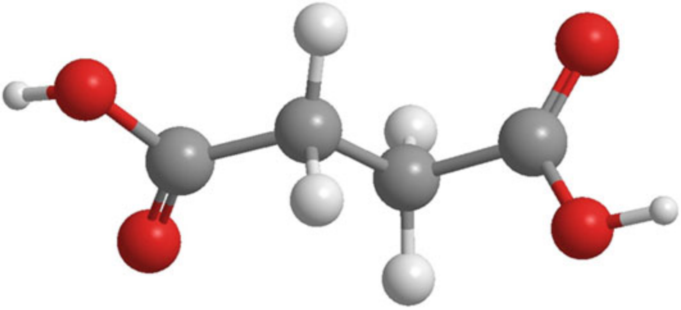

Fig. 1 Chemical structure of succinic acid

obtained from industrial lignocellulosic residues [19]. The synthesis pathway consists of three principal stages: (1) physical and/or chemical pre-treatment of the industrial lignocellulosic residues, (2) hydrolysis into sugars, and (3) fermentation of sugars using adequate microorganisms.

In the last 10 years, diverse research work has been carried out to synthesize succinic acid using different microorganisms and scaling-up the process to the industrial level [36–38]. Companies such as Bioamber, Myriant Technologies, BASF-CSM joint venture, DSM-Roquette Frères joint venture, and Mitsubishi Chemical Company-PTT produce succinic acid through biotechnological synthesis [39]. 1,4-Butanediol can be synthesized through the reduction of bio-based succinic acid, although an alternative process is that using *E. coli* to metabolize sugars into butanediol [40].

Table 1 lists some lignocellulosic residues and microorganisms used for the synthesis of succinic acid. It must be highlighted that almost any lignocellulosic residue can be used for the synthesis of succinic acid through fermentation processes, and the key factors are adequate microorganisms and a lignocellulosic source.

2.2 Synthesis of PBS

Poly(butylene succinate) is commonly obtained from succinic acid and 1,4-butanediol, following a polycondensation reaction via a two-stage melt process: esterification into oligomers and oligomers coupling into high molar mass PBS (Fig. 2). The reaction of 1,4-butanediol with succinic acid (esterification) or dimethyl succinate (transesterification) is achieved at 160–190 °C, taking care of removing the condensation sub-products (water or methanol). This reaction can be carried out either under stochiometric conditions or using a small excess of alcohol (5−15 mol%). The polycondensation reaction is carried out under melting conditions at 220−240 °C [40]. Direct polycondensation may be possible with the drawback of lower molar mass [56]. An alternative eco-friendly process to obtain PBS is through

Table 1 Lignocellulosic residues and microorganisms used for the biotechnological synthesis of succinic acid

Lignocellulosic residue	Microorganism	References
Arundo donax	*Basfia succiniciproducens* (BPP7)	[41]
Corn fiber	*Actinobacillus succinogenes*	[42, 43]
Sugarcane bagasse		[44]
Carob pods		[45]
Wheat bran		[46]
Corn stalk		[47]
Cotton stalk		[47]
Corn stover		[48]
Cane molasses		[49]
Fresh cassava root		[50]
Corn cob		[51]
Corn straw		[52]
Sugarcane bagasse	*E. coli* (BA305)	[53]
Corn stalk		
Oak wood	*M. succiniciproducens* (MBEL55E)	[54]
Orange peel	*Fibrobacter succinogenes* (S85)	[55]

microwave irradiation and using 1,3-dichloro-1,1,3,3-tetrabutyldistannoxane as catalyst [57]. This esterification process is achieved in short time in the absence of solvents, but the resulting molar mass is low (10 kDa) and scaling-up this process is complicated. PBS has also been synthesized using different organometallic catalysts of Ti, Zr, Sn, Hf, Bi, and metal-oxides of Ge and Sb. The catalytic efficiency resulted as follows: Ti > Ge > Zr ~ Sn > Hf > Sb > Bi. It should be considered that Ge and Zr derivatives can be an alternative for the Ti-based catalyst, which possesses the best catalytic performance but is involved in side degradation reactions [58].

2.3 Properties of PBS

PBS is thermally stable up to 300 °C and possesses a melting temperature (T_m) of around 115 °C, which is close to that of low density polyethylene (LDPE) (T_m = 108 °C) and high density polyethylene (HDPE) (T_m = 125 °C) [59]. Furthermore, PBS develops a semicrystalline structure that can be modified by inclusion of co-monomers like ethylene succinate and hexamethylene succinate [60]. PBS reaches a maximal strength (30–35 MPa) like that observed in polypropylene; additionally, PBS has an elastic performance (Young modulus at 300–500 MPa) that can vary with the crystalline/amorphous ratio. Two crystalline structures (α, β) are found in

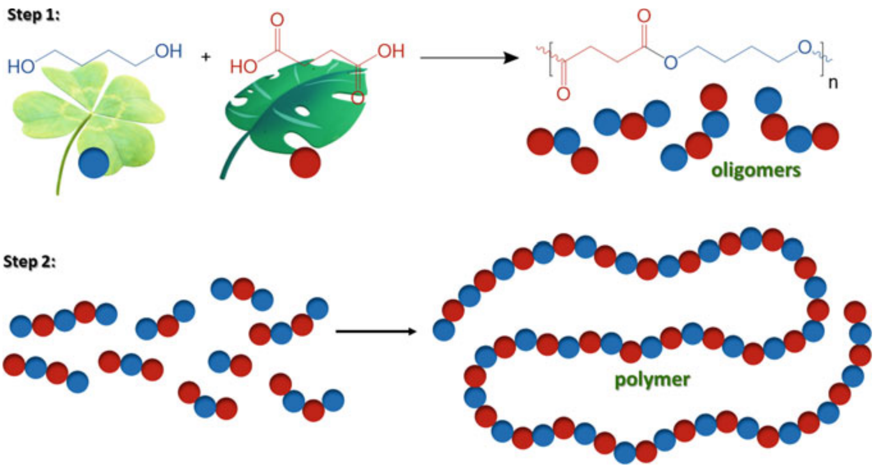

Fig. 2 Synthesis of PBS

PBS. The β-form is exhibited under strain conditions, and changes to the monoclinic α-form after removing the mechanical stimulus [61]. As PBS has a softness performance (elongation at break > 300%) and a poor gas barrier property, it is often blended with other compounds (fillers) to satisfy the application [62].

It is worth mentioning that PBS exhibits high ductility and a wide range of workability compared with other biodegradable polyesters (PHB, PLA, PHA and PHV). Its glass transition (−28 °C) and melting (115 °C) temperatures are favorable to the conventional polymer processing techniques like extrusion, injection molding, and thermoforming [56, 62]. High molar mass PBS shows enhanced mechanical properties suitable for food packaging and biomedical devices [56, 63].

Like for any other polymer, the mechanical and thermal properties of PBS can be improved through functionalization, copolymerization, chemical functionalization, and fillers addition. For instance, the maleic anhydride-grafted PBS (PBS-*g*-MA) has been prepared as compatibilizer in montmorillonite-filled PBS nanocomposites [64]. The incorporation of PBS-*g*-MA increased the crystalline degree from 57 (PBS-montmorillonite) to 66% (PBS/montmorillonite/PBS-*g*-MA). The rise in crystallinity was in turn reflected in the enhancement of both tensile strength and Young modulus, reaching values of up to 37 and 650 MPa, respectively. Another example involves PBS/PLLA immiscible blends with added hydroxyapatite; the resulting reinforced blend reached a tensile strength of up to 57 MPa [65]. Likewise, through PBS blending with acrylonitrile–butadiene–styrene (ABS), films with 30% more porosity than that of the raw PBS can be prepared [66]. Additionally, in comparing with PBS films, the PBS/ABS blends (70/30) exhibited increments of up to 188% in tensile strength, 99% in Young modulus, and 65% in elongation at break, making them attractive membranes for wastewater treatment applications.

Research works on the improvement of the barrier capabilities of PBS films has led to the inclusion of nanofillers such as nanocrystalline cellulose and chitin whiskers

[67]. Blends of PBS with these nanofillers prepared by melt extrusion developed higher crystalline degrees with no significant changes in the thermal properties of PBS. The addition of nanocrystalline cellulose has no significant effects on the tensile strength of PBS, although it improved its barrier properties. PBS films with 3 wt% of nanocrystalline cellulose showed a decrease in both the water vapor transmission rate (WVTR) and the oxygen transmission rate (OTR); reported values for WVTR go from 83 (PBS film) to 30.8 g/m^2/day (PBS/nanocrystalline cellulose film/compatilizer) while for OTR they go from 737.7 to 23.8 cc/m^2/day. Negative effects on the degree of crystallinity of PBS by the inclusion of nanofibrillated cellulose have been reported [68]. While the thermal performance of this polyester remains relatively unaffected by this crystallinity hindrance, the elastic modulus as well as tensile strength values resulted higher than those of the pure PBS. Moreover, it was found that the nanofibrillated cellulose accelerates the biodegradation process of PBS. In this study, it was observed that with the addition of 15 wt% of this nanofiller, the degree of crystallinity of PBS exhibited a reduction of around 20% which was reflected in higher degradation rates.

Some works report significant changes in the thermal properties of PBS. For instance, the use of palygorskite as a filler has proven to exert a significant influence over the glass transition and crystallization temperatures. Reported formulations of PBS/palygorskite nanocomposites indicate that 3 wt% of polygorskite is optimal to manage a higher PBS glass transition temperature (−11 °C) in comparison with that of the neat PBS (−20 °C) [69]. As for the crystallization temperature, the same content of palygorskite leads to an increment of around 20 °C due to the nucleating effect. The thermal stability of PBS is also improved by 14 °C as a result of palygorskite inclusion. Further crystalline phase modification and thermal stability enhancement for this polymer have been achieved through melt compounding of PBS with graphene nanosheets [70]. Although no significant changes in T_{m} resulted from the addition of this type of nanofiller, the degradation temperature of PBS became 35 °C higher with 5 wt% of graphene nanosheets. Likewise, a small decrease (from 39 to 30.7%) in crystallinity is noticeable in these nanocomposites, though β crystal structures are formed without inducing stress over the material, enabling a Young modulus enhancement of around 15%.

The thermal and electrical conductivities are two attributes of great importance in eligible materials for electronic, biomedical and packaging industries. Implementation of natural fillers as green alternatives for polymer modification, such as lignin and kenaf core fibers (KCF), has been tackled during recent years. These additives not only have low environmental impact but also can modify the thermal properties of polymeric materials. PBS/lignin/KCF composites have displayed a thermal conductivity increment of around 100% relative to the pure PBS [71]. Higher enhancements in this thermal property (up to 160%) have been reached through PBS/silicon carbide composites [72]. As for electrical conductivity, this property is conferred to PBS with fillers such as carbon black and carbon allotropes like graphene nanosheets and carbon nanotubes [70, 73, 74].

2.4 Biodegradation Features in PBS

The susceptibility of PBS to biodegradation is determined by its chemical and morphological features such as molecular weight, degree of crystallinity, and ester group periodicity [62]. Environmental conditions to which this polymer is exposed also have a major influence over its degradation. The main environmental factors are temperature, pH, presence of water, hydroxides and enzymes produced by microorganisms such as bacteria and fungi [75]. The main types of degradation by which PBS decomposes are classified in hydrolytic and enzymatic degradation, however, in specific fields of application, thermal and photo-degradation mechanisms are also considered a matter of study.

Hydrolytic degradation of PBS consists in the breakage of ester bonds when reacting with water or hydroxides, resulting in chain scission and formation of carboxyl group species, events leading to molecular weight reduction and pH level decrease, respectively [76, 77]. Moreover, increased porosity and abundant amorphous regions (more prone to degradation than crystalline regions) promote higher hydrolysis rates due to increased water permeation and contact area [78].

As stated, PBS is capable of undergoing degradation induced by several enzymes that are naturally generated by specific biological agents. This degradation mechanism starts with enzyme adhesion to the polymer surface, subsequently, liable ester bonds are hydrolyzed by enzymatic activity, causing surface erosion and cavities, eventually leading to overall PBS degradation; water and CO_2 are PBS degradation products obtained by enzymatic reactions [62, 79, 80]. According to numerous research works, *Pseudomonas fluorescens* (bacterium), *Candida rugosa* (fungus) and *Candida antarctica* (yeast) are examples of microorganisms that produce lipases, enzymes that efficiently degrade PBS under determined conditions [75, 81]. Other reported types of enzymes with similar hydrolysis capabilities over PBS are esterases and cutinases, which are synthesized by various actinomycetes and filamentous fungi [80, 82]. Therefore, in some cases PBS degradation is favored under enzymatic conditions rather than under a hydrolytic mechanism at neutral pH.

3 Polymer Nanocomposites

Polymer nanocomposites (PNCs) are an important category of engineering materials, either reinforced or functional. A PNC combines the advantages of both polymers and nanoscale particles (1–100 nm), thus allowing the development of new or performed chemical, physicochemical, biomedical, thermal, mechanical, thermophysical, and electrical properties [83–86]. The amount, aspect ratio, size, and state of dispersion of nanoparticles [87–89] as well as the interfacial interaction [90] are critical parameters as they determine the final properties. It is important to note that the nanoparticles are often difficult to disperse due to their strong tendency to self-aggregate into micro-scale clusters. Efficient mixing operations are therefore necessary to disaggregate and disperse them into the continuous major polymer phase [91, 92]. Surface modification and functionalization of nanoparticles or using compatibilizers are common strategies to improve the interfacial interaction, thus making the homogeneous dispersion of nanoparticles and the transfer of properties among the two distinct phases easier [70, 93, 94]. A wide variety of nanofillers (nanometals, nanoclays, carbon allotropes, etc.) has been explored with the aim to improve the properties of the neat polymer, and so make its range of applications wider [95–98]. Among them, the carbon allotropes have attracted great interest because they have shown to enhance the electrical and thermal conductivities, while providing environmental stability and good mechanical strength [87, 99]. Lightweight and flexible polymer/carbon allotrope nanocomposites able to exhibit electrical transport or heat dissipation capacities have potential use in a variety of applications [85, 100–102]. It must be taken into account that the ever-increasing power densities in electronic devices have made the efficient heat transport and dissipation extremely essential as overheating can cause premature failure [103–105]. Avoiding build-up of static charge in devices is also essential [106].

3.1 Biodegradable Polymer Nanocomposites (BPNCs)

A noticeable trend in polymer science is the development of BPNCs with specific functionality [107]. BPNCs have shown huge progress with the development of modern methods of synthesis of polymers, the refinement of processing technologies, the use of new characterization techniques, and the attracting potential applications [108]. BPNCs prepared from synthetic chemicals and biomass (also known as green composites [109]) are alternative advanced materials to the conventional PNCs whose degradation rate is extremely low [110–112]. Some attractive biodegradable polymers (BPs) for developing BNPCs are shown in Fig. 3. PBS polybutylene succinate, PHB polyhydroxybutyrate, PCL polycaprolactone, PBSA polybutylene succinate adipate, PHA polyhydroxy alkanoate, PBAT polybutylene adipate terephthalate, PHV polyhydroxy valerate, PLA polylactic acid.

The electrical and electronic industries demand the use of large amounts and wide variety of polymers. However, most polymers in these industries are non-biodegradable, generating serious environmental problems [113]. The industries´ must be drawn to BPs, which decompose in a short time once they are discarded or released into the environment [112, 114]. The use of BPs is perhaps the most innovative solution to the environmental problems caused by non-biodegradable fossil-based plastics [2]. Nowadays, BPs are successfully applied in packaging and biomedicine [115, 116], however, in other fields such as those requiring electro- and thermo-conducting materials they still remain as potential materials in future applications [117]. The addition of high conductive nanoparticles can make the BPs become BPNCs semiconductors [74, 118] or thermo-conductors [119]. The implementation

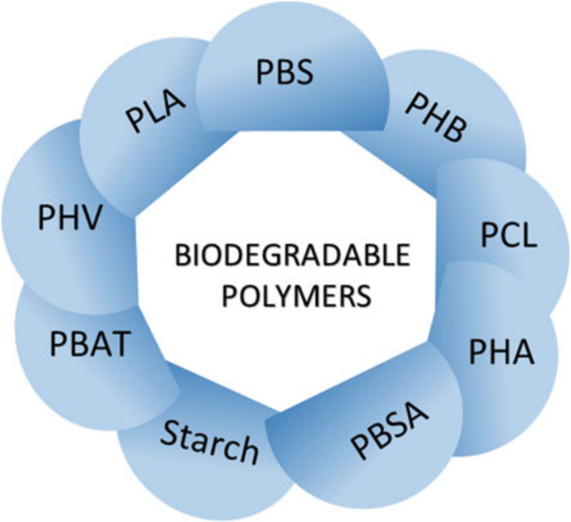

Fig. 3 Attracting biodegradable polymers for preparing BPNCs as substitutes of traditional non-biodegradable polymers

of electric and heat conducting BPNCs in devices is a very new approach in the electric, electronic, automotive, robotic, sensors/actuators, and aerospace industries [120–123].

3.2 Poly (Butylene Succinate) Nanocomposites

Poly(butylene)succinate is regarded as one of the most promising BPs due to its biodegradability, abundant renewable source, easy processing, reasonable production cost, and good performance characteristics [70, 74, 124]. This polymer can compete with polyethylene and polypropylene [125], but its low strength, low gas barrier, and low crystallization ability are frequently insufficient for various end-use applications [88]. The incorporation of nanofillers into PBS has been an effective way to enhance properties and make it more useful [70] like, for instance, in packaging [62] and biomedical applications [126]. The electrical and thermal conductivities of PBS can be enhanced by adding low amounts of conductive nanoparticles [97, 127]. A variety of carbon allotropes have been tested in PBS. They include carbon black (CB) [74], graphene nanoplatelets (GNPs) [128], reduced graphene oxide (G_{red}) [129], carbon nanofibers (CNFs) [130], and carbon nanotubes (CNTs) [131]. For instance, Ray et al. reported for the PBS/CNTs nanocomposite (10^{-3} S/cm) an electrical conductivity six orders of magnitude higher than that measured for neat PBS (10^{-9} S/cm) [131]. PBS-based nanocomposites with improved thermal, mechanical and/or electrical properties are listed in Table 2.

Table 2 PBS nanocomposites containing carbonaceous nanomaterials

Carbonaceous material	wt%	Processing method	Improved properties	References
Carbon black	0.5, 0.1	Extrusion	Crystallization rate, mechanical	[88]
Carbon black	0.5, 1, 3, 5, 10	Melt compounding/foaming (CO_2)	Electrical conductivity, mechanical	[74]
Carbon black	10	Melt compounding	Flame retardancy, mechanical	[132]
Carbon black	1, 3, 5	Extrusion	Mechanical, thermal, electrical conductivity	[125]
Carbon fibers	5, 10, 15, 20	Solution casting/foaming (CO_2)	Electrical conductivity, mechanical	[133]
Multi-walled carbon nanotube	0.5, 1.0	Melt blending	Crystallization rate	[134]
Carbon nanotube	0.5, 1.0	Extrusion	Crystallization rate	[88]
Carbon nanotube (Functionalized)	0.1, 0.3	Solution coagulation	Electrical conductivity, crystallization, mechanical	[135]
Graphene nanoplatelets	0.5, 1, 3, 6	Melt blending	Electrical, thermal conductivities	[128]
Graphene nanoplatelets	0.5, 1, 2, 3, 5	Extrusion	Thermal stability, electrical conductivity, mechanical	[70]
Graphene nanosheets	0.5, 1, 2	Solution	Thermal stability, electrical conductivity, mechanical	[124]
Graphene oxide Nnanosheets	3	In situ polymerization	Mechanical	[136]
Graphene oxide (modified)	0.1 to 1	Solution	Thermal stability, mechanical	[137]
Multi-walled carbon nanotube	0.5, 1, 2, 3	Melt compounding	Crystallization thermal stability	[138]
Multi-walled carbon nanotube	3	Melt blending	Electrical conductivity, mechanical	[131]

Carbon black

In recent years, static dissipative and conductive polymer composites have become part of the polymer world, and carbon black (CB) remains the major conductive filler as it offers the best performance at the lowest price [139, 140]. The addition of CB affects the melt viscosity and improves the flame retardancy as well as the thermal, mechanical, and electrical properties [74, 132, 141]. CB has already been tested for improving the mechanical and electrical properties of PBS. For instance, Ge et al. prepared PBS/CB nanocomposites and observed an electrical conductivity (10^{-6} S/cm) much higher than that measured for neat PBS (10^{-12} S/cm), indicating that the CB particles can connect with each other forming conductive pathways [142]. Chen et al. also prepared PBS/CB nanocomposites and found that CB disperses fairly well in PBS even at high contents [74]. These authors found that CB can have significant effects on the crystallization temperature, crystallinity, thermal stability, storage modulus, and complex viscosity properties.

Carbon nanotubes

Carbon nanotubes are single—(SWCNTs) or multiple-walled (MWCNTs) structures of rolled-up graphene sheets [143, 144]. Both types of nanotubes are considered ideal reinforcing fillers because of their high mechanical strength, excellent biocompatibility, and very high thermal and electrical conductivities [145–148]. Their high aspect ratio allows reaching the percolation threshold at very low contents (as low as 0.0025 wt%) [149]. CNTs have already been used to reinforce PBS [150, 151]. For instance, Shih et al. prepared PBS/CNT nanocomposites through melt blending and observed good dispersion of CNTs that reflects in enhanced thermal and mechanical (120% increment) properties [152]. They also found a marked decrease (> 10^9 fold) in the surface resistivity as compared to that of pristine PBS. Ali et al. also prepared PBS/CNT nanocomposites and found important effects in crystallization and tensile properties, as well as an important increase in electrical conductivity (10^8 fold) as compared to that of pristine PBS [153]. However, one drawback in reinforcing with CNTs is the inefficient dispersion within matrices [154], although functionalized nanotubes disperse quite well by traditional melt compounding procedures [155].

Carbon nanofibers

Carbon nanofibers exhibit good mechanical, thermal, and electrical properties [156, 157]. They are cheaper than CNTs [133], however, the major obstacle to the use of CNFs is the high filler loadings that are required to reach the percolation threshold. Kuang et al. found that, in incorporating CNFs in PBS, the crystallization rate increases and the tensile strength improves 5 times to reach 124 MPa, fulfilling thus the demand of engineering plastic [133]. They also observed an increase in electric conductivity at a relatively low loading (2.37 vol.%), evidencing the formation of conductive networks. These properties make this composite a promising material for the anti-static and EMI shielding areas.

Graphene Nanoplatelets

Graphene nanoplatelets are composed of few closely packed graphene layers. They exhibit exciting properties such as light weight, high aspect ratio, and high electrical and thermal conductivities, among others [158]. They are an attractive option to replace different nanofillers, including CNFs and CNTs. Dispersion of GNPs within biodegradable polymer matrices is possible by solution [159] and melt blending methods [160], among others. This nanofiller can form percolated networks as demonstrated by Platnieks et al. in preparing PBS/GNPs nanocomposites by melt-blending; the electrical conductivity, measured at different frequencies, reached a value of up to 10^{-5} S/cm [128].

3.3 Preparation of PBS Nanocomposites

Polymer nanocomposites can be prepared by three conventional processes: in situ, solution and melt blending processes. The first two afford good dispersion but their use at the industrial scale is limited because they involve the use of solvents. The third one is preferred because it is greener, economical and fast.

4 PBS Applications

In recent years, there has been a growing interest in PBS as a replacement for non-biodegradable materials, such as PE, PP and PET, in an attempt to reduce plastic pollution [62]. Biodegradability and good processability, which are considered two of the most attractive features of PBS, along with its good thermal and mechanical properties, have placed this aliphatic polyester inside several fields of application (Fig. 4), including agricultural, biomedical and environmental [56]. Moreover, through formulations of PBS with conductive nanofillers, particularly carbon allotropes, nanocomposites with enhanced thermal, mechanical and electrical properties can be created and implemented in electronic components [56, 161].

4.1 Agriculture and Environmental

Seed sowing and plant growth are two agricultural practices in which PBS has been employed to improve harvest quality and quantity. Mulch films whose control over soil temperature and moisture, among other parameters, increases crop yields, represent a great opportunity for PBS [62]. Mulch films have been elaborated with PBS and fillers such as potassium dihydrogen phosphate, salicylic acid, and starch, granting these films with fertilizer release control, plant growth stimulation, and increased

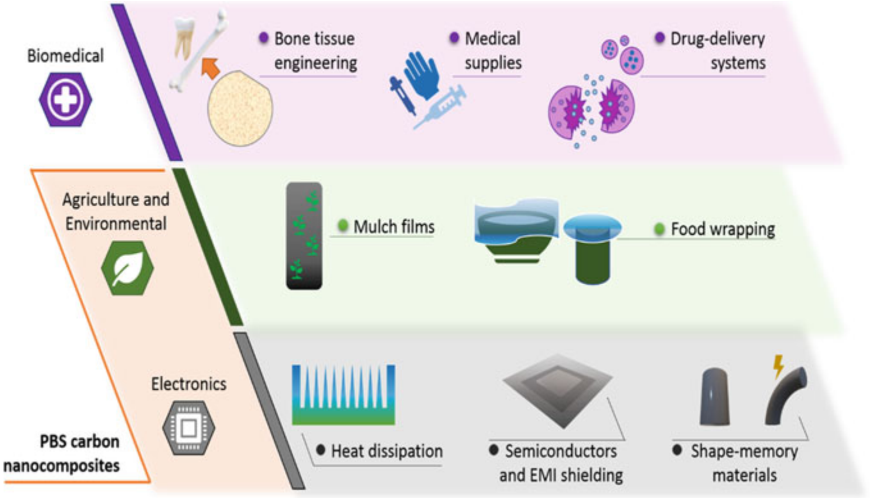

Fig. 4 PBS and PBS carbon nanocomposites applications

water permeability [162–164]. PBS is becoming relevant in the food packaging industry because, beside its biodegradability, it possesses adequate processability to create materials for food transportation and conservation. PBS/GNPs nanocomposites have shown superior oxygen barrier properties and reduced water vapor permeability than PBS [165]. Furthermore, properties such as impact and flexural strength, tensile modulus, and heat deflection temperature of PBS/GNPs nanocomposites can be improved when a third component is added, thus widening their applications inside the packaging industry [166].

4.2 Biomedical

Some of the current biomedical trends encompass tissue engineering, bioabsorbable devices, and drug-delivery systems [56, 167]. Taking advantage of PBS biocompatibility and non-cytotoxicity, the development of scaffolds for stem cell growth to promote osteogenesis has been achieved by combining PBS with polylactic acid (PLA) [168]. Other reported PBS-based nanocomposites useful for tissue repair include PBS/PLA/hydroxyapatite blends, which can also be used in bone fixation devices and periodontal applications [126, 169]. Likewise, antioxidant and antimicrobial properties can be conferred to PBS with the inclusion of lignin as filler; research regarding this composite showed adherence-resistant activity against the respiratory and skin bacterial pathogen *Staphylococcus aureus* [170]. Therefore, the implementation of PBS/lignin in medical devices would reduce the incidence of nosocomial infections [170, 171]. Concerning drug-delivery systems, reported drug-release formulations primarily made of PBS porous particles exhibit compatibility with numerous active ingredients. For instance, PBS microspheres have been loaded with levodopa to promote sustained release, succeeding as a potentially effective treatment for Parkinson's disease [172]. In other study, high drug loading percentage and controlled release rates of paclitaxel, an anti-tumor medication, have been achieved when incorporated into poly(butylene succinate-*co*-butylene dilinoleate) encapsulations [173].

4.3 Electronics

Thermal and electrical properties are key attributes to consider in the design of electronic devices. From semiconductors to electromagnetic interference shielding (EMI), PBS/CNTs nanocomposites have been recently introduced as substitutes of metals as they allow the manufacture of lighter and flexible conductive components with anticorrosive feature [174]. Heat dissipation requisites in electronic devices vary according to their size, generated heat, and limit working temperature. Several techniques for thermal management have been implemented, and these are commonly classified as direct and indirect contact cooling [16]. Indirect heat exchange to lower the temperature of electronic components can be carried out by heat sinks. Size, pins shape and addition of coolant inner microchanneling are features to tackle in heat sink design; nevertheless, substrate selection remains as one of the main characteristics to take into consideration [175]. Conventional heat sinks are made of metallic materials, such as copper and aluminum, while non-metals like silicon are also commonly used. Graphene and its derivatives are thermo-conductors and therefore they are suitable fillers to create heat-dissipating BPNCs [176, 177]. Potential BPNCs in heat sinks include PBS/GNPs, PBS/PLA/CNTs, and PBS/CB nanocomposites [125, 128].

In energy storage and conversion technologies, conductive foams have been proposed due to their low weight and easy moldability. Through batch foaming, using supercritical CO_2, PBS foams loaded with CNFs and expanded graphene can be obtained. These nanocomposites show enhanced electrical conductivity, granting them the capability of performing as low-density semiconductors with customizable morphology [73, 130]. Further studies of soft multilayered PBS-based nanocomposites have positioned these materials as potential candidates for EMI shielding. Taking advantage of the conductivity properties of MWCNTs [178], their incorporation into a PBS/thermoplastic polyurethane (TPU) blend has been performed. Achieved results point to the development of insulating nanocomposites consisting of a polymeric phase with a three-dimensional MWCNTs network that successfully weakens incident EMI by electromagnetic wave absorption and reflection mechanisms [179]. Additionally, a reported increase in the limited oxygen index (LOI) of these materials, induced by an increase of MWCNTs content, indicates the obtainment of EMI insulators with fire-retarding properties, mandatory traits for cellphone and computer parts, electronics for aerospace, and biomedical applications, among others [174, 179, 180].

Additive manufacturing, paving its way into the field of electronic devices currently represents an attractive approach for the construction of components with complex dimensions and specific thermal, electrical and mechanical properties. Moreover, through 4D printing, the creation of shape-memory polymers (SMPs) is attainable [181]. As SMPs response to thermal, electrical, magnetic or light stimuli depends on composition, reversible shape alterations of poly(butylene succinate-*co*-ethylene glycol) copolymers can be electro-induced when CNTs are added as nanofillers. These materials show high flexibility and shape recovery, which makes them functional in electromechanical systems where temperature control is difficult to obtain [182].

5 Conclusions

Poly(butylene succinate) is an aliphatic polyester that has found application in the major industrial sectors (ex. packaging) largely dominated by the traditional petroleum-based polymers. In recent years, PBS has attracted increasing attention due to the possibility of bio-based production, biodegradability, good processability, and excellent balance in thermo-mechanical properties. It is produced by reacting succinic acid with 1,4-butanediol in two subsequent steps: esterification into oligomers, and oligomers coupling into high molar mass PBS. These two monomers can be obtained at industrial level from both petroleum-based sources and bacterial fermentation of biomass derivatives. Great efforts have also been done in copolymerizing these two monomers with other dicarboxylic acids or diols to produce copolymers with properties varying in a wide range. PBS can also be blended with other biodegradable polymers like PLA. The thermal properties of PBS are favorable to the conventional polymer processing techniques like extrusion, injection molding, and thermoforming. However, PBS has some drawbacks, such as poor gas barrier property, which is not sufficient for some end-use applications. An actual trend in polymer science is developing biodegradable polymer nanocomposites, and PBS has been considered highly promising to produce high-performance and environmentally friendly nanocomposites. In numerous research works, it was reported that small amounts of nanoparticles in PBS can have significant effects in crystallization, thermal stability, and mechanical properties. Small amounts of nanoparticles can make PBS electrically and thermally conductive and thus suitable for application in areas such as electronic, sensors/actuators, robotics, etc. At present, much research work is being done to expand the capabilities of PBS-based nanocomposites to meet the rapid increase in the demand of improved biodegradable plastics. All new developments in PBS can make this bio-based polymer more and more present in all sectors of the polymer industry.

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Sustainable Approach of the Natural Rubber

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Abstract Elastomers correspond to a wide group of polymers, which are mostly obtained from non-renewable sources, such as crude oil. However, natural rubber is extracted from plant sources, such as *Hevea brasiliensis* and *Parthenium argentatum*, among others. In fact, several plants synthesize cis-1,4-polyisoprene through rubber transferase. Nevertheless, environmental, and social issues related with natural rubber difficult its integration in a circular economy approach. Therefore, this work is aimed to the understanding the fundamental aspects of the biosynthesis of cis-1,4-polyisoprene, as well as its biological degradation. Regarding the bacterial degradation, the use of bacteria genus, such as *Gordonia*, *Mycobacterium*, *Nocardia* and *Streptomyces* for degrading natural rubber, is boarded. Likewise, fungal degradation carried out by fungi species such as *Aspergillus*, and *Penicillium* genus is reviewed. The advances related to the isolation of Latex clearing protein (Lpc) and Rubber oxygenase A from Gram-positive *Streptomyces sp.* 30 and *Xanthomonas sp.* 35 Y, respectively, also are revised. Additionally, the processes, reactions and additives used in natural rubber for its use in different applications and how they can hinder the degradation of this material are boarded.

Keywords Natural rubber · Biosynthesis · Bacterial and fungal biodegradation

1 Introduction

Several polymers present elastomeric behavior at ambient temperature. There are several aspects that are inherent of this behavior, for instance, the glass transition temperature of elastomers is below ambient temperature, they can retract quickly after to be elongated, possess low or negligible crystallinity, and their large molecular weight allows to form chain networks, or it is needed to crosslink them by curing or vulcanization. As known, below the glass transition temperature (*Tg*) the

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polymer backbone are frozen, and their movements are restricted. Once the temperature increases above the glass transition temperature, the segmental movements of the polymer chains take place due to the thermal energy acquired by them. This also produces an increase of the free volume, consequently, the polymer backbones are less restricted to show segmental relaxations [1]. Below *Tg* elastomers are brittle, but above this parameter elastomers tend to be soft and flexible. The glass transition temperature is the most relevant aspect that influences on the macroscopic properties of polymer, but not the one. The main characteristic that produces that the elastomers can recover they shape after strain is attributed to the fact that polymer chains are crosslinked by dynamic or covalent bonds. The most extended type of crosslinking process used for obtaining elastomers is the vulcanization, where sulfide, disulfide and polysulfides acts as crosslinking between the polymer backbone. The vulcanization corresponds to a crosslinking process, which usually is carried out using sulfurs, activators, and accelerators, additionally, other additives are used to favor the processability and to enhance the performance of the mechanical properties. Further description of this process it is boarded on Sect. 4.1.

Cis-1,4-poly(isoprene), so-called as natural rubber, was the first elastomer used, and it is obtained mainly from plants of *Hevea brasiliensis*. However, this is not the only source where cis-1,4-poly(isoprene) that can be founded in nature. In fact, this polymer is bio-synthetized by several plants, because rubber is an end-product of one branch of the ubiquitous plant isoprenoid/terpenoid synthesis pathway [2]. *Castilla ulei, Manihot glaziovii, Hevea guianensis, Ficus elastica, Funtumia elastica, Landolphia kirkii, Euphorbia tirucalli, Cryptostegia grandiflora, Marsdenia verrucosa, Taraxacum kok-saghyz, Parthenium argentatum and Solidago spp.*, are some of the plants which bio-synthetize rubber [3]. Different authors have identified a high number of the plants that produce natural rubber.

Although *H. brasiliensis* crops are the most extended form to obtain natural rubber, *P. argentatum* (guayule) is being considered as an attractive alternative. This is because this perennial shrub produces an NR with a molecular weight comparable to that of *H. brasiliensis*, and guayule does not contain proteins related with the allergic reactions in human beings [4]. Moreover, the mechanical properties, such as a comparable strength, higher softness, and elongation to those of *H. brasiliensis*, highlight the NR obtained from guayule for medical applications [5]. Nevertheless, the production of NR obtained from guayule is discrete, and need to be grow up to be competitive with NR obtained from *H. brasiliensis*.

T. kok-saghyz, rubber dandelion, is another alternative to the *H. brasiliensis*, which correspond to an herbaceous plant. Its rubber present similar properties and quality to that of obtained from *H. brasiliensis*. However, there are several issues that should be solved before its production in crops. For instance, slow growth rate, high degree of heterozygosity, among others [6].

2 Biosynthesis of Natural Rubber

Cis-1,4-polyisoprene is biosynthesized by plants in proteolipid uni-lamella membranes, where the rubber transferase (RT-ase) complexes are linked, and catalyzed the cis-1,4-polyisoprene. Consequently, particles of natural rubber are yielded in the cytosol. RT-ase produces cis-1,4-polyisoprene by promoting a pathway where molecules such as of isopentenyl pyrophosphate and its stereoisomers, and dimethylallyl pyrophosphate (DMAPP) are involved (Fig. 1). The condensation reaction of these monomers generates several allylic pyrophosphates, which can act as rubber chain initiators, especially farnesyl pyrophosphate (FPP, C15). Further description of allylic pyrophosphates metabolic pathways can be found in the work reported by K. Cornish et al. [5].

3 Natural Rubber Latex

As mentioned, the formation of cytosolic natural rubber is promoted by the proteolipid uni-lamella membrane and RT-ase complex. Therefore, the natural rubber can be extracted as a colloidal suspension from its sources, such as *H. brasiliensis.* This suspension is so-called as natural rubber latex. The word latex, which come from Latin, indicates the fluid character of this substance, which is because the colloidal properties of this suspensions. Polymer colloids correspond polymer particles dispersed generally in aqueous medium, where particles sizes are in the range of 1–1000 nm [7]. The colloidal stability is imparted by the polar character of the moieties of proteolipid uni-lamella membranes and RT-ase which are on the surface of the rubber particles.

The colloidal stability of natural rubber latex can be explained by the DVLO theory, which describes particle interactions by combining electrostatic potential attributed to repulsive electrostatic double layer, and attractive London–Van der Waals interactions [8]. The coagulation is promoted by Van der Waals force, while the suspension is stabilized by the double layer force. In this regard, the enzymatic degradation of non-rubber content of natural rubber latex is promoted by bacteria, which putrefied the NR latex in hours after its extraction from tree [9]. In addition, the degradation of the non-rubber content of NR latex, namely, phospholipids and proteins, produces the decrease of the double layer repulsion [10]. As a result of the degradation, the surface charges which imparted by these polar moieties in natural rubber particles tend to disappear. Consequently, the rubber particles will coagulate. Therefore, it is necessary to use antibacterial agents to inhibit the degradation. As known, the ammonia is a mild bactericide, hence determined ammonia amount are added to NR latex to prevent the biological degradation of non-rubber content of NR latex.

The solid content of latex extracted and stabilized with ammonia is *ca.* 30% However, this concentration is not proper for processing or transport because the high

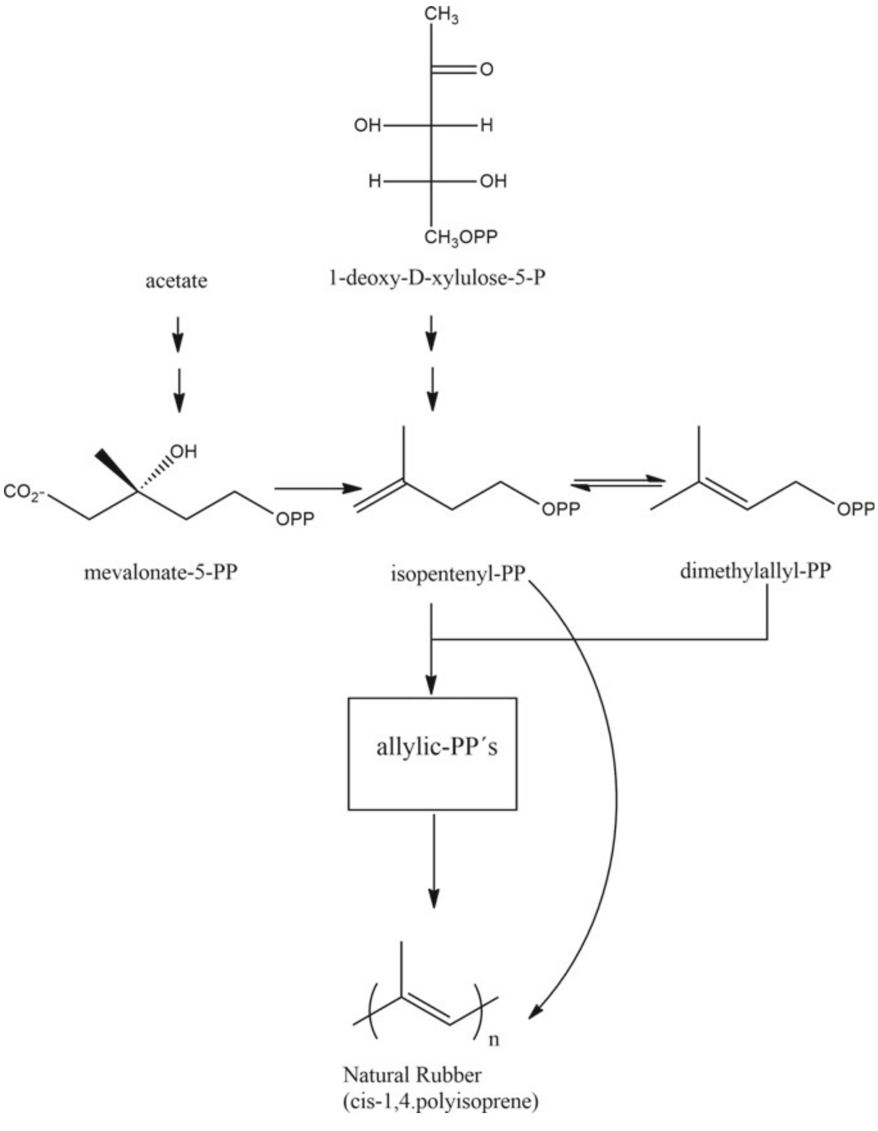


Fig. 1 Abbreviated biosynthesis of cis-1,4-polyisoprene

content of water. Therefore, the NR latex also is concentrated from field natural rubber latex. The American Society for Testing and Material (ASTM) defines in ASTM D 1076–21 five categories of NR latex, where the categories 1 and 3 corresponds to centrifuged *Hevea brasiliensis* natural latex stabilized with ammonia only, category 3 has low ammonia content (0.29% max.). category 2 is creamed *H. brasiliensis* natural latex stabilized with ammonia, while category 4 consists of centrifuged or centrifugated and creamed, and it has protein content less than 200 μg per gram dry weigh of latex. The stabilizer of category for can be ammonia or another hydroxide. category 5 is centrifuged *H. brasiliensis* natural latex treated with aluminum hydroxide or by other means, stabilized with ammonia only containing less than 0.5% non-rubber content. ASTM D 1076–21 also describes the methods for the characterization of most relevant parameters useful to the classification as well as its performance in industrial process or applications of *H. brasiliensis* natural rubber lattices.

Ammonia is not the only way to stabilize and preserve the natural rubber latex, other co-preserving agents also are used. For instance, natural rubber latex concentrated designed as LA-TZ contain 0.025% of tetramethylthiuram disulfide (TDMT) and Zinc Oxide (ZnO), and 0.05% of ammonium laurate. Other agents with multiple functions, such as, to impart stability to the natural rubber latex and to function as bactericide or enzymatic inhibitors, are used [9].

A widespread variety of natural rubber lattices is commercially available, this is due to the fact the NR latex can be submitted to different processes to obtain specialty NR lattices. For instance, the pre-vulcanized natural rubber latex corresponds to a specialty NR latex ready to use and its applications are in globes, condoms, catheters, etc. The pre-crosslinking of latex is possible because all accelerators and activators are added to NR latex before the dipping, so, when the dipping is accomplished the rubber particles start to coalesce, and the crosslinking takes place. This process occurs at ambient temperature and imparts mechanical strength to the film [9]. On the other hand, the deproteinized natural rubber corresponds to a latex which its protein was removed by chemical or enzymatic process, and as consequence of the lack of proteins in its surface surfactants are added to prevent its coalescence. Likewise, there are other chemically modified natural rubber lattices, such as epoxidized, methyl methacrylate grafted, cyclized and radiation vulcanized natural rubber lattices [9].

The natural rubber latex offers excellent properties and low viscosity to added it stabilized micro and nanoparticles. In this regard, it is possible to prepare masterbatch based on carbon black, nanocomposites containing, carbon-based nanomaterials, such as graphene, carbon nanotubes, or inorganic fillers, such as silica or ceramic nanomaterials [11].

4 Natural Rubber

Natural rubber is produced mainly by a conventional method which consists of the tree tapping and collection of latex, its filtration and after the coagulation of field NR latex with organic acids. This allows to obtain a gel in a shallow tray, which is squeezed, using roller to remove the serum, and washed to eliminate the chemical or biological remnants. The resulting sheet is dried by air, smoke or under the sun. several types and grades of natural rubber can be found available commercially, and therefore they are classified by using two systems. The International Standard of

Quality and Packaging is graded on the visual characteristics, while the Technically Specified Rubber (TSR) is graded on the technical specification of rubbers.

The rubbers produced by the industry can be grouped in: (i) Block natural rubber, (ii) Sheet natural rubber, (iii) Crepe natural rubbers and (iv) Specialty natural rubber. The global production of rubber increased one hundred per cent in the last two decades, from 6.8 million metric tons in 2000 to almost 13 million metric tons in 2020. A scheme of the industry-produced natural rubber is shown in Fig. 2. The block natural rubber is most relevant industry-produced natural rubber because it represents around 80% of the global production of all natural rubber traded internationally. The first developed block rubber was the Malaysia Standard rubber (SMR), obtained from *Hevea brasiliensis*. Other block rubbers are those produced by India, Indonesia, Thailand, Sri Lanka, Vietnam, designed as ISNR, SIR, STR, SSR, TVR, respectively. Sheet natural rubber are grouped as unsmoked sheet (USS), ribbed smoked sheet (RSS) and air-dried sheet (ADR). In the case of crepe rubbers, they are grouped mainly in thick pale crepe (TPC) and thin white crepe (TWC), which have less importance than the block rubber. Others natural rubbers of interest are specialty rubber, which also considers those rubbers chemically modified, among them it can be found natural rubber with lo protein content (DPNR), epoxidized NR (ENR), constant and low constant viscosity natural rubber (CVNR and LVNR, respectively), superior processing rubber (SP), liquid natural rubber (LNR), chlorinated NR (CNR) and tyre rubber [9].

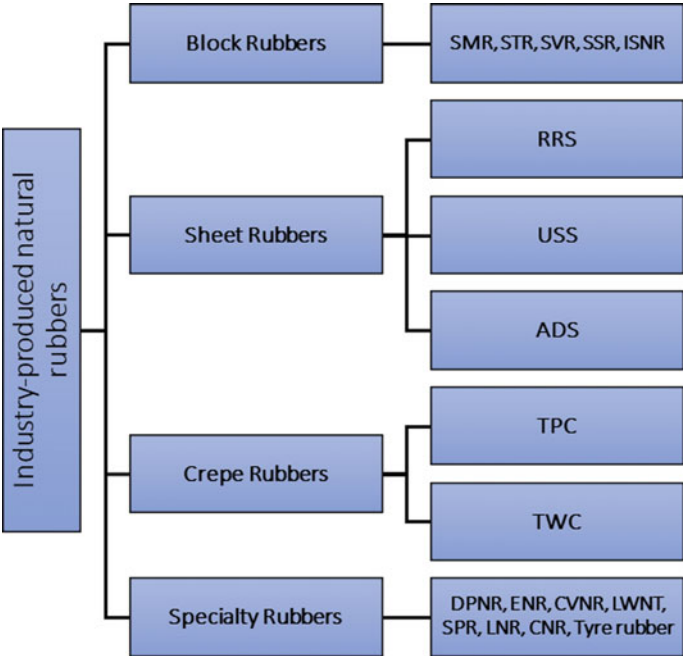


Fig. 2 Scheme of the industry-produced natural rubbers

4.1 Processing, Crosslinking, and Reinforcing of Natural Rubber

Raw natural rubber presents properties like thermoplastic polymer, the elastomeric properties are achieved by crosslinking, therefore, it is considered as thermosetting elastomer. The crosslinking promoted by sulfur is the most extended strategy to obtain natural rubber with elastomeric properties, and this process is so-called as vulcanization. As known, the vulcanization process was discovered by Charles Goodyear in 1839, however, the American original peoples already used crosslinking process of the natural rubber to obtain pieces such as balls used in rituals and games.

Currently, the technical background of rubber offers a widespread variety of reagent and formulations for crosslinking natural and synthetic rubbers. The vulcanization conventionally considers the addition of sulfur, activator, and accelerator agents.

The activators correspond to compounds which enhance the performance of accelerators. Zinc (II) oxide is the most extended, and also it is considered as the most efficient activator used in vulcanization. Although Zinc (II) oxide is hydrophilic compound, the mixing with fatty acids, such as stearate acid, allows to obtain a complex, Zinc stearate, ($ZnSt_2$), which increase Zn (II) solubility in natural rubber. Additionally, the presence of Zinc optimizes the crosslinking formation [12]. The activators are involved in the breakage of sulfur ring (S_8), instead, the accelerators promote the formation of sulfur intermediates, facilitating the occurrence of the crosslinking between sulfur and carbon double bonds [13]. Consequently, the accelerators are those compounds that increase the speed of the vulcanization, decreasing the time and temperature to obtain a more efficient vulcanization.

Guanidines, benzothiazoles, sulfonamides, thiurams, thiocarbamates are the most extended accelerators used in vulcanization [14]. Guanidines and benzothiazoles correspond to moderate accelerators, while sulfonamides are considered as fast accelerators. Instead, thiurams and thiocarbamates are ultrafast accelerators. Likewise, the type of accelerators will determine the crosslinking length, for instance, thiurams and thiocarbamates yield short crosslinking length, while the crosslinking length using guanidines and benzothiazoles are medium-long, and long medium, respectively. Additionally, the absence of accelerator produces a very long time of vulcanization, as well as the crosslinking length is quite long. It is important to mention that ASTM D 4818–21 classifies the accelerator in six groups: (i) Sulfonamides, (ii) Thiazoles, (ii). Guanidines, (iv) Dithiocarbamates, (v) Thiurams disulfides, (vi) Thiurams without disulfide function.

The content of accelerators and sulfur in rubber matrix and the ratio of these substance determine aspects such as length of polysulfide moieties which connect the cis-1,4-polyisoprene backbones, as well, as the density of crosslinking. The vulcanization system is classified in three types: conventional (CV), semi-efficient (SEV) and efficient (EV). CV system consists of a ratio of accelerator to sulfur between 0.1 and 0.7. In this system the content of sulfur in the rubber compound is in the range of 2.0–3.5 part per hundred rubber (phr), while the accelerator content is between

1.0 and 0.4 phr. CV system favors to increase the tensile and tear strengths, also impart a high compression set, and poor heat aging to the rubber compounds, and it yields polysulfide-based crosslinking. SEV system considers accelerator to sulfur ratio in the range of 0.7 and 2.5. The accelerator content in rubber is between 2.5 and 1.0 phr, and the content of sulfur is in the range of 1.0–1.7 phr. This system is characterized by conferring a balance of strength and heat aging resistance, and the sulfur crosslinking moieties are mixture of poly, di and mono-sulfide. EV system provides excellent heat aging resistance, low compression set, low tensile, and tear strengths, the mono-sulfide are the species resulting of the crosslinking. The accelerator and sulfur ratio in EV system are between 2.5 and 12.0, and the accelerator content is between 6.0 and 2.5 phr, the sulfur content is of 0.3–0.8 phr [15].

A concerning issue related to the vulcanization is the toxicity of some reagent such as accelerators. The high toxicity of benzothiazoles, such as 2-mercaptobenzothiazole (MBT) and 2-(2-hydroxyethyl-mercapto) benzothiazole (HMBT), have been demonstrated by carrying out studies in cells and mice [16]. Sulfonamides are also a kind of antibiotics, which frequently have been detected in environment [17], consequently, their release to the environment from rubber compounds can mean other ecological concern related to the rubber compounds. The use of thiurams is widely extended in vegetal crops as fungicidal [18, 19], as well as thiocarbamates are use as herbicides [20]. There are a wide types guanidines-based compounds, some of these are cytotoxic, antifungal, and antiviral, but also there are guanidines-based compounds that are used in the pharmaceutical industries [21, 22]. Consequently, the accelerators not only can be released from the natural rubber compounds, also can inhibit the proliferation of microorganisms.

Organic and inorganic fillers are other components used for obtaining rubber compounds. Carbon black is the most extended materials used as filler in rubber composites, these materials is obtained from pyrolysis of different hydrocarbon such as oils. The size, iodine or oil absorption, specific surface area, pour density among other features of carbon black are categorized in norm ASTM D 1765–21. For instance, the basic classification of carbon black consists in four characters, where the first indicates the effect carbon black in the curing rate, in this way, “N” indicates that the curing will not be affected, while “S” indicates that the curing rate will be inhibited. The second character indicates the average of surface area is from 0 to 9. Carbon blacks designed as 0 in its second character have a specific surface area higher to 150 m^2/g, those designed with “1” have a specific surface area in the range of 121–150 m^2/g. As the character increases from 1 to 9, the specific surface area decreases, reaching value between 0 and 10 m^2/g in the case of carbon black designed as “9”. The third and fourth characters in the ASTM carbon black system classification are arbitrarily assigned digits. As known, carbon black is a very stable material and impart properties which significantly enhance the properties or rubber compounds, such as abrasion resistance, hardness, mechanical properties, among others. Other fillers are uses, such as silica and clays, but the affinity of carbon black to conventional rubbers is excellent, superior to that of silica or clays [23].

The processing of solid natural rubber, such as block, sheet, or crepes, for obtaining vulcanizate rubber consists of the addition of different components, such as sulfur, activators, accelerators and filler to the rubber matrix, and their mixing using equipment such as Banbury and two roll mills. This approach for processing rubber compounds allows to obtain a homogeneous dispersion of the curing agents and filler in a relatively easy and fast way. After, the mixing is vulcanized by using pressure and heat to promote the occurring of vulcanization. The time required for curing the compound is an important matter for obtaining compounds with features as good as the formulation allows to achieve. In this regard, the curing curve, determined by using, for instance, a Rotorless cure meter (ASTM D 5298–19), which uses two specimen shaping members or dies to sense the torque or stress during strain application. The value measured by a torque transducer at the peak strain amplitude of the oscillating cycle represents the elastic response of the test material. This response is recorded in a curve of torque as a function of time, which is so-called vulcanization curve (Fig. 3). The vulcanization curve is an excellent tool to understand the vulcanization, which is a process that involves various reactions, which occurs sequentially or concurrently. Overall, these reactions start slowly, and after of the scorch time, occur rapidly. These reactions gradually deaccelerate until to reach a constant stiffness. Then, it is possible to observe the gradually reduce of the stiffness, process, which is so-called reversion, or to occur a gradually stiffness increase associated to a process designed as marching. By using this method, it is possible to estimate the time to reach a percentage of full cured by considering some percentage of the difference from the minimum to the maximum torque.

In addition, there are several methodologies to determine the features of the rubber compounds, such as, compression set, abrasion resistance, tensile strength, tear strength, resilience, among others, and all of these are essential to know, predict and estimate the performance of rubber compounds in a specific application.

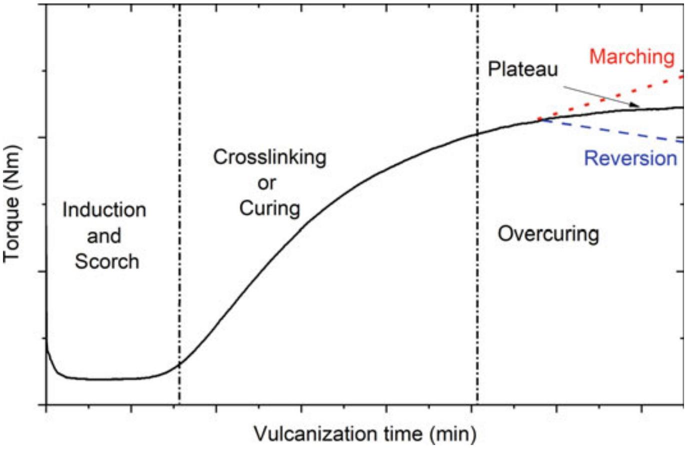
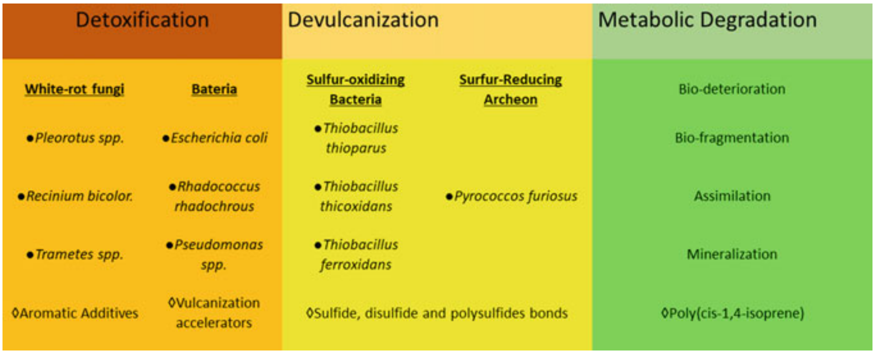


Fig. 3 Vulcanization curve of natural rubber

5 Biodegradation of Natural Rubber

The microbial susceptibility of Natural rubber (NR), as *Hevea brasiliensis* latex, was widely examined and reviewed to understand not only its degradation, but also ways to preserve it [24]. Nevertheless, most of pieces based on natural rubber are based in crosslinked natural rubber, filled with different materials to enhance their mechanical properties performance and lifetime. Consequently, the natural rubber-based composites consist of a highly unsaturated polymer, strongly susceptible to oxidative degradation. Besides, the molecular weights of *cis*-1,4-polyisoprene of natural rubber is in the range between 10^5 and 10^6, and due to the crosslinking network structure of the vulcanizates makes them insoluble in any organic solvent. In this respect, the organisms prefer to use as carbon and energy source the low molecular-weight fragments produced by a non-biological degradation [25].

To the best of our knowledge, and backed by the information available in the literature, the biodegradation of rubber compounds is a slow process since it requires, at least, three main stages, which must carry out by specialized and specific microorganisms (Fig. 4). The first stage corresponds to the detoxification of natural rubber compounds. In this stage the curing agents, such as accelerators and activators are removed and/or metabolized by white-rot fungi or bacteria. This stage is addressed to remove potentially toxic compounds to those devulcanizing and rubber degradation microorganisms. The devulcanization corresponds to that stage where the sulphide, di-sulphide or poly-sulphide bonds are degraded by action of sulphur-oxidizing bacteria or sulphur-reducing archeon. The third stage corresponds to the complex process of metabolic degradation, which involves four stages, namely, bio-deterioration, bio-fragmentation, assimilation, and mineralizing. These four stages are mainly discussed for biodegradable polymers such as poly(lactic acid), poly(caprolactone), among others [26–28].



Detoxification		Devulcanization		Metabolic Degradation
White-rot fungi	**Bateria**	**Sulfur-oxidizing Bacteria**	**Surfur-Reducing Archeon**	Bio-deterioration
•*Pleorotus spp.*	•*Escherichia coli*	•*Thiobacillus thioparus*		Bio-fragmentation
•*Recinium bicolor.*	•*Rhadococcus rhadochrous*	•*Thiobacillus thicoxidans*	•*Pyrococcos furiosus*	Assimilation
•*Trametes spp.*	•*Pseudomonas spp.*	•*Thiobacillus ferroxidans*		Mineralization
◊Aromatic Additives	◊Vulcanization accelerators	◊Sulfide, disulfide and polysulfides bonds		◊Poly(cis-1,4-isoprene)

Fig. 4 Schematic representation of the biodegradation of vulcanized natural rubber compounds consisting of 3 stages, namely, detoxification, devulcanization and metabolic degradation. Solid circle (•) represents the organisms described for the respective processes. Empty rhombus (◊) indicates the molecules or functions degraded in the respective stage

Usually, the fillers used are stable and non-biodegradable materials, which improve several properties, such as, tensile strength, permeability, abrasion resistance, among other characteristics. In the same way to that processes to obtain conventional natural rubber compounds based on those non-biodegradable fillers (carbon black, silica, clays), the degradation can be understanding as a complex process, which involves several stages. Currently, there are several groups which are developing sustainable fillers which can improve the properties of the natural rubber compounds as good as conventional fillers. The biodegradation of sustainable fillers is an important matter for the developing sustainable rubber compounds, but in this chapter, this is not discussed.

By considering exclusively the degradation of rubbers, overall, bacteria and fungi can degrade natural as well as synthetic rubbers and utilize it as carbon and energy source [29]. The first report related to biodegradation of natural rubber was done by Söhn-gen and Fot in 1914, who observed that the *Actinomyces spp.* such as *A. elastica*, and *A. fuscus*, usually present in garden soil, can assimilate caoutchouc hydrocarbon [30]. Likewise, several studies have showed that the Actinomycetes were almost the only organisms able to considerably decompose Natural Rubber and to use the rubber hydrocarbon as a carbon source [25, 31–33]. Furthermore, Jendrossek et al. published the article entitled "Bacterial degradation of natural rubber: a privilege of actinomycetes?" where they reported the interesting results obtained only for organism members of the Actinomycetes, which correspond to a large group of mycelia forming gram-positive bacteria [33]. Nevertheless, it is currently known that there are several bacteria and fungi capable of achieving rubber degradation with different mechanisms.

5.1 Bacterial Degradation of NR

The bacterial degradation of rubber compounds conducted by different strains has been widely studied for decades [34], as well as several studies have been carried out using similar methodologies, considering variations in the substrate, species/strains, pre-treatments, time, etc. This to evaluate, for instance, different stages of degradation, affinity, and efficiency of the strains.

Rubber degrading bacteria are divided into two groups, which use different strategies to degrade natural rubber [35]. The first group corresponds to Translucent halos forming bacteria, which excrete rubber-cleaving enzymes, when they are cultivated on solid media containing latex particles. Actinomycetes, of the following genera: *Actinomadura, Actinoplanes*, *Dactylosporangium, Micromonospora, Microtetraspora* and *Streptomyces*, are the most representative members of this group [29].

The second group corresponds to those organisms that neither form halos nor grow on NR latex films, by the contrast to the first group, they must be in direct contact with the polymer. These organisms grow adhered to the surface of rubber matrix in a liquid culture and lead the most potent rubber-degrading bacterial strains.

This group comprises Actinobacteria that excretes mycolic acid, and their genera are *Gordonia*, *Mycobacterium*, and *Nocardia* [29].

Due to the explosive interest on the biodegradation of natural rubber, it is important to know about the enzymes involved in the degradation of this polymer. In this respect, latex cleaning protein ($\mathrm{Lcp_{K30}}$) isolated for first time from *Streptomyces sp.* K30 and rubber oxygenase A (RoxA) isolated from *Xanthomonas sp.* 35Y, have been identified and characterized [36, 37]. The degradation conducted by RoxA cleaves poly(cis-1,4-isoprene) to 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (ODTD) as the core outcome. The structure of RoxA has been characterized, and several residues that were involved in oxygen stabilization and active site properties have been identified in the last decade [38].

In addition, Lcp enzymes are an important part of the protein complex responsible for clear zone formation on latex agar overly NR film by oxidatively cleave poly(cis-1,4-isoprene) to smaller products but with the same keto and aldehyde end groups as in RoxA-generated ODTD [39]. In a similar way, Aamer et al. [40] indicates that these two enzymes are essential for the degradation of natural and synthetic rubbers, which are obtained from Streptomyces sp. and Xanthomonas sp., respectively. These enzymes yield compounds such as aldehydes, ketones, and other carbonyl-type groups because of the degradation.

5.2 Fungal Degradation of NR

The first report on the ability of filamentous fungi to degrade natural rubber was done by De Vries in 1928, who described the biodegradation of rubber in liquid cultures of *Aspergillus sp.* and *Penicillium sp.* where the weight loss of natural rubber was 15% after 19 months of incubation. In the following years, several studies have been published confirming the possibility of degrading natural rubber by these fungal species and several other species [41]. Decades later, Kwiatkowska et al. carried out a simple but effective method by using soil burial tests on NR vulcanise sheets, obtaining a decrease on the sample weight up to 90% after 3 months [24, 42].

The work reported by G. Nayanashree and B. Thippeswamy in 2015 describes the degradation of rubber discs conducted by *Penicillium chrysogenum.* The degradation achieved was of 64.3% of weight loss. Besides, they identified enzymes belonging to manganese and laccase peroxidases complex [43]. In other work approached to compare two fungal species, *Alternaria alternata* y *Penicillium chrysogenum*, it was observed that *Alternaria alternata*, in an incubation of 65 days, was more effective in the degradation than *P. chrysogenum* [34]. In the case of *A. alternata* two methodologies were used, reaching a biodegradation of 4.3 wt%.

Likewise, enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), laccase and versatile peroxidase (VPs) of white-rot fungi are involved in the mineralizing of various recalcitrant aromatic compounds, consequently, these enzymes have the potential of degrade various rubber aromatic additives [27, 44].

6 Outlook and Perspectives

The development of new elastomer materials should be according to the approach based on circular economy and sustainability. The first advantage of natural rubber steams from its availability in nature, as several plants can produce it. Eventually, the genetic engineering will develop species with the capability to biosynthesize natural rubber in extreme conditions than that species such as *Hevea brasiliensis, Parthenium argentatum* and *Taraxacum kok-saghyz* are promising vegetal sources.

Besides, the natural rubber compounds that will be obtained by a sustainable approach should complain with the features of the conventional elastomeric materials. Therefore, it is essential to develop materials based on sustainable sources, such as biosynthesized and biodegradable fillers. Undoubtedly, the new sustainable elastomer compounds based on natural rubber must be designed considering their biodegradation processes.

In a similar way to the process for obtaining natural rubber compounds that involves several steps, the biodegradation of these compounds should be boarded as a collection of steps addressed to degrade sequentially the different components present in natural rubber-based compound. For instance, detoxification, devulcanization, rubber degradation, processes which carried out by specific and specialized microorganisms. Besides, the formulation of rubber compounds such as the curing system should be re-thinking to obtain safer system to the microorganisms.

In addition, it is possible to expect that the futures elastomers and their biodegradation will produce compounds useful in different areas. For instance, the degradation of these materials will produce fertilizing compounds, or promote the soil regeneration.

As more than one century, the natural rubber continues being a strategic material, but it is crucial to identify its place into the circular economy, therefore, multiply the efforts and the research addressed to this topic is essential to reach sustainable elastomer materials.

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Gum Based Green Nanocomposites and Their Applications

Suman Thakur

Abstract Research on biobased polymers has gained prominence to minimize the dependency on conventional petroleum-based polymers. Among the different types of biobased polymers, tree gums are one of the greener materials and are derived from a variety of tree genera. They fall into three categories based on their origin: plant exudate gums, microbial gums, and seed gums. Gums are abundant, cheap, harmless, biodegradable, and biocompatible. Gum nanocomposites are used in various industries, including packaging, tissue engineering, medicine delivery, and environmental applications. This chapter gives a state-of-the-art overview of gum-based nanocomposites and discusses how they may be used in various fields. First, various tree gums (Arabic, karaya, tragacanth, ghatti, and kondagogu), their chemical structure, and respective properties will be discussed. Gum nanocomposites with metal, metal oxide, and carbon based nanomaterials will then be described for their applications in a multifaceted arena, to deliver a milieu for future research.

Keywords Tree gum · Bio-based · Antibacterial · Biodegradable · Sustainable · Polysaccharides

1 Introduction

Throughout history, biopolymers have been utilized in various fields [1]. However, the utilization of biopolymers has been declined especially last two centuries due to the development of cost-effective and mass-produced petrochemical based polymers which rapidly established to be a better fit for society's demands [2]. In recent years, limited stock of petroleum and environmental concerns has inspired a rise in interest in biopolymers and sustainable techniques for producing them [3]. Numerous sustainable approaches and new biopolymers have been developed or explored by industrial and academic researchers to find the best alternatives to petrochemical based polymers [4].

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Amongst the biopolymers, gums are a relatively unexplored source of high-value natural products [5]. Gum polysaccharides are inexpensive, readily available, non-hazardous and eco-friendly versatile green materials with diverse structural and functional properties [6]. Many gum polysaccharides have been reported in the literature in recent years from a variety of plant sources with multifaceted applications. They are generally hydrophilic and can enhance the viscosity of aqueous solutions even at low concentrations [7]. Gums contribute as crucial components of cosmetics, paper, biomedical, therapeutic, fabric and other markets [7]. They have been utilized as emulsifiers and thickeners in food, cosmetics, and pharmaceutical formulations to control texture, and increase the viscosity as well as consistency of the final product. Gums also find multiple biomedical applications due to their unique structure and functionality. As tree gums are safe, edible and biodegradation, they are ideal materials for persistent drug delivery applications.

Even though gums are recently found suitable biobased materials for many applications due to their nontoxicity, biodegradability, and physicochemical properties, the use of pristine gums alone is limited in many applications too. This is due to their hydrophilicity, brittleness/low flexibility, lack of film-forming ability, and low tensile properties. To address the problem, different types of gum nanocomposite with various nanomaterials are developed and investigated for their suitability in many applications such as drug delivery, water treatment, gas barrier, etc.

In this chapter, different kinds of gums polysaccharides based on their sources are described along with their structure and physicochemical properties. Gum nanocomposites with different nanomaterials such as metal oxide, carbon and organic-based are highlighted with their applications, to deliver a milieu for future research.

2 Gums: Types, Structure and Properties

Gums are basically hydrocolloid, polysaccharides with complex structures of repeated glycosidic bonds and many hydroxyl groups [8, 9]. The complex chemical structure facilitates to form a network with metal ions. The main chemical functional groups found in most of the gums are hydroxyl, acetyl, ether, carbonyl, and carboxylic groups. Due to presence of lot of hydroxyl groups in the structures, it has interaction with water and form hydrocolloid. Therefore, gums are the ideal materials for food and biomedical applications.

Gum broadly classified into three categories based on their natural resources; tree gum (arabic, tragacanth, karaya, kondagogu and ghatti), seaweed gum (agar, carrageenan, and alginate) and microbial gum (dextran, xanthan, and gellan gum) as shown in Fig. 1.

There are many plant-based gums, among them; five gums are widely used in various applications. A brief discussion about those gums is provided below.

(a) Arabic or acacia gum is edible. It is generally derived from the branches and stems of *Acacia seyal* and *Acacia senegal*. It has a complex branched structure

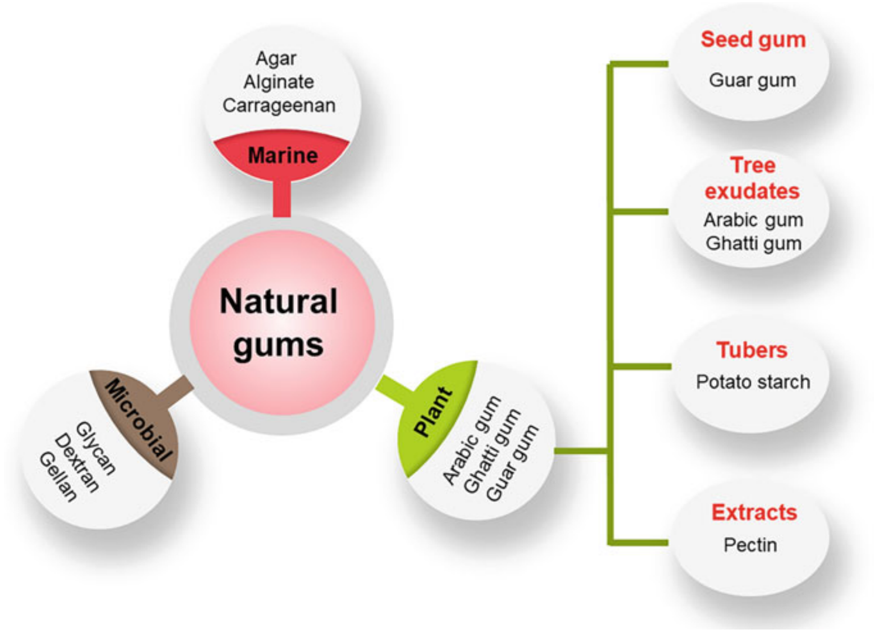

Fig. 1 Types of tree gum based on their origin

of polysaccharides and metal ions (calcium, potassium, and magnesium) and Arabic gum is either neutral or slightly acidic [10, 11]. It has a slight Newtonian viscosity even at high concentrations of gum. Its ability to intercalate with a wide range of molecular entities, such as proteins, minerals, and polyphenols, is attributed in great part to its distinct structural properties (carbohydrate-protein macrostructure). As a result, Arabic gum is widely used as an emulsifier, additive, or thickener. It is utilised as a food additive in the bakery, beverage, confectionery, flavour encapsulation and food emulsion sectors to prevent sugar crystallisation [12, 13]. It also has pharmaceutical applications as a tablet binder and emulsifying agent in oral/topical pharmaceutical formulations [14].

(b) Karaya gum is stems and branches derived exudate from species of *Sterculia,* especially *Sterculia urens* tree. It has a pinkish-grey coloured fine powdery appearance. Karaya gum has a branched structure of magnesium and calcium salt of partially-acetylated polysaccharide [15, 16]. It is made up of 60% neutral sugars (galactose and rhamnose) and 40% acidic sugars (galacturonic and glucuronic acids). Food stabiliser, denture powder, meat binder, dyes thickener for textile printing and bulk-forming laxative to treat constipation are the main uses for karaya gum [17].

(c) Ghatti gum is a polysaccharide derived mostly from the *Anogeissus latifolia* plant (Combretaceae, Myrtales). Ghatti gum consists of magnesium-calcium salt of various carbohydrate molecules such as D-xylose, D-mannose, D-galactose, L-arabinose, and D-glucuronic acid [18, 19]. It has a light yellow to brown coloured dust appearance and is available in various grades based on its solubility and viscosity. Ghatti gum is mainly utilized as a binder, emulsifier, thickener, and stabilizer [6].

(d) Kondagogu gum is exudate derived from the Kondagogu tree, which is mainly found in India. It is made of various types of acidic sugars (glucuronic and galacturonic acid) and neutral sugars (arabinose, glucose, rhamnose, galactose, and mannose) [20]. Due to its non-toxic, cost-effective, and biocompatibility, this gum is utilised as an emulsifying agent in the food and pharmaceutical industries.

(e) Tragacanth gum is an exudate taken out from the branches and stems of *Astragalus microcephalus* and *Astragalus gossypinus*. It is a branched anionic heteropolysaccharide. Seasonal changes and geographic location influence the chemical composition of tragacanth gum. It mainly contains different sugars such as arabinose, xylose, glucose, galactose, fucose, rhamnose and galacturonic acid [21]. This gum is extensively used in food industries (Fig. 2) in various forms as stabilizers, emulsifiers, protein replacers, fat replacers and so on [22]. This gum is used in many non-food applications due to its remarkable functional and structural properties. It is utilized in hydrogels, cryogel and xerogel blends, tunable drug delivery systems, bioplastics, micro-encapsulations, and edible blend films nanocomposites for heavy metal ions removal, and so forth [23, 24].

3 Gum Based Green Nanocomposites

The incorporation of various metals, metal oxides, and carbon based nanomaterials in gum provides green nanocomposites for multifaceted applications in the environmental, textile, packing, and biomedical sectors. In this section, different gum-based nanocomposites are described along with their special features and applications (Table 1).

3.1 Gum/silver Nanocomposites

Silver based nanomaterial is one of the most used nanomaterials for antibacterial and antiviral applications. To develop the antibacterial green nanocomposites, silver based nanomaterials are incorporated in various gums. This gum nanocomposite showed good antibacterial activity against Gram-positive and Gram-negative bacteria. Gum/silver nanocomposites are explored for wound healing, drug delivery, biosensors, and water treatment. Karina et al. introduced silver nanoparticles in carboxymethyl cellulose/cashew gum to fabricate an antibacterial nanocomposite [53]. In vivo wound healing ability of pristine cashew gum in a rat model was improved by introducing silver nanoparticles. Hussain and Jaisankar prepared

Fig. 2 Application of tragacanth gum and its derivatives in food industries. Reprinted from Ref. [22] with permission from the publisher

almond gum/poly(acrylamide)/silver hydrogel by radical polymerization and simultaneously Ag^+ reduction to Ag nanoparticles [26]. This hydrogel showed good antibacterial activities against *Escherichia coli*, *Staphylociccus aureus* and *Pseudomonas aeruginosa*. Antibacterial gum/silver nanocomposites are also used for water treatment. Mohammadian et al. prepared modified-gum tragacanth/poly(vinyl alcohol)/silver nanocomposite which was used as an adsorbent for Ca^{2+} and Mg^{2+} ions (Fig. 3) [25].

Carboxymethyl guar gum/silver nanocomposite films are used for food packaging applications. The nanocomposite films showed antibacterial properties against *B. subtilis* and *E. coli*. The tensile strength of those nanocomposite films is also enhanced than pristine carboxymethyl guar gum film. Kondagogu gum/silver nanocomposites show anti biofilm and antibacterial properties against Gram-positive and Gram-negative bacteria [29]. Antibacterial activity of the nanocomposite is further improved by the incorporation of antibiotics such as aminoglycosides or

Table 1 Gum-based nanocomposites: their antibacterial activities and applications

Gum nanocomposites	Antimicrobial activity	Applications	Reference
Poly(vinyl alcohol)/tragacanth gum/Ag	*E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*	Water treatment	[25]
Poly(acrylamide)/almond gum/Ag	*E. coli, P. aeruginosa, S. aureus*	Biomedical	[26]
Guar gum/Ag	Not reported	Gas sensor	[27]
Tragacanth gum/graphene oxide/Ag	*S. aureus*	Water treatment	[28]
Kondagogu gum/Ag	*E. coli, P. aeruginosa, S. aureus*	Antibacterial devices	[29]
Tragacanth gum/Ag	*B. subtilis, E. coli*	Wound dressing	[30]
Tragacanth gum/cotton fabric/Ag	*E. coli, S. aureus*	Wound dressing	[31]
Guar gum/chitosan/Ag	Not reported	Water treatment	[32]
Guar gum/chitosan/Ag	Not reported	Sensor	[33]
Tragacanth gum/cotton fabric/Ag	*E. coli, S. aureus*	Wound dressing	[34]
Guar gum/Ag	Not reported	Wound healing	[35]
Acacia gum/poly(acrylate)/Ag	*E. coli*	N.R	[36]
Guar gum/Ag–Cu	*Listeria monocytogenes, Salmonella typhimurium*	Food packaging	[37]
Xanthan gum/chitosan/Ag	*E. coli, S. aureus*	Wound dressing	[38]
k-Carrageenan gum/xanthan gum/gellan gum/TiO_2 NPs	*S. aureus*	Food and non-food industries	[39]
Gellan gum/TiO_2	*E. coli, S. aureus*	Wound dressing	[40]
Ghatti gum/modified montmorillonite/CuO	Not reported	Water treatment	[41]
Arabic gum/poly(vinyl alcohol)/TiO_2	Not reported	Drug delivery	[42]
Arabic gum/ZnO	*E. coli*	Medical and food	[43]
Acacia gum/alginate/ZnO	*P. aeruginosa*	Medical	[44]
Tragacanth gum/ZnO	*S. aureus, E. coli, Candida albicans*	Medical	[45]
Acacia gum/alginate/ZnO	*P. aerigunosa, Bacillus cereus*	Medical	[46]
Acacia gum/Au	*E. coli, S. aureus*	Medical	[47]
Olibanum gum/Au	Not reported	Catalysts	[48]
Kondagogu gum/Au	*E. coli, B. subtilis*	Medical	[49]
Ghatti gum/Pd	*S. aureus, P. aeruginona*	Medical	[50]
Kondagogu gum/Se	*B. subtilis, Micrococcus luteus*	Medical	[51]

(continued)

Table 1 (continued)

Gum nanocomposites	Antimicrobial activity	Applications	Reference
Guar gum/Al_2O_3	*S. aureus*	Water treatment	[52]

ciprofloxacin. An increase in intracellular reactive oxygen species (ROS) production in bacteria is the reason for the improved antibacterial activity. Tragacanth gum/silver nanocomposite hydrogel shows good potential as a wound healing material [30]. Tragacanth gum/silver nanocomposite hydrogel is also used to provide antibacterial properties in cotton fabrics [34].

3.2 Gum/TiO_2 Nanocomposites

TiO_2 nanoparticle is another excellent antibacterial material. Antibacterial activities of TiO_2 nanoparticles are ascribed to the formation of ROS-derived oxidative stress, which damages bacteria's site-specific DNA [54]. Gum/TiO_2 nanocomposites show antimicrobial properties [55]. The nanocomposites are good candidates for biomedical, water treatment, and food packaging for their biodegradable, antibacterial, anti-ultraviolet light, and photocatalytic properties.

Xanthan gum, k-carrageenan gum and gellan gum based TiO_2 hydrogel nanocomposites have been prepared by solvent casting as packaging materials [39]. The addition of TiO_2 nanoparticles to hydrogel nanocomposites increases their physico-mechanical properties. These hydrogel nanocomposite films are UV light resistant and possess partially antibacterial activities. They are used as ultraviolet light-shielding materials for food and non-food packaging. Gellan gum/TiO_2 nanocomposites biofilm which was prepared by solvent casting method showed good potential for wound dressing material [40]. Wounds treated with the gellan gum/TiO_2 nanocomposite films entirely recovered in two weeks, but wounds treated with pure gellan gum films only healed partially. El-din et al. fabricated acacia gum/polyvinyl alcohol/TiO_2 nanocomposites by γ-radiation for drug delivery as pH dependent release of the steroid prednisone [42]. Santoso et al. developed porous adsorbent materials using guar gum and TiO_2 [56]. A schematic illustration of the preparation of guar gum/TiO_2 hydrogel is shown in Fig. 4.

3.3 Gum/ZnO Nanocomposites

Zinc oxide nanoparticle is another widely used antibacterial nanomaterial and is used in many applications such as biosensors, photodetectors, electronics, solar cells, lasers, and cosmetics. Due to excellent antimicrobial activities against fungi, Gram-positive and Gram-negative bacteria, gum/ZnO nanocomposites are used in medical

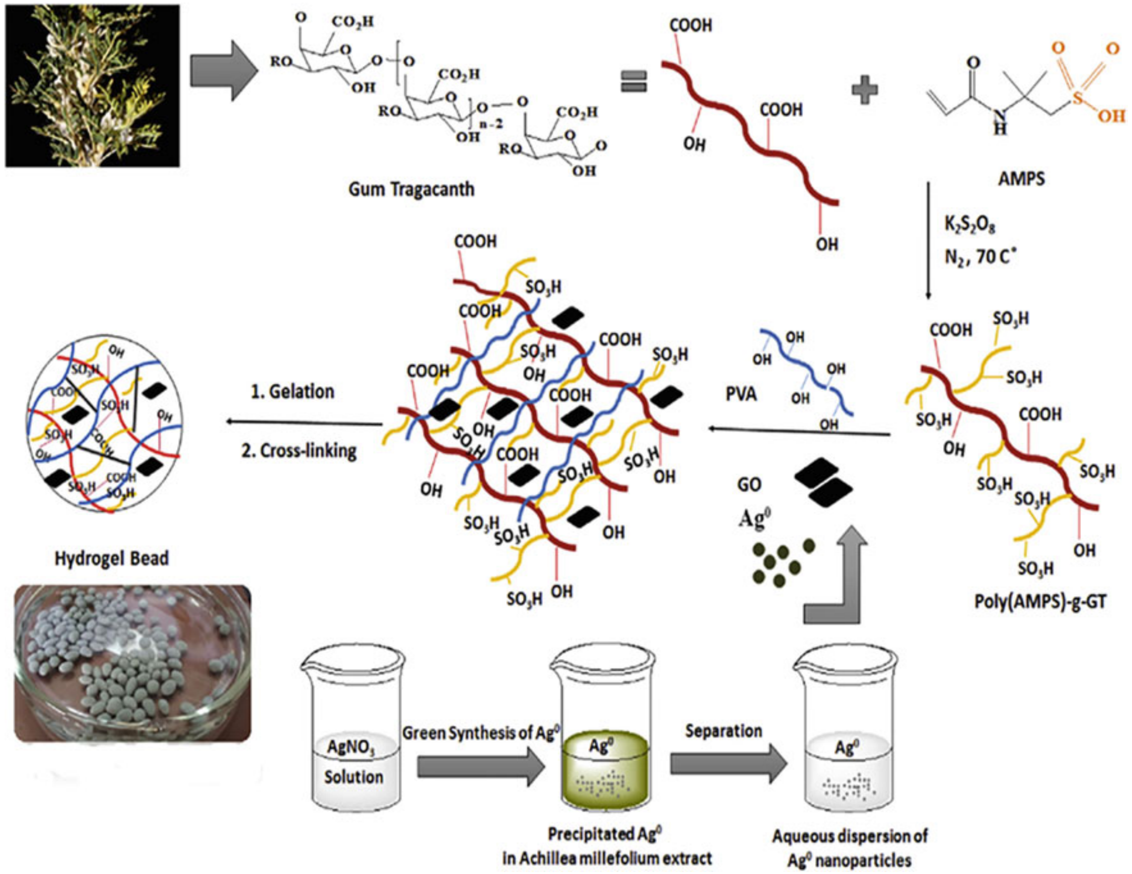

Fig. 3 The preparative route of polyvinyl alcohol/tragacanth gum/Ag/GO nanocomposite hydrogel. Reprinted from Ref. [25] with permission from the publisher

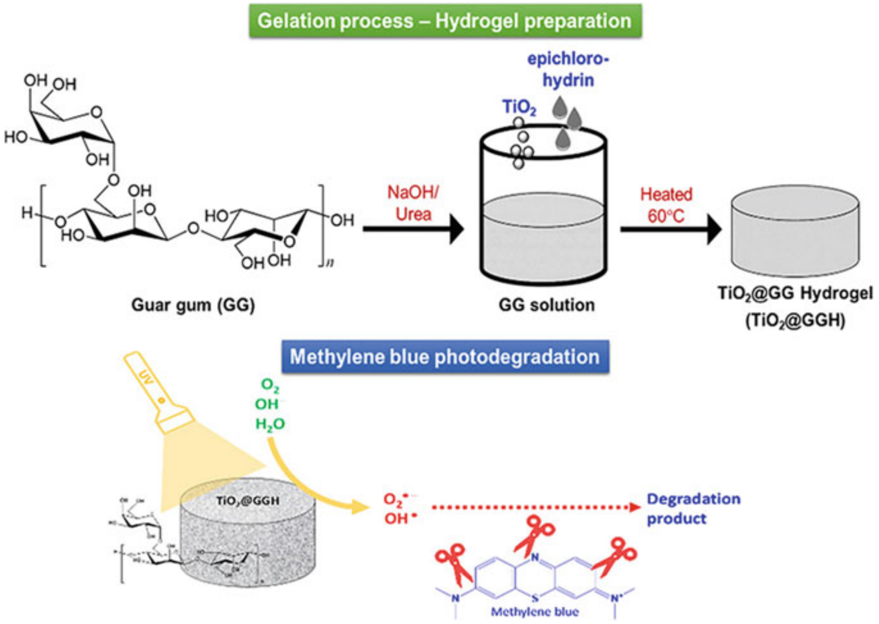

Fig. 4 The preparation of guar gum/TiO_2 nanocomposite hydrogel and their application for adsorption and photodegradation of dye. Reprinted from Ref. [56] with permission from the publisher

and food applications. Chopra et al. prepared acacia gum/sodium alginate/ZnO hydrogel nanocomposites which sustained release of the Zn^{2+} rather than burst [44]. The toxicity of ZnO nanoparticle-loaded hydrogels found significantly lower than control ZnO nanoparticles in cytotoxicity studies on the vero cell lines. Ghayempour and Montazer developed an antimicrobial textile for wound dressing using tragacanth gum and ZnO nanoparticles (Fig. 5) [45]. Composite coated cotton fabric showed good photocatalytic activity and antimicrobial properties.

3.4 Carbon Based Gum Nanocomposites

Carbon-based nanomaterials (expanded graphite, carbon nanotubes, graphene, fullerene, carbon dots) are gaining attention day by day due to their inimitable chemical and physical features. Mainly, carbon nanotubes and graphene are used to make gum nanocomposites.

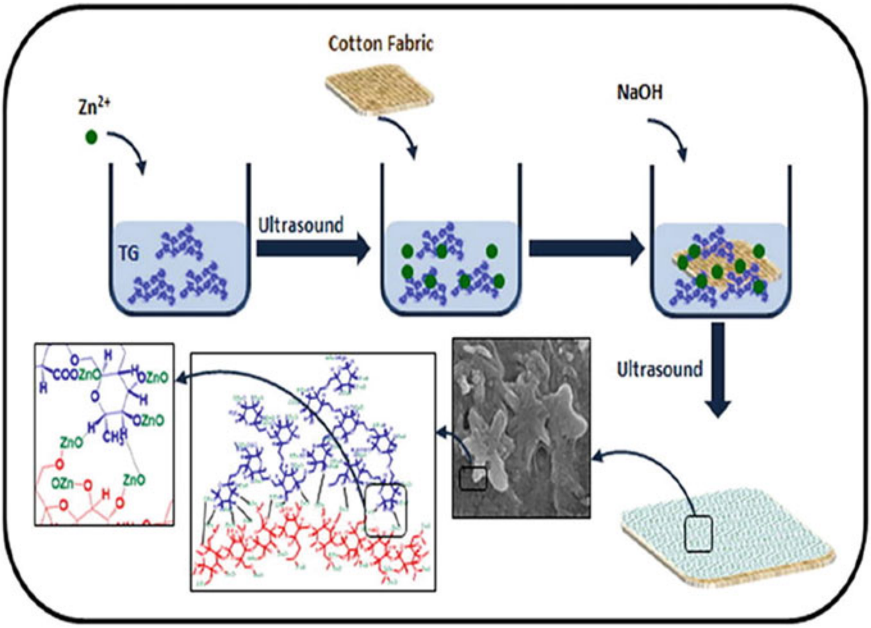

Fig. 5 Preparation of gum/ZnO nanocomposite coated cotton fabric for development of antimicrobial and photocatalytic textiles. Reprinted from Ref. [45] with permission from the publisher

3.4.1 Graphene Based Gum Nanocomposites

Graphene is a two-dimensional nanomaterial and carbon atoms are connected in the hexagonal lattice structure through sp^2 hybridization. The antimicrobial activity of graphene oxide (GO) against bacteria, fungi, and their biofilms is very well known. This excellent microbial property is ascribed to five potential mechanisms: changes in the morphology of the cell structure, leakage of RNA and cell electrolytes, oxidative stress, bacterial cells' trans-membrane potential changes and GO-induced lipid peroxidation damages bacterial cell membranes. Oliveira et al. prepared GO/Arabic gum nanocomposites to design a potentiometric pH sensor electrode [57]. Titanium dioxide functionalized GO/Xanthan nanocomposites showed potential in purifying water from polluted heavy metals [58]. Carboxymethyl tamarind gum/polyvinyl acid/GO nanocomposite film possesses slight antimicrobial activity against *E. coli* [59]. The antimicrobial drug has been incorporated into the nanocomposite films for potential use as cardiovascular implants or cartilage repair.

3.4.2 Carbon Nanotubes Based Gum Nanocomposites

Carbon nanotube (CNT) based gum nanocomposites have an ambiguous antibacterial activity. CNT and functionalized CNT based tamarind gum hydrogels exhibit no antibacterial activities [60]. Similarly, polyethersulfone functionalized multiwall CNT/acacia gum nanocomposite membranes have no antimicrobial effect [61]. The nanocomposites have an anti-quorum sensing effect, which inhibits the growth of biofilms. These CNT/acacia gum nanocomposites showed anti-biofilm and anti-adhesive properties against Gram-positive and Gram-negative bacteria. Tragacanth gum/carboxylic acid-functionalized multiwalled CNT hydrogels demonstrate improved tensile modulus and tensile strength compared to the pristine hydrogel [62].

3.4.3 Other Carbon-Based Gum Nanocomposites

Various other carbon based nanomaterials (carbon dots, carbon nanofiber, and fullerene) are also used in gum nanocomposites. Carbon dots, which are often described as quasi-zero-dimensional particles with a size below 20 nm, naturally exhibit fluorescence. Carbon dots are also incorporated in gum for ion sensing and biomedical applications. Gum olibanum resin derived carbon dots were synthesized along with carbon nanobeads (40–50 nm) by Gaddam et al. They found nanobeads are non-fluorescent, whereas carbon dots emit blue-green fluorescence at 300 nm [63]. Normal cell lines are biocompatible, whereas cancer cell lines are cytotoxic with both materials. The incorporation of carbon nanofibers in gum provides conductive properties and such gum nanocomposites are potential for the development of solar cells. Conductive gum nanocomposite could also use for cardiac and nerve tissue engineering.

4 Applications for Gum Based Nanocomposites

4.1 Biomedical

Gum nanocomposites are useful in a variety of biomedical applications, including diagnostics, drug delivery, bio-sensing and imaging. Due to their high water solubility, biodegradability, biocompatibility, and ease of delivering chemical drugs in water, gum nanocomposites based hydrogels are particularly attractive polymers in biomedical applications.

The development of alternative drug delivery systems to address the shortcomings in traditional pharmaceutical active ingredients has received a lot of attention in recent years, intending to achieve the optimal clinical efficacy. Biodegradable polymers having the ability to interact with living systems without causing harm have attracted their attention for this purpose. Various gum nanocomposites have been studied for drug delivery systems and found effective results. Low water solubility, bioreactivity in the body, and storage stability are all challenges that most drugs and biomolecules encounter. In comparison to traditional drug delivery, gum nanocomposite encapsulation offers two advantages; (a) protects drugs/biomolecules in the physiological environment from becoming unstable and inactive before they reach their targets or receptors and (b) biodegradability of gum nanocomposites in the human body. Core–shell Gum-Acacia/hydroxyapatite nanocomposites showed the potential to be a suitable material for sustained in vitro drug release behavior of naringenin [64]. Drug loaded nanocomposite has blood compatibility, and antimicrobial properties. Guar gum/nanosilica nanocomposite membranes also showed prolonged and efficient delivery of diltiazem hydrochloride (DH) through the skin [65].

Thousands of surgical operations are performed every day to replace or repair tissues damaged by trauma or illness. Tissue engineering combines body cells with very porous scaffold biomaterials that function as templates for tissue repair and direct the formation of new tissues to restore damaged human tissues. Poly (dialdehyde) modified acacia gum based biomimetic hybrid scaffolds have been developed by the selective oxidation of acacia gum and blended with collagen and melatonin [66]. The prepared acacia gum based scaffolds demonstrated noteworthy biocompatibility. Horst et al. designed a magnetite-based Arabic gum nanocomposite for hyperthermia therapy [67]. At 260 kHz and field amplitude of 52 kA/m, the highest specific absorption rate (SAR) of 218 W/g Fe was measured for this gum nanocomposite. These findings show their potential to be used in tumor ablation therapy. Raizaday et al. prepared karaya gum microparticles via spray drying technique using water as a solvent [68]. The in vivo results suggest their potency to use for treating many diseases like Ulcerative Colitis, chronic hypertension and Diverticulitis. Kumar et al. reported a highly porous xanthan gum nanocomposite hydrogels based scaffold [69]. They prepared gum nanocomposite with halloysite nanotubes (HNTs) and/or cellulose nanocrystals (CNCs) and the fabrication process of the scaffold by freeze-drying method is shown in Fig. 6. The gum nanocomposite scaffold showed improved thermal stability, mechanical properties, and cytocompatibility. This scaffold has the potential for bone tissue engineering applications.

4.2 Water Treatment

Various heavy metal cations and organic pollutants are highly hazardous to aquatic ecosystems and may be damaging to human health. A global environmental issue is the prevalence of these pollutants in effluents brought on by fast industrialisation and technological development. Two crucial techniques, namely adsorption and filtration, are frequently used to solve the serious environmental issue of toxic metal poisoning of water bodies. A number of natural biopolymers, such as lignin, chitin, chitosan, seaweed, wool wastes, biomass by-products, sugarcane bagasse, sargassum algae, agricultural wastes, etc., have shown to be quite effective in adsorbing pollutants. Gum-based nanocomposites membranes are more advantageous for environmental applications such the absorption and degradation of pollutants due to the ease of membrane fabrication, the variety of materials that may be used, and their ensuing

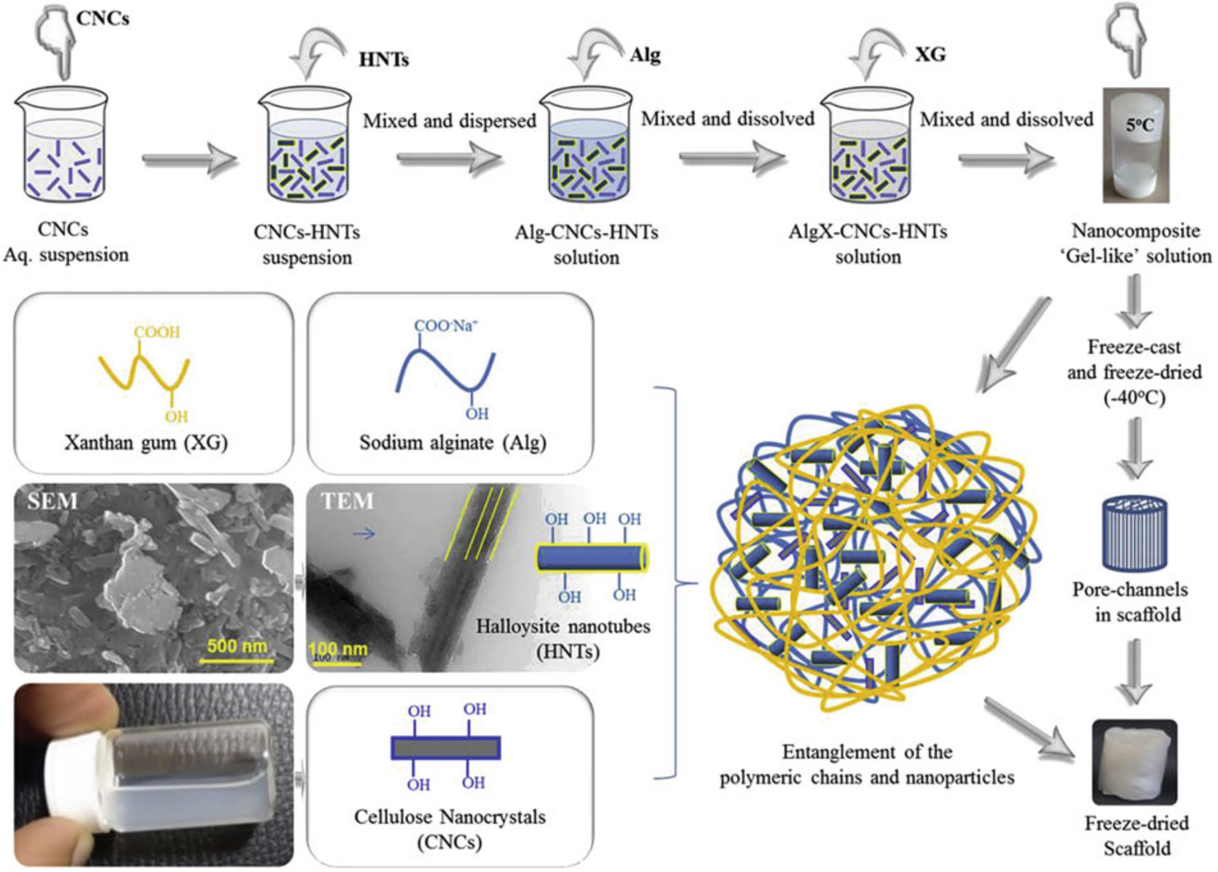

Fig. 6 Schematic representation of the scaffold preparation using xanthan gum, sodium alginate (Alg), cellulose nanocrystal (CNC) and halloysite nanotubes (HNT). Reprinted from Ref. [69] with permission from the publisher

Table 2 Gum based nanocomposites for water treatment

Gum-based nanocomposites	Type of contaminants	Adsorption capacity (mg/g)	Adsorption/removal mechanisms	Reference
Arabic gum/Fe_3O_4	Cu^{2+}	38.5	Complexation	[70]
Tragacanth gum/GO	Pd^{2+}, Cd^{2+} and Ag^+	142.50 (Pb^{2+}), 112.50 (Cd^{2+}) and 132.12(Ag^+)	Chemical reaction and mass transfer	[28]
Tragacanth gum/Fe_3O_4/poly(methyl methacrylate)	Cr^{6+}	7.64	Chemisorption	[71]
Kondagogu gum nanocomposite	U^{6+}	487	Precipitation, ion exchange, complexation	[72]
Kondagogu gum/Pd	G-250, methylene blue, methyl orange, and 4-nitrophenol	150 µM (G-250), 62.5 µM (methylene blue), 30 µM (methyl orange) and 100 µM (4-nitrophenol)	Catalytic reduction	[50]
Kondagogu gum/PAA-co-acrylamide/Fe_3O_4	Rhodamine B	654	Electrostatic attraction, and binding sites	[73]

architectures. Gum contains a number of functional groups, including ether, acetyl, hydroxyl, and carbonyl groups, which together make up the main interaction groups involved in metal biosorption. The findings demonstrate that gums might capture dangerous metals by chemisorption, ion exchange, functional group exchanges, surface adsorption, physisorption, and higher surface area. Adsorption tests were also conducted to examine the impacts of process factors such the concentration of primary metal ions, pH, sorbent concentration, and contact duration. The adsorption findings were closely fitted to the isotherm models of Langmuir, Freundlich, Tempkin, and Dubinin-Radushkevich, while the kinetic data was fitted to the pseudo-first order and second order models. Table 2 provides a summary of the research on gum-based nanocomposites for the removal of radioactive elements, heavy metals, and organic dyes from aqueous and industrial effluents.

4.3 Biosynthesis of Nanomaterials

Natural products, especially gums help as mediators and provide stabilization. Various gum based used for the synthesis of nanoparticles and the gum coated nanoparticles have tremendous potential for applications in many fields.

These nanoparticles have numerous potential advantages as antibacterial materials compared to the nanoparticles produced by utilizing various chemical reducing agents and/or other organic materials which are used as capping and stabilizing agents. For biotechnology and antibacterial performance, several nanoparticles (Ag, Au, Cu, ZnO, and CuO) or organic substances like dodecenylsuccinic anhydride (DDSA) have been combined with tree gums. The micro broth dilution methods and the well diffusion method (zone of inhibition) were mostly used to study the antibacterial susceptibilities of the bacterial strains against the impacts of the nanoparticles. Gum based nanoparticles and membranes both exhibit strong antibacterial activity against both Gram classes of bacteria. Due to their potential antibacterial efficiency and surface flexibility, these nanoparticles can readily be modified to be biocompatible or cytotoxic in a number of biomedical and pharmaceutical applications.

4.4 Packing

The food industry heavily depends on food packaging to improve food safety and protection. For food safety and extending the shelf life of packaged foods, materials with the appropriate physicochemical properties are needed. Gum nanocomposite films exhibit intriguing characteristics, including biodegradability, non-toxicity, colourlessness, and odorlessness, as well as demonstrating an excellent oxygen barrier. In the gum nanocomposites based packing materials; the main role of nanomaterials is to improve mechanical and barrier properties. Therefore, two-dimensional nanomaterials such as graphene oxide, clay are used to get antibacterial food packing materials. Venkateshaiah et al. developed gum–graphene oxide nanocomposite films [74]. Three different nanocomposites films were fabricated using Arabic, karaya and kondagogu gum with GO (0.5 and 1 wt%). The brittle gum film was further transformed by the addition of GO into a free-standing film with significant mechanical strength. Along with better oxygen gas barrier properties, the layered nanocomposite films also showed reduced water vapor transmission. For food packaging, Janani et al. developed gum-based nanocomposite films using Tragacanth gum, polyvinyl alcohol, ZnO nanoparticles, and ascorbic acid [75]. They used glycerol as a plasticizer and citric acid as a cross-linker.

5 Conclusions and Future Outlooks

Several vital properties and abilities of gum nanocomposites have been discussed in the earlier sections of this chapter. Gums are highly nontoxic, biocompatible, non-inflammatory, cheap, and abundant. They are used in various industrial applications, including pharmaceuticals, tissue engineering, oil and petrol, food and food packaging, paints and emulsions, cosmetics, bakery, beverages, etc. Many unique properties, such as pseudo-plastic nature, shear-thinning, high viscosity, and thermal, make gum beneficial over other bio-polymers. Every merit is accompanied by a certain demerit, and gums are no exception. The low mechanical properties and processing of gum restrict its applications in the area of tissue engineering and food packing. Imminent usage of gums will also need the development of advanced membranes and three-dimensional porous networks that are incredibly light, extremely strong, biodegradable, and tuneable. Such gum-based porous networks could form an integral part of various fields such as biomedicine (tissue engineering and drug delivery), catalytic remediation, water treatment, and safer bioplastic as food packaging and disposable materials. Despite being biocompatible and promising candidates for enzyme immobilization, there are very few studies on the use of gum nanocomposites as biosensors. Since gums are not electrically conductive, they may not be suitable for biosensors. However, this can be overcome by modifying the chemical structure of gums or incorporating an appropriate conductive nanomaterial (carbon-based or metal or metal oxide) in the appropriate amount. Green chemistry can help with biomedical sensor applications, including biosensors, which must be both safe and biocompatible.

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Nanocomposites Based on Biodegradable Polymers for Biomedical Applications

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Abstract Regenerative medicine has sought new alternatives of materials capable of being used in humans for different purposes, with low or no cytotoxicity, ability to adapt to the body and mainly biodegradation. In this sense, polymeric nanocomposites based on biodegradable polymers have shown a high level of acceptance for this application. Among the most promising biodegradable polymers are polylactic acid (PLA), polyvinyl alcohol (PVA), polyhydroxybutyrate (PHB), polyhydroxyalkanoates (PHAs); which have served as a support or matrix, nevertheless, they lack molecular signals to respond to stimuli from the receiving organism, due to this, different biopolymers have been used such as chitosan, chitin, cellulose, collagen, alginate and combinations of them, to improve the biological response and generate a synergy effect. Biodegradable nanocomposites have been a sensitive topic due to the implications involved in the incorporation and subsequent release of by-products of the degradation of the polymeric material, therefore, it is of great interest to study the possible mechanisms of biodegradation in in vitro and in vivo environments and the response at the cellular level due to the presence of metabolites from the biodegradation of the support matrix, on the immune response within the organism.

Keywords Nanocomposites · Biodegradation · Regenerative biomedicine · Biopolymers

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1 Introduction

The development of new polymers currently presents new challenges in different aspects; On the one hand, it seeks to meet the needs demanded by the population, and it is desirable to manufacture them at affordable costs without losing their attributes. Nevertheless, in the same way, it is sought that its production does not imply environmental consequences. Regenerative medicine is among the areas that present the greatest interest in developing new technologies based on the creation and improvement of properties of polymeric materials with potential biomedical applications. In this sense, the study of a category of polymers, known as biodegradable polymers, which, in addition to exhibit versatile characteristics associated with polymers, has recently drawn attention; they are an alternative for environmental care. Among the main advantages of these materials, we can mention: their biocompatibility with the human body, easy adaptation, and ability to combine with different agents that help tissue regeneration; in addition, once they have fulfilled their function, they are degraded by different metabolic pathways without the need for surgical intervention [1]. Another of the strategies of interest is based on the use of nanocomposites, which allow the interaction of different materials at molecular levels within the organism, thus allowing a better capacity for drug interaction, success in therapies, and tissue regeneration at the cellular level [2].

Nanocomposites are currently being developed that have promising characteristics in the biomedical field. For them, polymeric matrices and nanofillers are used [3]. Among the preferred polymers for these applications we have polylactic acid (PLA), polyvinyl alcohol (PVA), polyhydroxybutyrate (PHB), polyhydroxyalkanoate (PHA); also, in order to provide a suitable environment for cell growth, biopolymers are often incorporated such as cellulose, collagen, and chitosan, among others; while for nanofillers they are mainly investigated: metallic nanoparticles, carbon nanostructures, nanohydroxyapatite, nanocellulose and nanolignin [1].

1.1 Problematic

Different challenges and associated problems arise in the field of research and the development of new polymer-based nanomaterials. Firstly, considering the raw material, the current environmental problems related to the use of plastics that have a long life must be considered; this is associated with properties such as high resistance to corrosion, water, as well as bacteria. Furthermore, over the years, there has been an increase in their production and consumption due to the ease of processing these materials, causing damage to the environment. On the other hand, the industries dedicated to producing these plastics are dependent on oil, so they are currently at risk in terms of costs and supplies [4].

Regarding the use of applied nanomaterials for tissue regeneration, the challenge is to understand the composition, processing, and properties of polymer-based nanocomposites, as well as the mechanisms of interaction within the body. Likewise, the characteristics of both the matrix and the filler must be considered, mainly paying attention to their degradation mechanisms and their in vitro cellular responses to guarantee their correct incorporation and thus achieve their function as a biomaterial. On the other hand, care must be taken that the materials used do not present rejection reactions or cytotoxicity, as well as studying their degradation rate so that it does not interfere with the regenerative capacity [5].

Finally, since studies regarding nanomedicine and the use of biodegradable polymers in bioengineering are relatively new, higher production costs must be considered since different processes and equipment are used concerning polymers from petroleum, adding to this the cost of characterization studies to guarantee the application of said material.

1.2 Alternatives

Recent research has successfully developed biologically based and biodegradable nanocomposites, so new perspectives should point to the analysis and improvement of these nanocomposites. First, attention should be focused on the use of biodegradable polymers as alternatives for the creation of matrices; this is based on the characteristics of these materials to decompose entirely after a period of useful life, in addition to the fact that a human organism can easily assimilate their degradation products; on the other hand, the advantages of incorporating nanofillers in biomaterials have been investigated, among which are: reinforcing effects and barrier properties, as well as their influence on the rate and speed of degradation, this associated with the molecular weight of the polymer, as well as its interaction with bacteria [6].

In creating these materials, it must be considered that the nanofillers' distribution must be homogeneous to take full advantage of their properties; additionally, geometry, performance, and manufacturing cost must be considered [7]. Some of the most suitable uses and conformations for the use of these materials in tissue engineering are:

- Porous nanocomposites: 3D structures can be developed with the help of electrospinning, leaching, or phase separation methods
- Nanocomposites with organic fillers: dressings and films can be achieved with the incorporation of keratin and cellulose nanocrystals
- Nanocomposites with antibacterial fillers: to avoid the formation of biofilms, the use of antimicrobial fillers in the polymer matrix can lead to antibacterial responses at low concentrations of nanoparticles.

2 Biodegradable Nanocomposites

The use of biodegradable polymeric nanocomposites has been the research subject in recent years for different pharmaceutical and biomedical applications. These nanocomposites present a multifunctional behavior and adaptability for various applications such as sutures, wound scaffolds, cartilage and bone replacement, and tissue regeneration, among others. Polymers of natural origin, such as polysaccharides and proteins in combination with biodegradable synthetic polymers, as well as the incorporation of different nanoparticles, have shown the ability to promote wound closure in vitro and in vivo environments. Currently, dressings for wounds with different levels of damage can contain collagen (CLG), alginate (ALG), chitosan (CHS), chitin (CH), and cellulose (CL), which provide different benefits such as inhibition of the growth of microorganisms and leaching of exudates and cell differentiation.

2.1 Biodegradable Synthetic Polymers

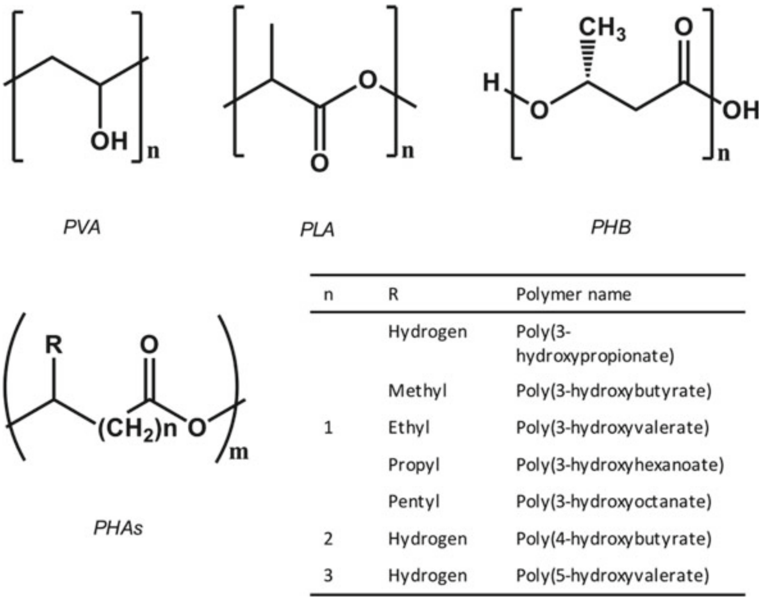
Among the biodegradable synthetic polymers that have most attracted the attention of the scientific community are PVA, PLA, PHB, PHAs, etc. In addition to being biocompatible, biodegradable, and has good mechanical properties, they have proven to be adequate for controlled drug release. They also provide an adequate platform for cell growth, migration, and differentiation when used in dressings and skin grafts, and are processable by simple and reproducible techniques at the laboratory level.

2.1.1 Polyvinyl Alcohol (PVA)

PVA is a semi-crystalline copolymer based on vinyl acetate and vinyl alcohol, their chemical structure is shown in Fig. 1. It is non-toxic, non-carcinogenic, biocompatible, hydrophilic, and can absorb a large amount of water. PVA can be blended with other polymers to improve their physical or biological properties. It can be cross-linked with glutaraldehyde or succinyl chloride to increase its flexibility. PVA mixed with CLG improves the quality of healing tissue and improves the strength and flexibility of cells at the wound site. The mixture of ALG, dextran, and CHS improves its biological properties [8–10].

2.1.2 Polylactic Acid (PLA)

PLA is a thermoplastic polyester resulting from the polymerization of lactic acid, which is present in a wide variety of natural sources that contain starch, their chemical structure is shown in Fig. 1. Because its degradation can be controlled, mainly by molecular weight, this polymer has been the subject of extensive research for



n	R	Polymer name
1	Hydrogen	Poly(3-hydroxypropionate)
	Methyl	Poly(3-hydroxybutyrate)
	Ethyl	Poly(3-hydroxyvalerate)
	Propyl	Poly(3-hydroxyhexanoate)
	Pentyl	Poly(3-hydroxyoctanate)
2	Hydrogen	Poly(4-hydroxybutyrate)
3	Hydrogen	Poly(5-hydroxyvalerate)

Fig. 1 Chemical structures of PVA, PLA, PHB, PHAs, adapted from [8, 11–13]

biomedical applications. In vivo tests of PLA implants showed mild inflammatory responses that may be associated with acid degradation byproducts. They currently find applications in manufacturing sutures, materials or osteosynthesis equipment. Finally, PLA is one of the biopolymers most used as a raw material in 3D printing for biomedical and other applications [11, 14, 15].

2.1.3 Polyhydroxybutyrate (PHB)

PHB is a natural thermoplastic polyester produced by bacterial fermentation and completely degrades the environment without toxic products, their chemical structure is shown in Fig. 1. PHB is a water-insoluble polymer with high crystallinity, biocompatibility, biodegradability, permeability, and processability. PHB is potentially useful for biodegradable materials such as mesh, absorbable surgical sutures, controlled drug release, and tissue engineering, among others. However, these potential applications of PHB have been limited due to its severe brittleness, which is caused by its low crystallization density, high brittleness, and poor toughness [16–18].

2.1.4 Polyhydroxyalkanoates (PHAs)

PHAs are biopolyesters of microbial origin. Recent biotechnology and genetic engineering advances have led to producing toxin-free PHAs, their chemical structure is shown in Fig. 1. Due to their biocompatibility and biodegradability, PHAs do not have acute and chronic effects when used in in vivo applications. So, they can be used for developing surgical meshes, medical devices, orthopedic pins, surgical sutures, stents, repair patches, heart valves, staples, and screws as they are well tolerated by the immune system. Besides, as a packaging material for cells and tablets [12, 13, 19, 20].

2.2 Biopolymers

Regenerative medicine has recently used natural polymers to develop scaffolds and materials for wound dressings due to their immense biocompatible and biodegradable properties. Due to their similarity to macromolecules recognized by the human body, natural polymers such as polysaccharides (CL, ALG, CH, CHS) and proteins (CLG, gelatin) are widely used in the treatment of wounds and burns. The formation of nanocomposites of these biopolymers with matrices of inorganic origin has given rise to the formation of scaffolds for the growth and regeneration of bone tissue, with outstanding biocompatibility, porosity, and interconnectivity.

2.2.1 Cellulose (CL)

CL is a natural polysaccharide with repeating glucose-based units connected by β-1,4-glycosidic bonds, as shown in Fig. 2. It has been used in regenerative medicine as a wound healing scaffold for damaged skin due to its excellent physicochemical properties, nanostructure, biodegradability, mechanical strength, antimicrobial property, and biocompatibility. Its use as an alternative dressing material for superficial burn wounds has also been reported. In addition, CL derivatives have also been used as film coatings, hydrogels, bioadhesives, a base material for the controlled release of drugs, and for tissue regeneration applications [21–25].

2.2.2 Alginate (ALG)

ALG are suitable for wounds that release a large amount of exudate since they can absorb up to 20 times their weight, allowing a moist environment in the wound and avoiding microbial contamination. Their chemical structure, is shown in Fig. 2. Dressings containing alginic acid from seaweeds are bioavailable as calcium ALG, calcium/sodium ALG. When placed on a wound, the sodium and calcium ions interact with the serum to form a hydrophilic gel. These dressings are especially effective for the treatment of pressure/vascular ulcers, surgical incisions, wound dehiscence, tortuous tracts, skin grafts, exposed tendons, and infected wounds [29–31].

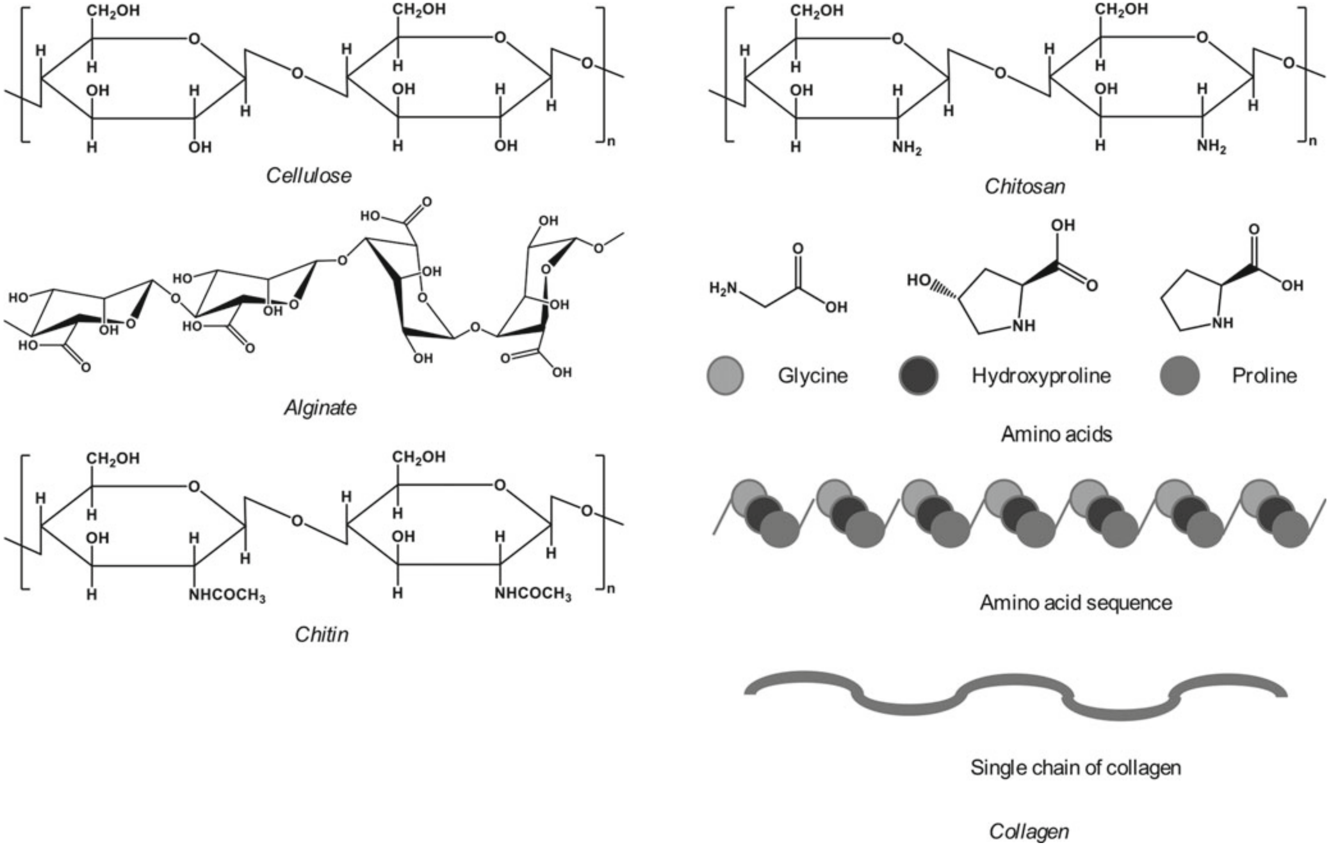


Fig. 2 Chemical structures of CL, ALG, CH/CHS, CLG, adapted from [26–28]

2.2.3 Chitin/Chitosan (CH/CHS)

CH is the second most abundant biopolymer after CL and is the main constituent of the exoskeleton of animals, particularly crustaceans, mollusks, and insects. The deacetylation of CH produces CHS, which makes it insoluble at neutral or basic pH due to its free amino groups, as shown in Fig. 2. In contrast, in acidic pH, the protonation of the amino groups leads to their solubility in water. CHS provides a substrate for cell attachment due to its polymeric structure that shows similarities to glycosaminoglycans (GAGs) which are the main component of the extracellular membrane (ECM). Due to the above, its applications include surgical sutures, artificial skin, and controlled drug delivery devices. CHS has intrinsic antimicrobial properties on bacteria, algae, and fungi and is also hemostatic. Furthermore, its abundance can be a low-cost solution to manufacture effective dressing materials for different types of wounds [32–35].

2.2.4 Collagen (CLG)

CLG is a substrate from natural proteins, which function for cell growth, proliferation, and differentiation. CLG is an excellent dressing material due to its negligible immunogenicity, biocompatibility, and mechanical stability, their basic chemical structure, is shown in Fig. 2. CLG dressings promote the deposition and organization of newly formed CLG, creating an environment that promotes healing due to the chemotactic properties of the dressings on fibroblasts in the wound. In addition, it can stimulate and recruit specific cells throughout the healing cascade to enhance wound healing and provide moisture or absorption, depending on the delivery system. Currently, CLG formulated from bovine, porcine, or avian sources is used to treat partial or full-thickness wounds with minimal to moderate exudates. They include CLG suspensions for dermal injection, topical hemostatic agents, materials for CLG bandages, sutures, and catguts, CLG sponges for hemostasis and joint coverage, artificial skin substitutes for the treatment of severe burns, and CLG-rich dressing materials for the regeneration of deep wounds [36–40].

2.3 Methods for Obtaining Nanocomposites

Both biodegradable synthetic polymers and biopolymers can be processed into a polymeric nanocomposite by using different techniques solution mixing/solvent casting, in-situ polymerization, sol–gel method, among others. Unlike what happens with non-biodegradable polymer composites (petroleum based), that be processed by a wide variety of processing techniques and stored for the next transformation process (like melt extrusion to obtain films, profiles, bottles, fibers and non-woven fabrics, injected parts, etc.), biodegradable nanocomposites must be processed in a second step as soon as possible to make the scaffold, mat or dressings, and to preserve the integrity of both components. Due that, the preparation method of biodegradable nanocomposites for biomedical applications is very linked with the preparation method of the scaffold; in order to preserve their bioavailability. The most used methods in obtaining scaffolds for biomedical applications are briefly described below; for a more detailed review, see [41–43].

- Solvent Casting and Particulate-Leaching: a combination of solvent dissolution and particle leaching methodologies is widely used to fabricate 3D porous structures successfully. This is a process based on salt dispersion in a polymer dissolved in an organic solvent. The solvent is removed, resulting in the solidification of the polymer. The salt crystals are then filtered out using water to form the pores of the scaffolds. The pore size can be controlled by the size of the salt crystals and the porosity by the salt/polymer ratio.
- Freeze-Drying: also known as lyophilization, it is a dehydration technique that allows a material to be frozen and then vacuum dried. First, a polymer dissolves in a solvent with the addition of water, followed by freezing at different temperatures and rates, forming ice crystals, and forcing the molecules to aggregate in the interstitial spaces. The frozen material is then dried at low temperature under reduced pressure to remove dispersed water and solvent, thus leaving a porous polymer structure.
- Electrospinning: this technique, has received considerable interest for use in tissue engineering to produce nonwoven membrane scaffolds of nanometer-order polymeric fibers with large surface areas and superior mechanical properties. During the electrospinning process, a polymer solution is held on the tip of a needle by surface tension. The applied electrostatic force opposes the surface tension, causing the initiation of a jet of solution that produces the fibers. As the jet travels, the solvent evaporates, and the nanofibers settle on the collector.
- Rapid prototyping: also known as additive manufacturing, is an advanced technique that uses computer-generated data, such as computer-aided design (CAD) data, to design objects with complex shapes. The manufacturing process involves a 3D design of the scaffold, which is directly produced layer by layer. As a result, this technique allows for precise control of pore size, geometry, and interconnectivity, allowing the infiltration and behavior of cells in the scaffold to be tuned.

3 Mechanisms of Degradation

3.1 Biodegradation

The term biodegradation is used generically to refer to the degradation that occurs in a biological environment and is used in fields as diverse as ecology, waste management, environmental remediation, etc. [44, 45]. In simpler words, biodegradation is related to the decomposition of substances by the action of microorganisms, which leads to the formation of smaller organic compounds, CO_2, water, and salts [45, 46]. The studies on biodegradation have allowed a better understanding of the process. They have shown that, contrary to what one might think, it is a complex phenomenon, significantly when the material that degrades is a polymer or a composite polymeric material [46]. In recent years, the study of the degradation of composite materials loaded with different types of nanoparticles has gained momentum since these nanomaterials have begun to be made from biodegradable polymers, such as PLA, PHAs, polycaprolactone (PCL), etc., filled with nanoparticles of CH, CHS, or with CL or CLG nanofibers, among others.

The biodegradation of polymeric materials, and therefore of the nanomaterials prepared with them (mainly if these constitute not only the matrix of the compounds but also their dispersed phase) is carried out in several stages, and these can be carried out concatenated, or they can stop at the end of each of the phases [46].

The first stage, called "biodeterioration", consists of the combined action of microbial consortia, decomposing organisms, or abiotic factors, which break down biodegradable materials into smaller fractions. In this sense, it should be mentioned that, although abiotic factors are not of biological origin, they do intervene in the biodegradation process. We can mention sunlight, temperature, pH, and oxygen among them.

Subsequently, the phase called "depolymerization" takes place, a stage in which the microorganisms secrete catalytic agents such as enzymes, and free radicals, among others, to break the polymer chains, reducing the molecular weight of these macromolecules and giving rise to compounds such as oligomers, dimers, monomers, etc. The generated molecules can follow two paths; some can be recognized by the receptors of some microbial cells and cross the cell membrane, or they can remain outside, in the extracellular environment, to be subject to different modifications. Finally, the molecules transported into the cells are integrated into their metabolism to produce energy, metabolites, or new biomass. This step is known as "assimilation". Simultaneously, the generated metabolites, or their salts, can be excreted together with other molecules such as CO_2, H_2O, CH_4, etc., and reach the extracellular environment, is completely oxidized. This stage is known as "mineralization" [46].

The biodegradation mechanism followed by each polymer (biodegradable or non-biodegradable) depends on its chemical structure, crystallinity, molecular weight, and conditions to which it is subjected [46]. For example, many biodegradable polymers used in biomedical applications, such as PLA, PHAs, and PCL are polyesters susceptible to hydrolytic degradation, especially at high temperatures [47, 48].

Nanomaterials prepared from biodegradable polymers have been used for different biomedical applications such as platforms for structural stabilization, filler material, drug delivery, and tissue engineering since they can reduce the complications of polymers or materials non-biodegradable, which are perceived by the body as permanent foreign objects. However, being biodegradable materials, one of the aspects that must be fully studied is their biodegradation mechanism, since all the decomposition products will remain inside the human body, which could generate toxicity problems.

It is a widely known fact that there are two ways to study the biodegradation mechanisms of materials: in vitro and in vivo experiments. The first are experiments carried out in a laboratory, in a controlled manner (outside a living organism), simulating the conditions to which the material will be subjected. In contrast, in vivo experiments are carried out inside a living organism, usually an animal model. Both methodologies have advantages and disadvantages; for example, in vivo degradation is more complex than in vitro, but in vitro assays are rarely an adequate measure of biomaterial behavior [49].

3.2 In Vivo Biodegradation

The biodegradability of a polymer-based biomedical product is especially advantageous when it has been designed for temporary use, and its elimination (resorption) represents a benefit because it reduces infections, pain, and cost. Therefore, some experiments have been designed that subject the material to in vivo experiments, where it is inserted into a tissue (bone, cartilage, etc.) of a living organism (animal model) in such a way that it can know the mode and degree of degradation of the polymer (or composite material), to carry out these studies. The morphology of the materials used, ranging from pins, discs, plates, screws, bars, etc., which are inserted into tissues through surgical interventions (preferably minimally invasive). Undoubtedly, the most studied nanomaterials, through in vivo tests, are prepared with biodegradable polyesters such as PLA, PCL, and PHA's, especially in bone regeneration applications. The degradation of the inserted material can be monitored by diagnostic equipment or histological tests [50].

3.3 In Vitro Biodegradation

In vitro biodegradation studies of nanomaterials prepared based on biodegradable polymers for medical applications are generally carried out in phosphate buffer solutions at a temperature of 37 °C (body temperature), a physiological pH of 7.4, monitoring both the loss of mass or properties (mechanical, molecular weight, etc.), over time (days or months) [51]. The identification of decomposition products is also essential since these could be potentially toxic to people. The reaction that usually takes place in this type of experiment is hydrolysis.

Another way to study the biodegradability of nanomaterials is using enzymes, which can hydrolyze polymers to give rise to low molecular weight compounds. For example, esterases are the enzymes responsible for the hydrolytic degradation of biodegradable aliphatic polyesters such as PLA, PHAs, PCL, etc. Like for polyesters, there are enzymes such as cellulase to degrade CL or chitinase to degrade CH, polymers that are used as reinforcing/filling agents in nanomaterials for biomedical applications. Because they are not only biodegradable polymers but also improve the biological response of the materials prepared with them [47].

4 Biological Aspects

Nanocomposites based on biodegradable polymers have promoted various medical advances because they are compatible with the human body and disappear without requiring surgical interventions after fulfilling their function. Furthermore, as detailed above, these nanocomposites exhibit adjusted physical, chemical, and mechanical properties for each tissue or organ and even have predictable degradation behavior from their design. However, the selection of a particular nanocomposite for a given application requires a detailed understanding of the tissue and cellular response that the stay of these compounds will trigger in organisms. Therefore, this section will analyze the biological effects of nanocomposite-cell interaction, mainly the effects on cell morphology, adhesion, proliferation, and differentiation.

4.1 Effects on Morphology

Cellular morphology reflects intracellular metabolic activity in response to an external or internal stimulus. In this sense, contact with biodegradable nanocomposites based on PCL, PHB, CL, PLA and PHAs, CLG, PVA [17, 18, 52–55], which have been designed for biomedical applications in dermal, neural, bone and cartilage regeneration, have shown that the topographical arrangement of nanocomposites can affect cell morphology. The last effect has triggered the effects on cell stretching (i.e. the arrangement of the cytoskeleton components) and the generation of cellular projections such as filipodia [56]. According to the contact guidance theory, which focuses on explaining the relationship between cell morphology and fiber arrangement or a defined pattern [57], the topographical characteristics of nanocomposites could guide the arrangement and migration of cells through promoting adhesion interactions and a rearrangement of the cytoskeleton [57–59]. Studies on PCL nanofibers have shown that fibrillar alignment promotes the presence of elongated nuclei and cytoskeleton aligned to the fiber axes in neuronal regeneration [52]. This contact orientation effect has been observed mainly in muscular and neural regenerative processes [60–62]. Recent findings on the phenotype of chondrocytes have shown that actin rearrangement defines and controls the phenotype of this cell line through the activation of signaling pathways that involve the modulation of pro-inflammatory cytokines, growth factors, and various factors associated with cytoskeleton such as RhoA/ROCK/Rac1/mDia1/mDia2/Cdc42, among others [56].

4.2 Effects on Cell Adhesion

Cell adhesion represents the initial phase of the cell-nanocomposite interaction, triggering numerous biochemical and cellular responses [63, 64]. Studies carried out with nanocomposites with various topographies, wettability and alignments showed that topography and alignment positively affect cell adhesion [52, 65]. Nanocomposites based on polysaccharides (CL, CHS, ALG, hyaluronic acid (HA)), proteins (CLG, silk, elastin, fibrinogen), bioabsorbable materials (hydroxyapatite, β-tricalcium phosphate), or synthetic polymers (PVA), have been shown to induce greater cell adhesion and extension, as well as the presence of filipodia in osteoblastic [42, 66–68], dermal [69, 70] and brain cells [52]. On the other hand, in the case of nanofibers, they promote a rearrangement of the cytoskeleton in a manner parallel to the orientation of the same. These effects on cell adhesion can be explained through the modulation of focal adhesion complex maturation [71] and mechanotransduction [72]. Recent studies carried out on corneal epithelial cells, cultured on silk films and FLC, suggest that changes in cell adhesion begin with the mechanosensation of biophysical signals or ligands offered by the nanotopography of the composites [73], activating cell receptors integrin-like, small GTPases (Rho and Rac), and Cdc42, initiating a cascade of signaling events that includes activation of ROCK and MYPT, leading to the formation of the ARP2/3 complex that induces actin nucleation, expression of vinculin and the formation of filopodia [71].

4.3 Effects on Cell Viability and Proliferation

Cell viability and proliferation, which refers to the number of healthy cells present on or in direct contact with the material at a given time or during a given kinetics time [74], considers aspects related to the morphology, adhesion, permeability of the membrane and cell metabolism [75]. Viability can be affected by the structure, composition, mechanical properties, and topography of nanomaterials [76]. Nanocomposites based on ALG, CLG, PHB, CL, CHS, HA and gelatin, with applications in bone regeneration [54, 67, 68, 76, 77], as well as nanocomposites based on ALG, PCL, and halloysite with applications in dermal tissue engineering [69] have been shown to induce an increase in cell viability and proliferation concerning cells not exposed to the nanocomposite in question. This induction is probably due to the activation of signaling pathways related to Mitogen-Activated Protein Kinases (MAPK), such as PMK-1/p38, and miR-27a, which are expressed in response to biopolymers such as CLG, CHS, and ALG [78, 79], accelerating the cell cycle and stimulating cell proliferation [80]. Other factors such as the molecular weight and the charge of the polymers that make up the nanocomposites can also participate in the modulation of cell viability and proliferation. Charged polymers such as CHS and ALG have been shown to promote ionic interaction with cell membranes, promoting cell viability and proliferation and even triggering the functionality of many medical materials [81]. On the other hand, a harmful modulation of the charge of the polymers that make up the nanocomposite can cause damage to the cell membrane, with the consequent activation of necrotic processes [82].

4.4 Effects on Cell Differentiation

Cells are susceptible to nanocomposites' structure, diameter, and orientation, which affect both function and cell differentiation [83]. Aligned PCL and poly(lactic-co-glycolic) acid (PLGA) nanofibers promote neuronal maturation and the expression of early myelination markers in neuronal regeneration studies [52, 61]. Nanocomposites of ALG, CHS, Carrageenan, clays, gellan gum, CL, gelatin, CLG, HA, and fibroin, induce biomineralization and the expression of alkaline phosphatase (ALP) both in in vitro and in vivo models of bone regeneration [43, 67, 71, 84–87]. The chemical composition is another factor that induces cell differentiation. It has been observed that components such as CLG and HA promote osteoblastic differentiation through signaling pathways that include the activation of CBFA-1, the master gene of osteogenesis, as well as of various proteins and factors related to the osteoblastic phenotype and genotype, such as osteocalcin, osteopontin, RANK, RANKL, OSTERIX, VEGF, as well as an increase in pro-osteoblastic cytokines and a decrease in anti-osteoblastic cytokines [88–91]. The induction of cell differentiation is also related to the mechanical properties of the nanocomposite [73, 92]. In this sense, materials with varying degrees of rigidity have shown clear effects on differentiation; for example, ALG-based soft scaffolds induce adipogenic differentiation of mesenchymal stem cells (MSCs), while rigid scaffolds favor osteogenic differentiation [93], probably through mechanotransduction [73, 74]. Anisotropy is another factor that impacts cell differentiation; for example, MSCs cultured on aligned CLG nanofibers spread along the fibers exhibiting morphology and myogenic markers such as Myosin-II; however, when the fibers are cross-linked, the MSCs show no orientation preference and undergo osteogenic differentiation (expression of laminin and ALP) [94].

5 Conclusion

The characteristics of the various types of polymer nanocomposites depends on the intrinsic properties of the polymers (natural or biodegradables) used for their preparation. The resulting products may be used individually or in combination with another component, to absorb exudate, combat odor and infection, relieve pain, promote autolytic debridement and/or provide and maintain a moist environment, i.e. at the wound surface. Unfortunately, no single material can accomplish all these goals. Thus, the selection of the appropriate polymer composite and constituents to healing a specific wound type, is a difficult task and depends on factors related to the composite itself, the patient's health status, wound type and location and economic considerations.

The challenges posed by regenerative medicine are increasingly complex and specialized since it requires the design, characterization, and testing of in vitro and in vivo environments for the new materials intended to be used in this field of medicine. In addition, it is considering that these materials are friendly to the environment. Biodegradable polymeric nanocomposites have proven to be a viable alternative to meet these challenges since biodegradable polymers and biopolymers (natural polymers) have been successfully tested in vitro and in vivo studies. Among the advantages exhibited by these nanocomposites, we can highlight biocompatibility, (controlled) biodegradation, adaptation to different geometries and conformations, incorporation of bio-functional additives, affordable and reproducible processing methods, versatility to be used in different applications such as tissue engineering and regeneration of bone tissue.

Regarding the biodegradability that these materials present, it can be mentioned that various in vitro (controlled laboratory environment) and in vivo studies (with animal study models) show slightly different biodegradation processes than those exhibited outside an organism. However, they go through various stages of degradation, such as the decrease in molecular weight, loss of mechanical properties, absorption, and mineralization, associated with the participation of various specialized enzymes for both the polymeric part and the biopolymers that are added to the nanocomposite. This behavior suggests that these nanocomposites can be grafted intentionally and directed, achieve successful regeneration, and be reabsorbed by the body without causing toxicity during biodegradation.

On the other hand, the cellular response exhibited by these biodegradable polymeric nanocomposites is highly associated with cell adhesion, viability, permeability, and it is related to various aspects involved in the synthesis of the nanocomposite, the proportion or quantity of each component, and the mechanical properties, particularly the morphology of the scaffold, play an essential role in cell differentiation or the growth of different cells since they can grow in an ordered or random fashion, with or without the required cell differentiation.

There is no doubt that more studies are still needed to understand the behavior of this new class of materials intended for tissue and bone regeneration, as well as a greater depth of knowledge in the mode of biomaterial/cell interaction as part of the learning process involved in the advancement of biomedicine. With the generation of new materials, processing methods, and combinations between them, there is a vast field of research for future generations aimed at developing new methods that can be scaled (continuous, less generation of waste (solvents), use of green technologies, reduction of preparation times, better and greater use of raw materials) that lead to a standardization of processes and obtain the best cost/benefit balance for the patient and society.

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Review and Analysis of Biological Tests on Nanomaterials to be Applied in Biological Areas

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Abstract Currently, we can find various types of nanomaterials applied in the design of medical devices or as biological nanomaterials, in contact with living organs. However, (little is known) It is hardly unknown about the tests or analyses that these materials must undergo before being used in biological environments. The toxicity or cytotoxicity of nanomaterials can be determined by various tests or analyzes that are regulated by several international standards such as ASTM F756 and ISO 10993-10. In this review, we provide an approach that combines the analysis and review of standards and methodologies tests carried out on nanomaterials or devices that allow us to determine if they can be considered biological nanomaterials. Some examples are interaction with blood (hemolysis), sensitization, implantation, toxicity, immunocitotoxicity, genotoxicity, and carcinogenicity, resulting in reliable and comparable data to determine whether or not a nanomaterial is toxic in a biological setting. In addition, supply some examples of research on how different biological assessments have been applied in recent years.

Keywords Biological test · Nanocomposites · Medical applications

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1 Introduction

Nanotechnology has advanced in great strides in recent years, resulting in the production of a large number of nanomaterials, these nanomaterials resemble biomolecules in size and their structures, therefore, they can be used in various fields such as biology, medicine, biotechnology, and health care [1]. The design of new nanomaterials plays an important role in the development of treatments and implants for medical uses. These nanomaterials may have ideal properties such as bioactive, biodegradable, and bifunctional. However, they must be biocompatible. Biocompatibility could be interpreted as the biological acceptability and inert behavior after carrying out the study and evaluating the interaction of the nanomaterials with the susceptible tissue, after a certain period, is for this that the nanomaterials have to be evaluated before being used in biomedical and biological applications.

Zhang et al., in 2016, defined the concept of a device medical, as an instrument, apparatus, implement, machine, gadget, implant, reagent, or another similar item, which is intended to be used, in diagnosis, or cure, mitigation, treatment, or prevention of diseases or other conditions, in man or others animals [2]. In this review, different tests are discussed biological to determine if the nanomaterials can be considered biocompatible, the procedure, and the most relevant examples will be summarized. This review can be used as a general guide to determine the type of test or tests that must be performed on the nanomaterials to be evaluated.

2 Hemolysis

The hemolysis test is one of the most widely used due to its simplicity and low cost, it is essential to determine if they are toxic or hemolytic, nanomaterials, or devices in contact with blood [3]. The presence of hemolytic nanomaterial tends to destroy blood cells, causing a sudden release of hemoglobin (Hb), from the red blood cells, resulting in high levels of plasma hemoglobin (Hbp) [4]. This can induce toxic or other effects that can damage the kidneys and/or nearby organs. To perform the hemolysis test, it is necessary to know the ASTM F756 standard for the evaluation of the hemolytic properties of nanocomposites.

The test could be performed on either the nanocomposite or some liquid extract that could be released from these nanocomposites, using PBS as the extract medium. In summary, the procedure consists of blood collection and its contact with the nanocomposite for 3 h at 37 °C for incubation. Subsequently, the cell button or erythrocyte is obtained by washing and centrifuging the sample. The resulting color in the supernatant or precipitate will indicate whether the nanomaterial is hemolytic or non-hemolytic. However, these results must be confirmed by determining the absorbance, at a wavelength of 540 nm. If the absorbance measurement exceeds the value of 2, the test should be repeated as there is a background error. Taking all the absorbance measurements of each supernatant, determine the hemoglobin

Table 1 Hemolysis index

Hemolytic index above the negative control	Hemolytic grade
0–2	No hemolytic
2–5	Slightly hemolytic
>5	Hemolytic

concentration using a calibration curve. The percentage of hemolysis (% hemolysis) is calculated using Eq. 1, and the test results should be compared with the results of the negative control, using Table 1 as a guide.

$$\%hemolysis = \frac{supernatant\ hemoglobin\ concentration \times 100}{total\ hemoglobin\ concentration} \tag{1}$$

The selection of the test or optimal to evaluate the biocompatibility of a specific nanomaterial will depend on the intended application. The Food and Drug Administration (FDA), is a US agency, which has established American (ASTM), European (ISO), and OECD guidelines, for biological tests on nanomaterials and thus ensures their safety and efficacy [5, 6]. Since ISO 10993 is the most widely used, however, there are various tests to determine if a nanomaterial can be considered a bionanocomposite.

Some examples where the hemolysis test is implemented in nanocomposites is the research work by Li et al. [7], they performed the hemolysis test on biocomposites based on poly(ε-caprolactone)/keratin S-nitrosylated, as vehicles in the release of nitric oxide, as possible cardiovascular treatments. The authors highlight the great importance of these green nanocomposites in medical areas, however, for this application, it is necessary to determine their blood compatibility first [7].

In addition to the previous work, Rabab et al., in 2022, used gamma radiation, as a green route, for the synthesis of biocomposites based on graphene/strontium oxide. In this investigation, hemocompatibility was also determined, as a result, it was obtained that the nanocomposite is promising for biomedical applications [8].

3 Sensitization

Sensitization tests are necessary regardless of the tissue and the duration of contact involved in the application [9, 10], even minimal amounts of nanomaterials can cause allergies or sensitization reactions. These studies are generally carried out in laboratory rodents, guinea pigs are a widely used species for being almost as sensitive to dermal sensitizers as humans [11, 12] and it should be established where it will take carry out the exposure of the nanomaterial to be evaluated; skin, eye, mucosa; and nature; degree, frequency, duration and conditions of exposure of the nanomaterial for clinical use (gloves, bandage, electrodes). These tests focus on determining the biological response to leachates present in medical nanomaterials

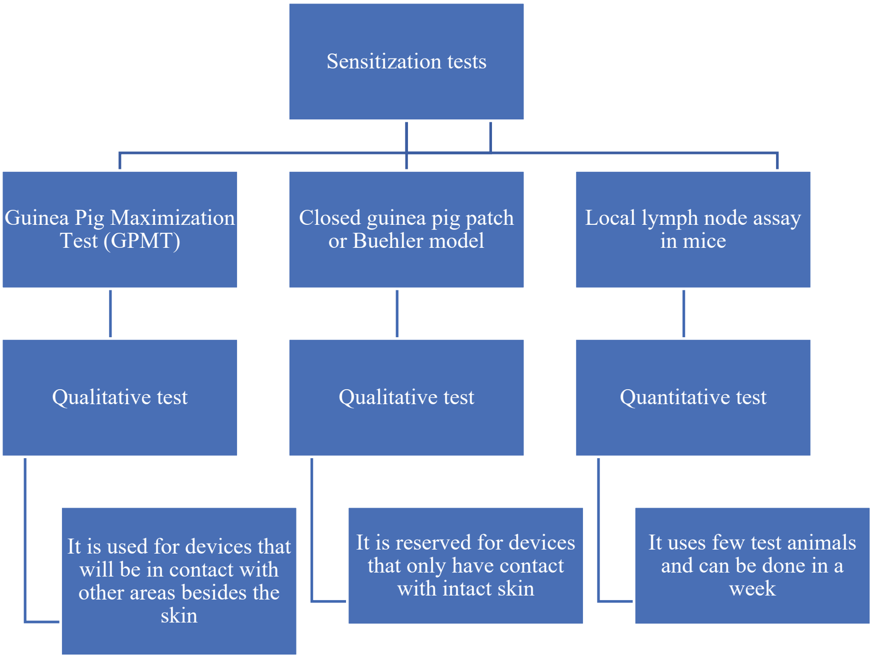


Fig. 1 Sensitization tests for medical nanomaterials or devices

or devices [12]. The basic methodology of these sensitization tests is described in ISO 10993-10 and it is similar to the methodology described in the Organization for Economic Cooperation and Development (OECD 406) [10, 11, 13]. Sensitization tests for medical nanomaterials or devices are presented in Fig. 1 [10].

3.1 Guinea Pig Maximization Test (GPMT)

The standard indicates that positive control nanomaterials be tested at least once every six months, using a weak sensitizer [10, 14]. The study is carried out in three phases:

- Intradermal induction. Animals receive three pairs of injections on the scapular region (Fig. 2): Site (A) Emulsion with a 50:50 ratio of the solvent vehicle (saline and vegetable oil)/test extract; and Freund's complete adjuvant, intended to enhance the immune response [10, 11]. Freund's complete adjuvant was developed by Freund and Col, in 1936, it is an emulsion of water in mineral oil, which contains dead mycobacteria, and it is used to boost the immune response against a simultaneously administered antigen [15]. Site (B) Test animals are injected with the undiluted test extract and control animals are injected with solvent only. Site

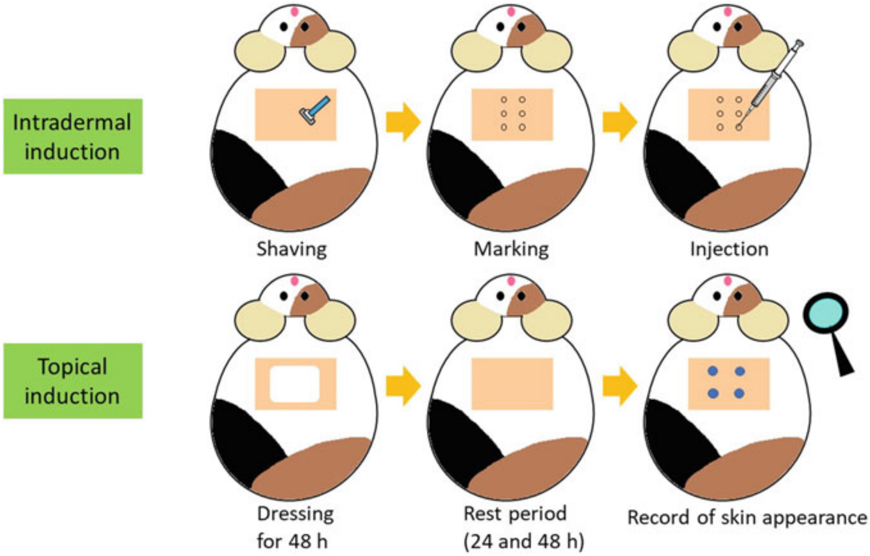


Fig. 2 Phases of intradermal induction and topical induction in the guinea pig maximization test

(C) Emulsion with a 50:50 ratio of the extract/test solution and Freund's complete adjuvant [10, 13].

- Topical induction. It occurs about one to two weeks after the intradermal injection. One day before starting the test, the area of the previous injection site is trimmed to remove excess hair, and 0.5 mL of 10% sodium lauryl sulfate is applied to cause mild irritation that will improve topical absorption. After 24 h, the sodium lauryl sulfate is removed, and a filter paper or absorbent pad is applied with the extract/test solution and covered with an occlusive dressing [10, 11]. The patches are removed after 48 h (Fig. 2). After topical induction, hair is trimmed on the back. For test animals, the test extract/solution is applied on the right side, and the vehicle/control is applied on the left side; in control animals, it is the opposite, the vehicle/control is applied on the right side, while the test solution is applied on the left side. Subsequently, the resulting erythema and edema are punctuated at 24 and 48 h, after removal of the patch; if there is no visible change it is considered 0 points, for discrete or irregular erythema it is 1 point, for moderate and confluent erythema it is 2 points and for intense erythema or swelling 3 points are considered; scores of one or more are considered positive to sensitization. The overall response of test animals is compared to the overall response of control animals [10].

3.2 *Closed Patch or Buehler's Model*

Skin irritation is a local non-immune inflammatory reaction caused by external stimuli [16–18]. The animal hair is trimmed in the application area, a 25 mm × 25 mm section of the sample to be evaluated, is applied over the trimmed area and is left there for a period of 6–8 h [10, 11]. Repeating the procedure 3 times a week for 3 weeks, this period is known as the induction phase. In control animals, a cotton pad or placebo patch is used as a negative control [9]. After the 3 weeks of induction, there are 2 weeks of rest (recovery phase), subsequently, the animal's hair is trimmed, and it is passed to the final exposure where a topical challenge is applied, which again exposes the animals to the bionanocomposite [19, 20]. The evaluation for resulting erythema and edema is the same as in GPMT.

3.3 *Mouse Lymph Node Assay (LLNA)*

The methodology for this test is the same for both chemicals and extracts of nanomaterials or devices. Unlike conventional sensitization tests, the animal should not be fully sensitized in this assay. Lymph node assay requires 12 young female mice (8–12 weeks) not pregnant. Four mice are used per test for chemical products, while 5 mice per group are used for extracts of medical nanomaterials or devices since a single dose is generally used. 25 μL of the test extract or control solution is applied to the dorsal surface of both ears of the mouse (Fig. 3), and this procedure is repeated for 3 consecutive days [10, 11]. 72 h after the last application, the mouse is intravenously injected with 250 μL of sterile phosphate-containing saline containing the radioisotope H-methyl thymidine [21]. The isotope is rapidly incorporated into dividing cells. 5 h after intravenous injection of the isotopes, the mice are sacrificed, and the lymph nodes are removed. The assay measures lymphocyte proliferation during the induction phase of skin sensitization [9]. If the test extract is a sensitizer, the production of lymphocytes in the lymph nodes that drain the ears will increase and the levels of radioactivity will be higher. The level of radioactivity in test animals is divided by the background level of radioactivity of control animals to determine a stimulation index. If the stimulation index is >3, the substance is considered a sensitizer. For positive control, it is considered to use a weak sensitizer such as hexyl cinnamic aldehyde, mercaptobenzothiazole, and benzocaine.

4 Implantation

The nanocomposites based on carbon nanotubes, with ceramic or polymeric matrices act as efficient vehicles in the administration of active substances in tissue engineering.

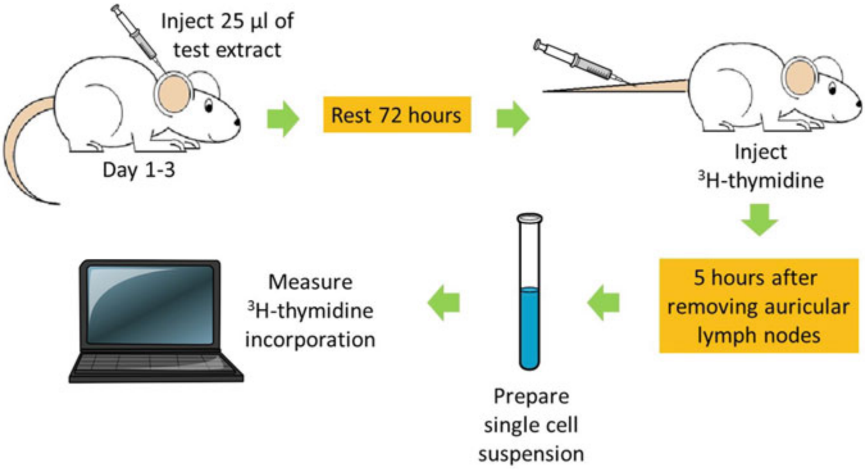


Fig. 3 Lymph node assay in mice

These nanocomposites are mostly implanted for their effective administration, having great benefits such as a decrease in toxicity and sustained release. However, the effectiveness of using nanocomposites in this context requires that they pass the implantation test among many [22–24].

ISO 10993-6: 2007 regulates the methodology for evaluating local effects after implantation, and can be applied to nanomaterials or medical devices, which are applied topically or as grafts in some body tissue, however, it should be considered that the shape of the nanomaterial to be evaluated directly impacted the response of the tissue when implanting [25].

The implant site is very diverse, from implant to muscle, bone, brain, ocular, dental, and mucosal sites [26, 27]. Several authors agree on the most appropriate site to perform the graft, it must be the same one where the nanomaterial and medical device foresee its final application [26, 27].

The animals used in the test will depend on the type and size of the nanomaterial or device and the evaluation period. The duration of the test will depend on the intended application of the nanomaterial to be evaluated.

For the biological evaluation of the test samples compared to the control sample as a function of time, it is necessary to determine the macroscopic and histopathological responses of the effect of the implant on the tissue and determine if there are alterations [25, 28].

All these histological responses receive a score according to the system established in the standard, in addition to the evidence obtained by photomicrography, and must be included in the report together with the description of the test nanomaterials, such as shape, size, surface weight, justification of the lesson of the control sample, information on the animals used and the conditions under which the implantation test was performed.

5 Toxicity

Toxicology plays an important role in the development of a bionanomaterial to ensure that the nanomaterials selected for use in medical applications are safe for their intended application, it is essential to identify and mitigate potential security problems [29, 30]. The response of the host tissue to an implanted nanomaterial is due to the sequential activation of cytokines, which direct inflammatory or curative reactions [31]. Periprosthetic tissues become potential sites of local toxicity, due to the presence of waste products released from the implanted nanomaterials, for evaluation. The wear residues activate endothelial cells at the implant/tissue site and express adhesion molecules as P selectin. ICAM-1, VCAM, and CD44. Granulocytes, macrophages, lymphocytes, and monocytes are attracted to these adhesion molecules; macrophages phagocytize waste particles and trigger the secretion of proinflammatory cytokines such as interleukin 2 (IL 2), IL-8, IL1-β, IL-6, chemokines, growth factors, prostaglandins, degradative enzymes, among other factors. The osteoclasts generated by the union of the macrophages adhere to the surface of the implant, causing osteolysis, which can result in the loosening of the implant (Fig. 4) [32].

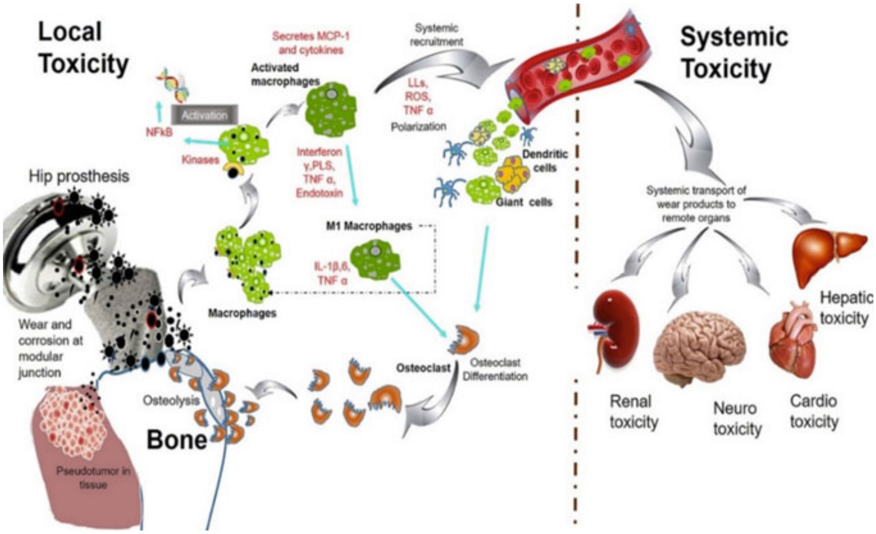


Fig. 4 Scheme of the adverse tissue reaction in the region of the implanted tissue and systemic toxicity. The particles generated by the implant induce a macrophage-mediated inflammatory reaction, and the macrophages absorb the degradation products thanks to phagocytosis, diffusion, and the membrane-mediated transport mechanism. Particulate wastes passing into the circulatory system can cause distal toxicity in different organs. [32]. Reused with permission from Elsevier

5.1 Systemic Toxicity

These tests assess generalized organ and tissue toxicity after exposure to nanomaterials to animal models [10, 12]. The toxicity observed after a single exposure is often different from that observed after repeated exposure [33]. This toxicity is due to the biological and immunological response caused by the degradation of products [32]. The term "systemic" means that the breakdown products of a medical device or nanomaterial are absorbed in one site and distributed throughout the body through the lymphatic or circulatory system causing a deleterious effect at another site. Systemic toxicity tests are divided into periods; acute, subacute, subchronic, and chronic [10].

5.2 Acute Toxicity

Acute toxicity is the adverse effect that could be generated after single or multiple exposures to a test sample for one day, within the first 72 h [12, 33–35]. This test should be performed on implanted and externally applied nanomaterials or devices, as well as surface-applied devices that come into contact with the skin, regardless of the contact time [35]. This study uses a limited number of animals (5 rodents). Mice can be dosed with saline/nanomaterial intravenously, or intraperitoneally with a non-saline solution (Fig. 5). Observational parameters such as clinical signs and symptoms, such as lethargy, hyperactivity, seizures, and body weight, are evaluated [10]. After the observation period for at least 3 days or up to 7 days to identify the late effects, the animals are euthanized and clinical pathology, gross pathology, and histology can be assessed [35].

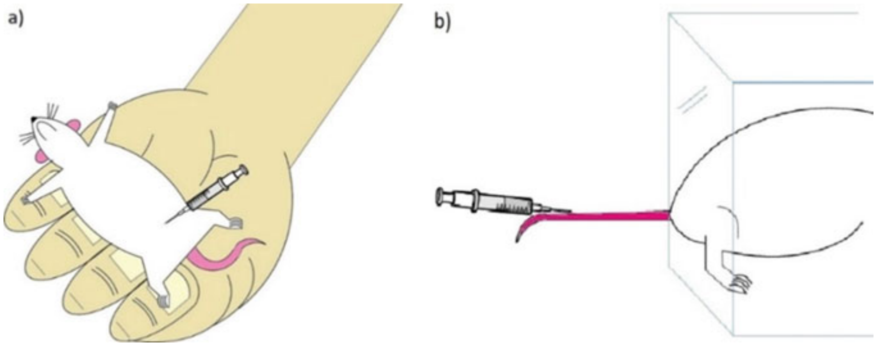


Fig. 5 **a** Intraperitoneal injection, **b** intravenous injection

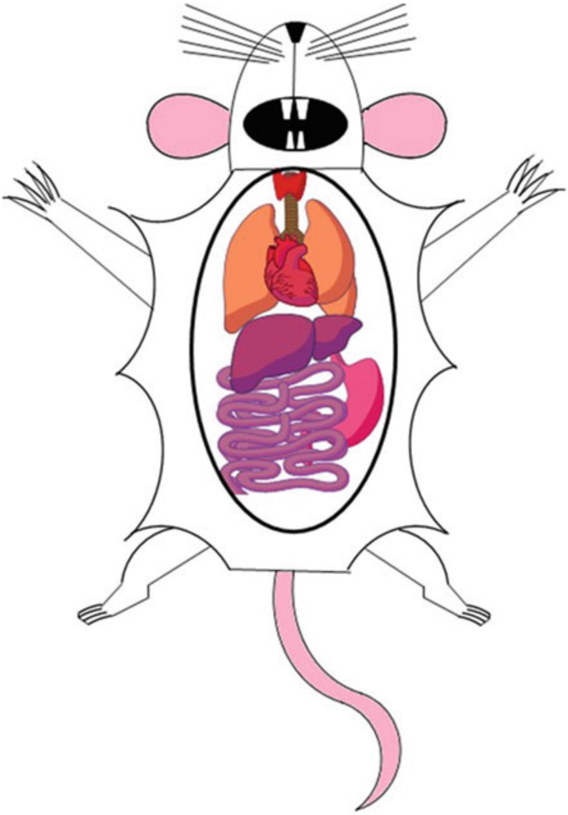


Fig. 6 Necropsy of the rodent, at the end of the subacute toxicity test

5.3 Subacute Toxicity

Subacute toxicity is the adverse effect after single or multiple exposures per day, of test nanomaterial, for a period of 14–28 days [12, 33]. This test requires 10 rodents (5 per sex). Exposure to the nanomaterial can be by intravenous or intraperitoneal injection. Observations of clinical signs and symptoms are recorded daily, body weight can be taken per week. At the end of the test, a blood sample is collected for pathological analysis, the animals are sacrificed, and a necropsy is performed (Fig. 6) [10].

5.4 Subchronic Toxicity

Subchronic toxicity, is the adverse effect after a single exposure or multiple exposures, of a test sample for one day, during a part of the useful life, generally 90 days, but not exceeding 10% of the animal's useful life [12]. In choosing between the subchronic test, the subacute test, the nature of the exposure, and a more extended period of exposure, which implies the intended application of the evaluated nanomaterial, must be taken into account. The same methodology and the same exposure routes of the subchronic test are followed. Rodents (10 per sex) are used. During the bioassay, clinical signs, and symptoms, the change in body weight should be observed; in addition to evaluations of neurotoxicology, immunotoxicology, and cardiovascular function [33].

5.5 Chronic Toxicity

Chronic toxicity is the adverse effect, after single or multiple exposures, of a test sample for one day, for at least 10% of the animal's lifespan, generally more than 90 days in rats [12]. The study design and the evaluated endpoints are the same as the subchronic toxicity test, however, the number of animals per treatment group is higher (30 animals, 15 for each sex) because possible losses during the study are considered study [10].

Toxicogenomics is a branch of toxicology that studies the effects of nanomaterials on gene expression, through a study of cellular transcriptomics, using microarray-based gene expression assays [36].

6 Immunocitotoxicity

Cell viability assays are used as tests to study the effect that a given nanomaterial may have on cell proliferation, as well as the direct immunocytotoxic effects that eventually lead to cell death. The most commonly used immunocytotoxic tests are; the MTT assay, neutral red uptake assay, and the kenacid blue binding assay [37–39].

6.1 Cell Proliferation Assay (MTT)

This is a colorimetric assay based on the metabolic reduction of tetrazolium components (MTT), carried out by the mitochondrial enzyme succinate dehydrogenase, resulting in a blue-colored compound (formazan), allowing to determine the mitochondrial functionality of viable cells [40, 41]. The amount of color produced is proportional to the number of living cells. The ability of cells to reduce MTT is an indicator of the integrity of the mitochondria and their functional activity is interpreted as a measure of cell viability and allows obtaining information about the toxicity of the nano-compound being evaluated (Fig. 7) [38, 42].

The MTT test, according to ISO 10993-5: 2009, establishes that initially, the cells must be kept under sterile conditions. The period of exposure to the substance or test

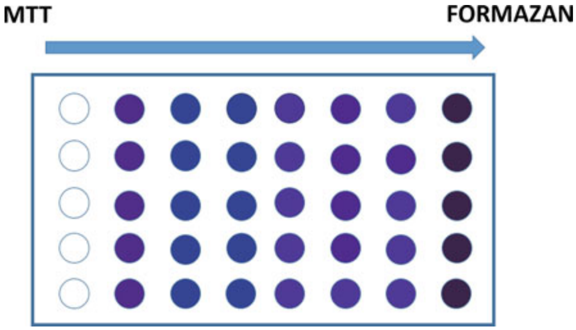


Fig. 7 Representation of MTT metabolism to a formazan salt by viable cells as shown in a cell culture plate

nanomaterial varies, can be from short periods of between 1 and 2 h of treatment, up to long periods of 24–72 h. Up to 6 concentrations of the nano-compound must be evaluated, reaching a concentration of 1000 μg/mL or up to the maximum limit of solubility of the product in the medium. If this concentration is reached and no toxicity is observed, then it is necessary to increase the concentration range to 100,000 μg/mL or the maximum soluble concentration of the nano-compound in the medium. It should be borne in mind that if the product being evaluated precipitates in the culture medium, these results must be discarded. A medium control, and a solvent control should be used in the assay, and positive control is recommended [39, 43].

The results should be expressed as a percentage (%) of living cells, according to the following Eq. 2:

$$\%Cell\ viability = \frac{\text{D.O. of the treated cells}}{\text{D.O. of control cells}} \times 100 \quad (2)$$

where D.O. refers to the optical density or absorbance of the sample or controls.

Balu et al. [44], evaluated the in vitro biocompatibility of carbon and silver nanomaterials, doped with hydroxyapatite, using an MTT assay. The test was performed at various concentrations of the nanomaterial, suggesting that the nanomaterials did not affect cell viability and could be safe for in vivo testing [44].

6.2 Neutral Red Uptake Test

The neutral red uptake assay (Fig. 8), is a measure of the toxicity of nanomaterial at short or long exposure time and is determined by the release of neutral red dye due to loss of cell viability [39]. This test is based on the fact that a nano-compound or nanomaterial is cytotoxic regardless of its mechanism of action if it interferes with the process of cell division and multiplication. It was resulting in the reduction of the speed of cell growth, reflected in the number of cells present in the culture. The degree of growth inhibition related to the concentration of the nano-compound being

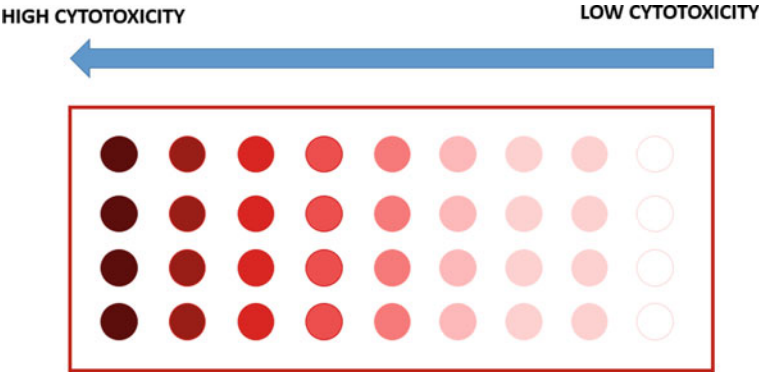


Fig. 8 Neutral red uptake decreases with increasing cytotoxicity (from right to left in the plate) [46]

evaluated is an index of toxicity. Lysosome and endosome cells capture neutral red, the lack of color, implies that the cell loses viability, due to the nanomaterial being evaluated. Next, the amount of neutral red that remains after exposure within the cell is determined, and the concentration that produces the 50% inhibition of cell growth is calculated [37, 45].

For the quantification determine the absorbance reading at 540 nm. For the quantification it should be read at 540 nm Absorbance values should range from 0.2 to 1.0 to give an adequate correlation between the number of cells present and the observed optical density. Most of the authors suggest that up to 6 concentrations of the nano-compound should be evaluated, reaching a concentration of 1000 μg/mL or up to the maximum limit of solubility of the product in the medium. If this concentration is reached and no toxicity is observed, then it is necessary to increase the concentration range to 100,000 μg/mL or the maximum soluble concentration of the nano-compound in the medium [39, 47, 48].

Under normal conditions, it must be assumed that there is no neutral red output. The obtained optical density (D.O.) is taken as an index of the original amount of neutral red present in the culture. The calculation of the percentage (%) of inhibition of cell growth is performed using the following Eq. 3:

$$\%\ of\ inhibition = 100 - \frac{\text{D.O. treated cells}}{\text{D.O. control cells}} \times 100 \tag{3}$$

6.3 *Kenacid Blue Binding Assay*

The kenacid blue binding assay determines the variation in the total protein content in the cell in contact with the nanomaterial to be evaluated for cytotoxicity, which reflects cell proliferation. If a compound is cytotoxic to the cell, it should affect at least one or more processes involved in cell proliferation such as DNA synthesis, the proper functioning of mitochondria, and lysosomes, or may cause an impairment of membrane integrity in protein synthesis [49, 50]. As cell growth is affected, the number of cells present in the treated culture must be reduced concerning the control. Therefore, the concentration of proteins present in the culture constitutes an index of toxicity. In this assay, the cells are exposed to the nanomaterial that is evaluated for a time or period of 72 h, and the cells are subsequently exposed to the dye, which binds to the cellular proteins. Finally, the amount of kenacid blue retained by the cells must be terminated, and the percentage of inhibition of cell growth must be quantified [51, 52].

7 Genotoxicity

Nanocomposites applied in the medical area, mainly as implants with long lifespans, must be evaluated through the genotoxicity test, where the possibility of genetic damage is determined.

Genotoxicity is the relative ability of an agent to cause damage to genetic material, causing adverse biological effects. It is also described as the property of a chemical compound or of particles to alter the genetic information of a cell [53, 54]. This can occur through different mechanisms such as genetic mutations and structural or numerical chromosomal aberrations. Such alterations can have serious consequences.

Genotoxicity testing in nanomaterials consists of the application of a series of tests that cover genetic mutations, and structural and numerical chromosomal aberrations, generally starting with an in-vitro test, if the nanomaterial evaluated has no effect, it could be classified as "non-genotoxic" however, generally require in-vivo follow-up research. On the other hand, a positive result in the in-vitro test may also require confirmation by appropriate in-vivo tests [55].

The in vitro genotoxicity tests approved by the REACH legislation are; the bacterial reverse mutation test, also known as the Ames test from the Guideline For Testing Of Chemicals (OECD TG 471), the mammalian genetic mutation test or the HPRT test (OECD TG 476 and 490), the mammalian in-vitro chromosome aberration test, also known as the CAT assay (OECD TG 473), also, the in-vitro mammalian cell micro-nucleus test MNT (OECD TG 487) [56].

7.1 Bacterial Reverse Mutation (AMES)

The Ames assay [57] (Fig. 9) is a biological assay to assess the mutagenic potential of nano-compounds. The procedure is described in a series of documents from the early 1970s, written by Bruce Ames and his group at the University of California, Berkeley [57–60]. This test uses several genetically altered strains of the *Salmonella*

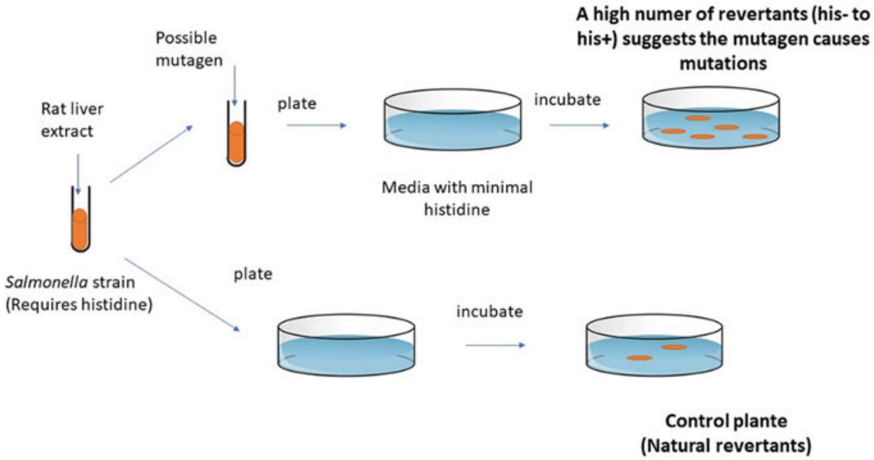


Fig. 9 AMES test

typhimurium bacteria to present mutations in the genes involved in histidine synthesis. Because of such mutations, these bacteria require an external supply of histidine for growth. The assay tests the ability of the mutagen to cause a genetic alteration in bacteria that allows growth to return on histidine-free support.

Liver enzymes (from rat liver extract) are also added in the trial to simulate the effect of metabolism. The bacteria are spread on an agar plate with a small amount of histidine and the nano-compound whose mutagenic effect is to be tested. This small amount of histidine in the culture medium allows bacteria to grow for the initial time and have a chance to mutate. When histidine is depleted, only bacteria that have mutated to obtain the ability to make their histidine will survive and multiply. In the experiment, the plate is incubated for 48 h, and the mutagenicity of the substance will be proportional to the number of colonies observed on the plate, after the end of the incubation time [61–65].

7.2 Mammalian Genetic Mutation (HPRT)

The HPRT gene mutation assay (Fig. 10) is a remarkable tool that detects genotoxic substances and allows the isolation and detection of types of inducible mutations. This HPRT gene mutation assay is considered an essential tool as set out in the guidelines for mammalian genetic mutation testing (OECD 476) [66–68].

The hypoxanthine phosphoribosyltransferase (HPRT) gene is on the "X" chromosome of mammalian cells. The assay can detect a wide range of nanomaterials capable of causing DNA damage and leading to genetic mutations, these are detected using eukaryotic cells [69, 70].

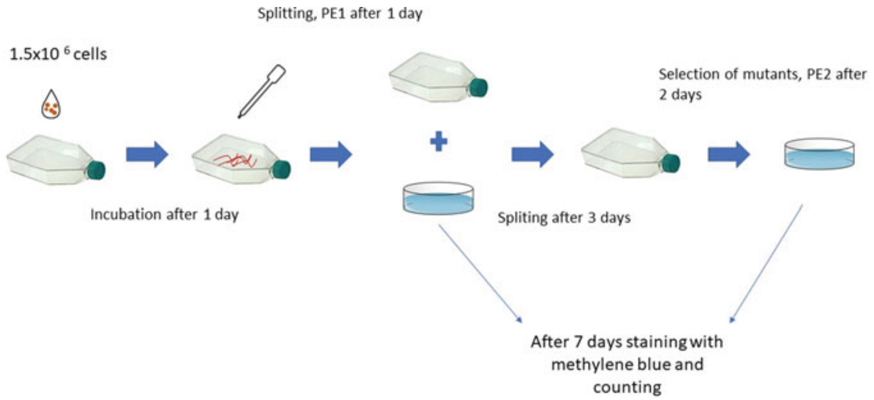
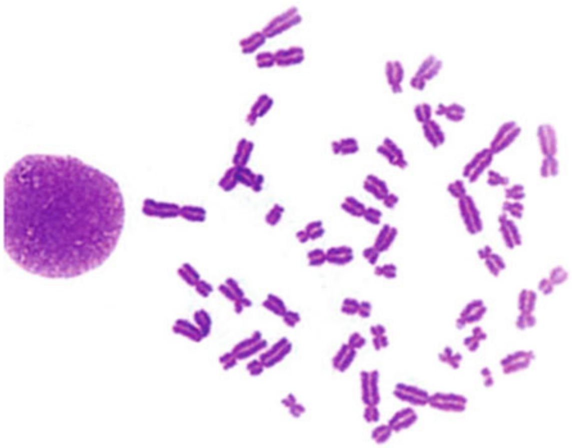


Fig. 10 HPRT method

7.3 *In-Vitro Chromosome Aberration or CAT*

The in-vitro mammalian chromosome aberration test (Fig. 11) (OECD 473), is used to identify substances that cause structural chromosomal aberrations in cultured mammalian cells. This test is designed to evaluate the potential of test nanomaterial and to induce structural chromosomal abnormalities such as breaks and exchanges [71]. This test can be performed on primary human peripheral blood lymphocytes (HPBL) or established cell lines, such as Chinese hamster ovary (CHO) cells. The cultures are incubated with various concentrations of the test nanomaterial for 3–4 h in the presence and absence of metabolic activation, a statistically positive result, characterized by a significant increase in the dose of aberrant cells, which exceeds the limits of the negative control [72, 73].

Fig. 11 Representative image of the in-vitro chromosome aberration test [71]. Reused with permission from Elsevier



7.4 In-Vitro Test of Micro-nuclei of Mammalian Cells (MNT)

The MNT in-vitro assay (Fig. 12) is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells. This test is used to evaluate if a nanomaterial can be carcinogenic and cause alterations in the genetic material of eukaryotic cells [74]. The micro-nuclei are detected at the interface, smaller and with the same morphological characteristics as the cell nucleus, and are of value in diagnosing genotoxicity [75–77]. These micro-nuclei appear in cells that have completed a nuclear division and are in the bi-nucleated phase of the cell cycle.

8 Carcinogenicity

Genomic alterations that include the activation of oncogenes and the inactivation of tumor suppressor genes are responsible for carcinogenesis [79]. Carcinogenesis is a multi-step process from the transition from healthy cells to cancer cells through a sequence of stages and complex biological interactions [80]. Carcinogenicity tests determine the tumorigenic potential of nanomaterials, and medical devices that are in contact with a test animal for a period longer than 30 days [13].

The ISO 10993-3 standard lists various situations where it is suggested to carry out carcinogenicity tests, even if there are previous tests, and these have been negative; i. when nanomaterials are slowly reabsorbed into the body (over 30 days), ii. when nanomaterials are introduced into the body for 30 days or more; except when the nanomaterial has a significant history where it has been used in humans [81].

For the carcinogenicity study, rodents have become the most widely used mammalian model, they have physiological characteristics similar to humans, they

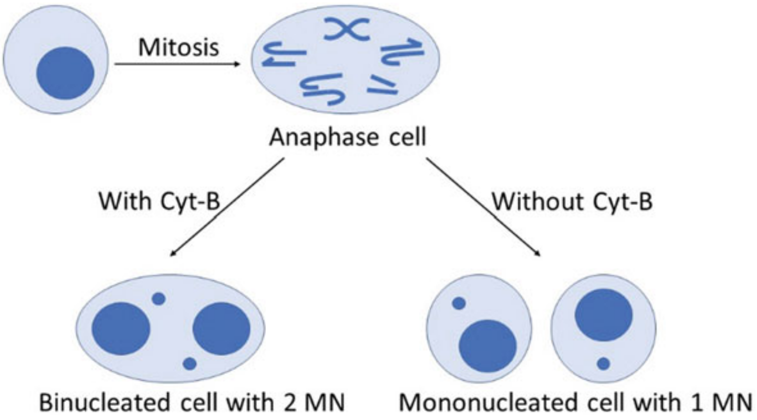


Fig. 12 Micronucleus formation due to loss of an entire chromosome and chromosomal fragments in the mitotic phase. Adapted from Ref. [78]

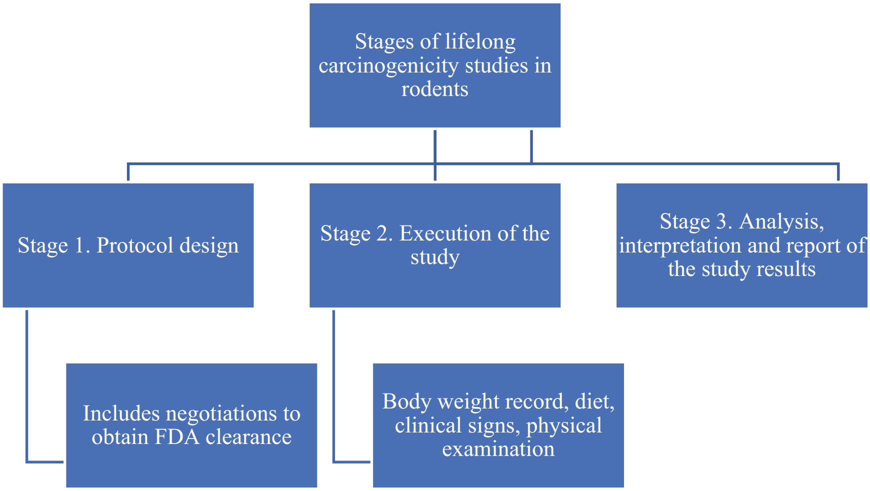


Fig. 13 Stages of lifetime carcinogenicity study in rodents

present genes homologous to human genes; and they frequently develop similar diseases [82, 83]. The traditional rodent bioassay in rodents and the rasH 2 transgenic mouse model are currently used for the carcinogenicity test. In Fig. 13, the stages of the carcinogenicity study when using rodents are presented [84].

8.1 Traditional Bioassay in Rodents

In the 1960s, tests were carried out to identify carcinogens to reduce or eliminate them from the environment. Since then, the 2-year bioassay in rats and mice has been considered “the gold test”, for evaluations of carcinogenicity [84]. It is a slow trial since the required period is at least 18 months for mice and 24 months for rats. The confidence of the bioassay is based on the fact that it can efficiently detect human carcinogens [85]. However, predicting cancer in humans is the subject of considerable debate because the carcinogenic effects are estimated to be produced by typically high doses used in rodent studies and the uncertainty associated with extrapolation of rodents to humans [79, 86]. The disadvantage of this bioassay is the large number of animals used and the high cost [86]. To carry out the traditional rodent bioassay study, two groups are required; for the negative control and test group, it is recommended to use more than 50 animals of each sex per group. At the end of the test, a valid negative result is obtained if more than 50% of each negative control group survives the test [84]. Rodents are prone to developing tumors surrounding implanted nanomaterials within 8 to 9 months after implantation and are known as the Oppenheimer effect or solid-state carcinogenesis. The rat strains that are commonly used in carcinogenesis tests are Sprague–Dawley rats and derivatives such as the CD rat, the F344 (Fischer) rat, and Wistar rats [87].

8.2 RasH 2 Transgenic Mouse Model

Transgenic mouse models are possible alternatives to the bioassay with long-lived mice. Transgenic mice are more sensitive to carcinogenic risk [86]. This model was created in the early 1990s, it has the human counterpart of the Harvey rat sarcoma virus oncogene with its promoter region in addition to the endogenous murine Ha-ras oncogene. In these rodents there is a higher incidence of malignant tumors after treatment with genotoxic carcinogens, on the contrary, do not demonstrate significant tumor induction when exposed to non-carcinogens [81]. The advantages of working with transgenic rasH2 mice are the short duration of the study (6 months), low cost and reduced use of animals, and a direct interpretation of the results. For positive control, various carcinogens such as ferric nitrilotriacetate (Fe-NTA) in rats, and *N*-methyl-*N*-nitrosourea (MNU) in mice [81] can be used (Fig. 14).

9 Conclusion

The purpose of this review was to develop biocompatibility tests for medical devices or bionanomaterials. Whenever you want to implement a new medical or bionanomaterial device, you should carry out a series of biocompatibility tests that assess the biological risk of the bionanomaterial.

Before identifying which, biological tests are necessary for bionanomaterial or medical device to be tested, it must first be identified what its application will be, which tissues it will encounter, and the duration of its use. The information gathered from the selected tests will provide the possibility that the medical device or bionanomaterial could be used successfully in patients or if it caused any damage. Failure in the biocompatibility tests of bionanomaterials does not necessarily reject the use of the bionanomaterial. Still, a detailed evaluation of the risks or adverse responses compared to the benefits that the use of the medical device or bionanomaterial should have should also be carried out with additional biocompatibility tests and a supported justification for continued use. The ISO 10993 standards for the biological evaluation of medical devices provide a series of standards that must be carried out to evaluate the toxicity of a bionanomaterial or medical device in vitro and in vivo. In vivo tests have the limitation that animal species do not always react in the same way that humans would, however, in combination with in vitro tests they will provide enough information to determine if a bionanomaterial would result in adverse reactions when used in patients. In recent years, there is a growing development of medical devices and bionanomaterials with biocompatibility and biodegradability properties to be used in biomedical applications. In this review, the standards used for risk assessment are

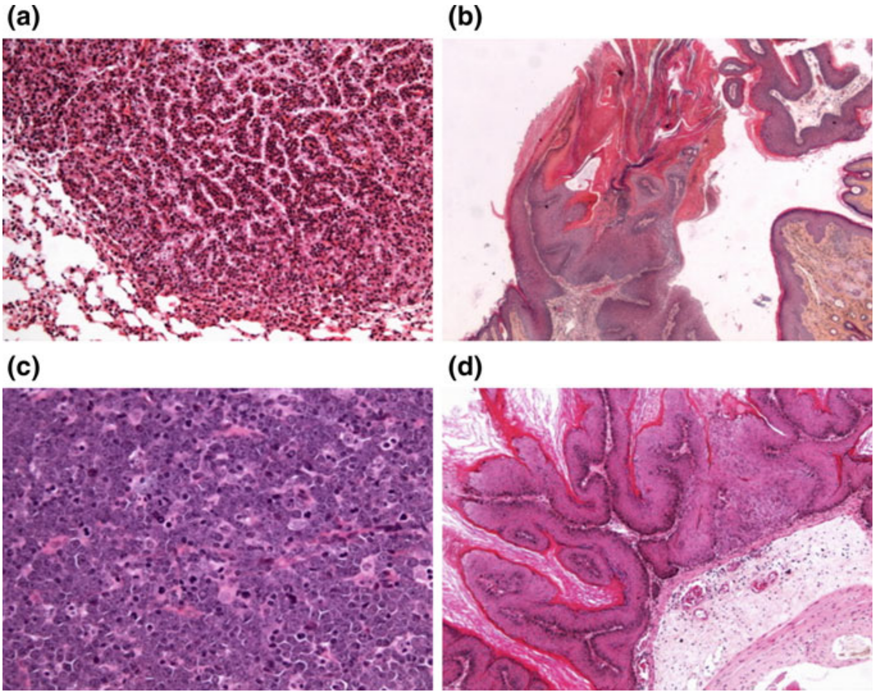


Fig. 14 **a** Low incidence of pulmonary adenoma in an MNU positive control rasH2 mouse. **b** Cutaneous squamous cell papilloma with a high incidence in the positive control group of rasH2 mice. **c** Thymic lymphoma with high incidence in the positive control group of rasH2 mice. **d** Squamous cell papilloma of the stomach (non-glandular mucosa) with a high incidence in the positive control group for MNU. Hematoxylin, eosin, and saffron. [81] Reused with permission from Elsevier

the most current, but it must be emphasized that they are continually evolving to ensure the lowest biological risk in medical devices and bionanomaterials.

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Harnessing the Potential of Fibrous Polyester Composites Meant for Bioactive Medical Devices

Graciela Morales, Heriberto Rodríguez-Tobías, Victoria Padilla-Gainza, Karen Lozano, and Daniel Grande

Abstract Fibrous-based composite polyester mats have gained importance in the biomedical area due to their morphological characteristics and mechanical performance. These materials can be modified by incorporating different inorganic micro- or nano-particles, thus leading to promising potential applications as medical devices with antimicrobial and/or bioactive properties. In this regard, this chapter reports a compilation of different studies related to the development of sub-micron fibers based on two important polyesters for biomedical use, namely poly(3-hydroxybutyrate) and poly(lactic acid). Studies focus on fibers developed through hydrodynamic techniques, namely electrospinning, electrospraying, and centrifugal spinning. The incorporation of zinc oxide or hydroxyapatite on the polyester-based fibers results in materials that inhibit bacterial growth or induce bone cell proliferation. Studies have reported on the effect of the fabrication method as well as the concentration of nanoparticles on the antimicrobial effectiveness against bacteria such as *Escherichia coli* and *Staphylococcus aureus*. Particularly, the development of polyesters and their corresponding composites have emerged using a novel and scalable technique named Forcespinning®. These recent studies report on the influence of morphology and chemical nature on cell adhesion and proliferation and more notable on the ability to mass-produce fibrous-based polyester composite systems with practical applications.

Keywords Electrospinning · Electrospraying · Forcespinning · Polyesters · Bioactive composites

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1 Fibrous Scaffolds for Biomedical Applications

During the past decades, scientific advances in molecular and cellular biology, as well as a progressive understanding of tissue regeneration and wound healing processes have stimulated the development of a novel and multidisciplinary field of research known as *Tissue Engineering*. This research field applies engineering and life science to design *biodegradable and bioactive materials* with specific structural properties that allow for the simulation of the extracellular matrix (ECM), with the purpose of promoting its regeneration or function [1, 2]. Most often, the ultimate goal is implantation in the body to repair an injury or replace the function of a damaged organ. Critical functions can be structural (bone, cartilage), supportive, transport (skin, blood vessels), secretory, and biochemical (liver and pancreas). The concept of *scaffold* in tissue engineering is to mimic the functions of native ECM [3]. Therefore, the properties of engineered scaffolds are related to the ECM functions in native tissues and are connected with their structural, biological, and mechanical features [4].

The morphological features associated with *fibrous 3D materials* closely mimic the natural microenvironment constituted of fibrous proteins present in native ECM [5]. In combination with their porous nature and high surface area to volume ratio, these qualities have made micro-/nano-fibrous scaffolds attractive not only from the academic point of view but also in terms of applied science to be used in tissue engineering applications. In this sense, they have been used as supports for drug delivering agents, dressings for wound healing, and porous scaffolds for the regeneration of tissues, including skin, blood vessels, nerves, tendons, bone, and cartilage [6–8]. In the field of bone tissue regeneration, efforts are being made to address diseases, such as osteogenesis imperfecta, osteoarthritis, osteomyelitis, osteoporosis, traumatic injuries, and orthopedic surgeries (i.e., total joint arthroplasty, arthrodesis of the spine, fixation of implants, etc.). These efforts have produced a remarkable clinical and economic impact in the treatment of bone defects. For this reason, the demand for bone implants has been increasing exponentially, until it has become the second tissue with the highest requirement for implants [9, 10].

1.1 Polymer Matrices

A polymer matrix for medical applications has to be categorized as a biomaterial, i.e. a material able to interact with a biological system to perform a specific action of regeneration or replacement of a tissue, organ, or body function [11]. A biomaterial has to be biocompatible, namely, it must have the ability to develop, with an appropriate host, a specific response without causing a cytotoxic effect. There are a wide variety of factors that affect the tissue response in contact with an implant and are mostly related to its biological and physicochemical properties, as well as to its shape and structure [3].

Some of the most important properties that should define a biodegradable material are listed below [12]:

- Prevent a sustained inflammatory or toxic response after implantation in the body.
- Possess an acceptable duration of action, which should coincide with the healing or regeneration process.
- Possess mechanical properties appropriate for the indicated application, where the variation of mechanical properties with degradation should be compatible with the healing or regeneration process.
- Provide non-toxic by-products and those should be capable of being metabolized and eliminated by the body.
- Present feasible processability for the intended application.

Polymeric biomaterials are currently used for biomedical applications as their mechanical and degradation properties are suitable for mimicking various tissues. Especially, scaffolds based on polyesters (poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PLG), poly(lactic-*co*-glycolic acid), and poly(hydroxyalcanoate)s (PHAs) among others) have gained increasing attention given their biodegradability, biocompatibility, and ease of processing [13].

1.2 Production Techniques for Fibrous Scaffolds

1.2.1 Fiber Production Techniques

Several methods have already been applied for generating micro-/nano-fiber, such as melt-blown [14], three-dimensional (3D) printing [15, 16], phase separation [17], template synthesis [1], and self-assembly [2, 18]. However, the selection of materials that can be produced by these methods is restricted, limiting their practical application [19]. On the contrary, electrospinning [20–22] is an extensively studied and widely applied method for nanofiber production from a remarkable range of organic and inorganic materials. Compared with traditional nanofiber preparation technologies, electrospinning can produce fibers with high specific surface areas, uniform pore sizes, and high porosity, which significantly improves the performance of nanofibers. In terms of adaptability, the versatility of the electrospinning technique has allowed for producing a vast range of materials. Moreover, it enables the fiber diameter to be adjusted from nano- to micrometers [23].

In general, spinning technologies for the production of fibers have been based on an extrusion process, which allows for the continuous production of materials with one or more filaments. The resulting product exhibits different properties depending on the used process and selected parameters [24]. Polymeric fibers can be obtained from melts, solutions, and emulsions. The polymer melt process offers unique advantages as it avoids the use of solvents and possesses economic advantages given the lack of solvent recovery steps. On the other hand, using solvent-based methods possesses the advantage of increasing the selection of materials to be used while also offering lower energy consumption and the potential to develop smaller fiber diameters. Emulsion spinning is a method that is selected to produce multiphase polymer fibers with different morphologies, active component encapsulations, etc. [25, 26].

Electrospinning typically consists of a syringe and needle through which a polymer solution is passed, and the flow is controlled by a metering pump. A power source applies several tens of kilovolts on the needle, and therefore on the polymer solution. The high applied voltage causes a significant density of charges on the protruding polymer droplet (Taylor cone) at the needle tip, to a point where the repulsive forces of the charges overcome the surface tension and a jet of polymer is generated that goes to the grounded collector, the solvent is evaporated during the path from the needle tip to the collector. The final morphology of the materials depends not only on polymer molar mass, viscoelasticity, electrical conductivity, and surface tension [27], but also on the voltage applied during electrospinning, the distance from the tip to the collector [28], and the feeding speed [29]. By adjusting these parameters and determining the appropriate values, electrospun micro/nanofibers with desirable morphologies can be obtained.

On the other hand, electrospinning and electrospraying are two electrohydrodynamic processes in which a suspension or solution can be spun or sprayed upon applying a high direct current voltage to generate fibers or particles, respectively. However, when nanostructures have to be incorporated into fibrous materials, it is difficult to uniformly disperse and distribute fillers within the electrospun fibers. In this regard, electrospraying is a flexible and effective method for fabricating nano-/micro-structures composites by spraying a solution in the form of droplets or beads. The simultaneous electrospinning and electrospraying technique is a combined strategy that has typically been used in which fibers are formed by electrospinning, while nanoparticles are deposited on the fiber surfaces through electrospraying.

Despite the versatility and effectiveness of electrospinning, there are still some challenges that need to be addressed, including the accurate and reproducible control of fiber morphology, structure, and uniformity as well as the slow collection speed, the need for a high voltage electric field, the sensitivity in the conductivity of the solution, and the difficulty in manufacturing three-dimensional (3D) structures. There are some strategies that allow for expanding the versatility of electrospinning [30, 31]; however, there is still a need for techniques allowing for easy and fast generation of sub-micron polymeric fibers [32].

A new process called Forcespinning® has been developed to obtain nanofibers from a wide variety of polymers, at a high production speed. This method uses centrifugal force, as opposed to the electrostatic force used in the electrospinning technique. Furthermore, through Forcespinning®, the fiber can be obtained from a polymer solution or from a molten polymer [33]. Conductivity and/or electrostatic charges are not relevant parameters for the properties of the precursor solution; therefore, the spectrum of materials that can be spun is broader [34]. One of the important features of the centrifugal spinning process is its high production speed. The average production rate is 50 g/h and meters per minute on industrial scale systems [34, 35], which is at least two orders of magnitude higher than typical laboratory-scale electrospinning processes. This advantage makes this centrifugal spinning system a low-cost, large-scale approach to deliver practical applications of fiber-based systems [36].

During fiber formation, the polymeric fluid is placed in the spinneret which contains multiple nozzles around the side walls. When the rotation speed reaches a critical value, the centrifugal force overcomes the surface tension of the polymer fluid (polymer solution or molten polymer), causing fine streams of polymer fluid from the tips of the spin head nozzles. The centrifugal force together with the frictional force of the air elongate the liquid thread and lead to the nanofiber formation. The material is deposited on the surface of a collector, forming a non-woven mesh of nanofibers. The polymer concentration and the associated viscosity of the precursory solution, temperature, the rotational speed of the spinneret and the collection distance are parameters influencing the morphology of centrifugally spun fibers.

1.2.2 Design Consideration of Fibrous Scaffolds

For a 3D scaffold construction, the mass transport is an important factor to consider. Oxygen and nutrient delivery, waste removal, cell migration and penetration are some of the processes involved in a natural ECM environment. These processes are associated with the morphological architecture of the scaffold, which is mainly characterized by its pore size, total porosity, pore interconnectivity and surface area [37]. Pore size controls the tissue in-growth and the internal surface area available for cell attachment. If pores are too small for the cell type of interest, they could be blocked by cells preventing cellular penetration, extracellular matrix production and vascularization into the internal region of the scaffold, where diffusion limitations have been one of the mayor obstacles in the production of 3D engineered tissues [38]. There is controversial information about the optimal pore size needed for cell growth and tissue formation. It has been reported optimal sizes between 5 and 15 μm for fibroblast, 20–125 μm for keratinocytes and, 200–400 μm for osteoblasts [39–41]. Additionally, highly porous structures fully interconnected with a large surface-to-area volume ratio are required to allow for cell ingrowth and facilitate neovascularization. An adequate diffusion and enough surface area for cell-polymer matrix interactions have been found in porosity ratios higher than 90%. However, high porosity has a negative effect on the mechanical strength of a scaffold [42, 43]. Therefore, the scaffold design should be optimized to get the maximum total porosity with appropriate mechanical needs based on the particular application.

Moreover, fiber diameter also plays an important role in cell behavior. Nanofiber scaffolds have resulted suitable for enhancing cell differentiation, adhesion, and proliferation [44]. However, the small pore sizes, associated with nanoscale fibrous scaffold, limit cell migration, thus producing a cell monolayer on the scaffold surface. On the other hand, microfiber scaffolds could be potentially advantageous due to their large pore sizes, enabling cellular infiltration and nutrient diffusion. These results suggest that a multiscale scaffold should be ideal to promote an accurate environment for tissue regeneration. Pham et al. [45] developed a bimodal scaffold using a sequential electrospinning technique producing multiscale layers of different morphologies. They evaluated the fiber diameter effect on the cell morphology, observing a larger number of cells (mesenchymal cells) spread throughout the nanofiber membrane. Additionally, the authors obtained a significant reduction in infiltration distance of cells when the nanofiber layer increased compared with other systems. Shalumon et al. [46] produced a 3D PLA multiscale fibrous scaffold using a layer by layer method combining nano- and micro-fibers in an optimum ratio. They found cell (MG63) penetration through the macropores and a well spread by taking advantage of available nanofibers within them. Deepthi et al. [47] designed an electrospun multiscale fibrous scaffold combining aligned and non-aligned microfibers. They used a dual electrospinning setup producing random PCL nanofibers and aligned microfibers at the same time. Their results showed improvement in cell attachment (Rabbit ligament fibroblast) and infiltration within the random microfiber systems.

In the case of composite fibrous materials, the concentration and morphology of micro- or nanoparticles incorporated to the polymeric matrix have to be considered as main factors affecting the morphology and final performance of the obtained composites, which will be addressed in the following sections.

2 Composite Fibrous Scaffolds for Biomedical Applications

2.1 Tailoring the Antimicrobial Properties of Fibrous Polyester Composites

Materials based on polymer fibers have suitable morphological characteristics that make them attractive for applications in the area of tissue engineering since they promote cell adhesion, proliferation, and migration, which are necessary mechanisms for tissue regeneration. However, their morphological characteristics could also promote the adhesion of bacteria (cells of pathogenic microorganisms), eventually leading to the formation of a biofilm.

In this context, composites materials based on polymeric matrices and metal oxide antimicrobial nanoparticles, have been extensively investigated over the last decade. The resulting findings have evidenced the interactions between the composite materials and microorganisms, which have to be circumvented to minimize the adhesion and proliferation of the pathogenic microorganism. As a summary, a sequence of interactions is explained below:

i. Firstly, bacteria are transported near the fibrous material surface by physical forces, such as gravitational and Brownian forces, or by protein-based organelles called flagella;
ii. Then, the attraction between microorganism and fiber surface takes place by physico-chemical interactions (Van der Waals and hydrogen bond) or chemical reactions, which can lead to reversible and irreversible adhesion, respectively;

iii. After anchoring on the material surface, the microorganism synthesizes the biofilm matrix which protects and facilitates its proliferation.
iv. Finally, biofilm maturation is achieved and subsequent detachment occurs.

Considering the aforementioned composite material-microorganism interactions in conjunction with the well-known antimicrobial mechanism of metallic and metal oxide nanoparticles, it could be hypothesized that the antimicrobial activity of fibrous polymeric composites derived from different spinning techniques could be tailored by modifying the morphological features of fibers and distribution/dispersion of antimicrobial agents. For example, Shalumon et al. [48] reported ternary fibrous composites consisting of sodium alginate, poly(vinyl alcohol) (PVA), and several concentrations of ZnO nanoparticles. The ability of the mats to inhibit the growth of both *S. aureus* and *E. coli* strains was proportional to ZnO concentration, and interestingly ZnO did not cause a detrimental effect on the adhesion of fibroblast cells. These materials have proven suitable for wound dressing or cell scaffold applications. Biodegradable and/or biocompatible fibrous mats have been developed utilizing other matrices, such as chitosan (which has an inherent antibacterial activity) [49], cellulose acetate, and PCL containing ZnO nanoparticles which showed excellent antimicrobial activity against *Candida albicans, E. coli*, *S. aureus*, and *Citrobacter freundii* [50]. Augustine et al. [51, 52] found the existence of an optimal ZnO concentration to induce inflammation in animals and inhibit the growth of bacteria; furthermore, in the whole range of ZnO concentration, PCL/ZnO membranes showed an excellent fibroblast attachment, which is very important for the application as a wound healing materials.

The combination of electrohydrodynamic techniques represents a versatile approach for tailoring morphological features and consequently the antimicrobial properties of fibrous composites. For instance, Rodríguez-Tobías et al. [53, 54] investigated the influence of the electrohydrodynamic technique employed and nanoparticles concentration on the antibacterial activity and mechanical performance of biodegradable polyesters (PLA or PHB) fibers containing nano-ZnO. In the first instance, it was evidenced that increasing the ZnO concentration (from 1 to 5%) the viscosity and conductivity of polyester precursory solution remains in the same order of magnitude, thus leading to fibers with similar fiber diameter ranging from 0.7 to 0.8 microns, porosity from 40 to 50%, and pore sizes from 10 to 300 microns. In spite of the homogeneity in morphological features, some aggregates into the fibers and semi-exposed on their surface were detected by SEM/TEM. Figure 1 gathers SEM and TEM images of the PLA electrospun fibers containing 5 wt% ZnO nanoparticles, in which it could be observed a homogeneous fibrous morphology with a wide diameter distribution, and the ZnO aggregates near the fiber surface. Similar trends were also found in the case of the electrospinning of PHB/ZnO solutions, but the formed fibers were thinner due to the lower viscosity and higher conductivity compared with PLA solutions.

Concerning the combination of electrohydrodynamic techniques, it was reported [53–55] the tandem process based on electrospinning of polyester solutions and simultaneous electrospraying of ZnO dispersion with the aim to obtain ZnO-coated fibrous materials and determine its antibacterial activity as a function of nanoparticles

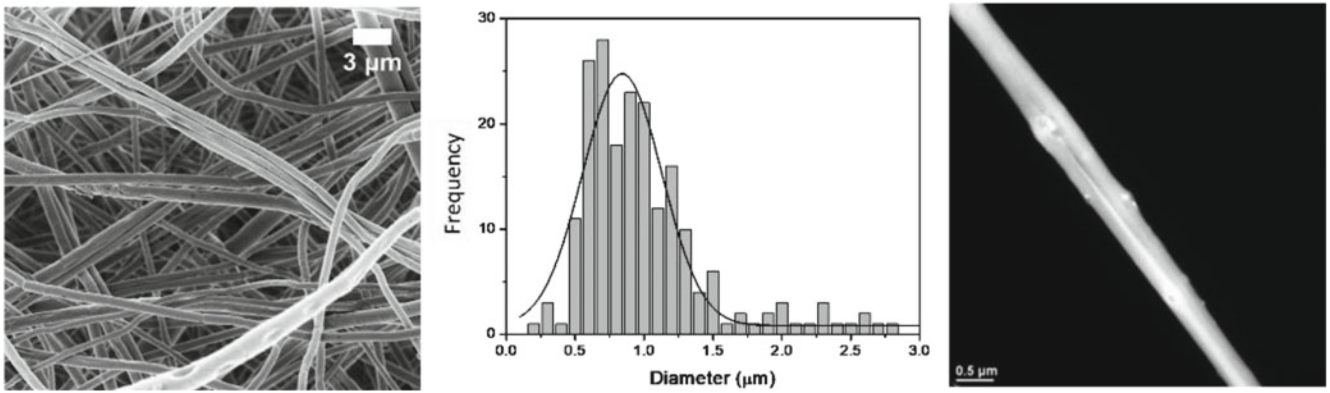

Fig. 1 SEM image of PLA/ZnO electrospun fibers (left), their fiber diameter distribution (center) and TEM image showing the inner and semi-exposed ZnO nanoparticles in one PLA fiber (right). Reprinted from Rodríguez-Tobías et al. [53] Copyright (2014), with permission from Elsevier

concentration. It was found that fiber diameter remains unchanged due to the absence of ZnO nanoparticles in the precursory solutions, and the electrospraying technique leads to a deposition system of ZnO aggregates onto the fibers surface, which ranged from several nanometers to 2–5 microns.

The evaluation of mechanical performance of fibrous composites materials is of paramount importance since they could be subjected to tensile forces not only during their handling but also once exposed to tissues by the corresponding movements. Rodríguez-Tobías et al. [53–55] demonstrated that the mechanical performance of PLA or PHB fibrous composites is dependent on the electrohydrodynamic technique as well as the ZnO concentration. Taking into account the ZnO concentration, both fibrous materials, i.e. electrospun and electrospun/electrosprayed polyesters, exhibited an increase in tensile strength when increasing ZnO concentration up to an optimal concentration of 3 wt%. Higher ZnO concentrations caused a deterioration of mechanical performance probably due to the presence of a high concentration of aggregates, which eventually acts as failure points.

Regarding the main topic of this section, antimicrobial properties of the aforementioned polyester fibrous materials were evaluated against *E. coli* and *S. aureus* strains by immersion tests during 24 h. In general, antibacterial activity was dependent on the type of bacteria, ZnO concentration, chemical nature of the polyester and the electrostatic technique used. Considering the bacterium, *E. coli* is more resistant to the fibers containing different concentrations of ZnO nanoparticles, which has been attributed to the ability of this bacterium to metabolize low concentrations of Zn^{2+} as an oligoelement in conjunction with the nature of its cell wall structure, which is more complex than that presented by *S. aureus*, and their components impart a partial negative charge. These findings have been reported in several research works thus demonstrating the importance to evaluate the fibrous composite materials against several microorganisms to ensure a suitable antimicrobial performance and increase the viability to scale up to medical devices.

On the other hand, the ZnO concentration has an optimal value (3 wt%) above which no longer antibacterial activity is observed. This behavior is associated with the agglomeration trend at high ZnO concentration, thus lowering the surface area prone to attack the bacteria. As previously mentioned, the chemical nature of the polyester can influence the antimicrobial performance of the corresponding fibrous materials since the polyester chain polarity can promote the attraction or repulsion of the bacteria as a function of its cell wall composition. The effect of nanoparticles agglomeration on antimicrobial performance has also been detected for a wide range of polymers and inorganic nanoparticles, so that the research scope for these materials has been to determine the optimal concentration to lead a good balance in mechanical and antimicrobial performance.

Korina et al. [56, 57] investigated the combination of different electrohydrodynamic techniques, thus evidencing that the use of electrospraying and electrospinning leads to the metal oxide-coated fibrous material with high surface area prone to attack more efficiently to microorganisms. In our research works, when electrospinning/electrospraying was used with at least 1 wt% ZnO nanoparticles, values higher than 94% of growth inhibition were achieved. On the contrary, fibrous composites materials derived from mere electrospinning exhibited a moderate antibacterial activity, which could be related to the low degree of ZnO dispersion/exposition and consequently minor interactions between nanoparticles and bacteria.

Recently, Padilla-Gainza et al. [58] have taken advantage of the Forcespinning® technique to obtain polyester/ZnO fibrous materials with potential to suppress *E. coli* and *S. aureus*. The authors evaluated the antibacterial efficiency of PHB- and PLA-based fibrous composites containing ZnO. In the case of PLA, at a concentration of 5 wt%, an inhibition of bacterial growth for both strains exceeded 97%. On the other hand, PHB showed an excellent antibacterial performance against both microorganisms, observing a slightly higher efficiency at a concentration of 1 wt%. It is noteworthy that the presence of ZnO did not affect the osteoblasts viability, thus increasing the potential use of this mats in bone regeneration.

2.2 Tailoring the Bioactive Properties of Fibrous Polyester Composites

In many cases, for the design of polymeric fiber scaffolds, it is necessary to consider the incorporation of substances that would promote cell-scaffold interactions. Scaffold bioactivity in bone tissue regeneration can be increased by incorporating ceramic biomaterials, such as laponite nanosilicates, bioactive glasses, calcium phosphates, biphasic calcium phosphate, among others [59, 60]. Nevertheless, hydroxyapatite (Hap) [$Ca_{10}(PO_4)_6(OH)_2$] is the most commonly used bioactive component, since it is the main inorganic constituent of the bone matrix; specifically, around 69% of the human hard tissue bone is Hap [61]. Hap has a Ca/P molar ratio of 1.67 and mostly made up of a hexagonal crystal structure. The different crystalline structure of Hap allows it to have compositional variations in a biological system. In different reports [62], it has been shown that Hap contributes to important biological processes for the regeneration of bone tissue, such as osteoconduction [50] (bone growth at the superficial level), osteoinduction [63] (pluripotent cells are stimulated to develop bone-forming cells, process that induces osteogenesis), and osteointegration (stable anchoring of an implant, obtained by direct contact between the bone tissue and the implant) [64]. However, the understanding of how Hap stimulates the mechanisms of cell adhesion, proliferation and differentiation is a controversial issue that is still under investigation. Currently, different fibrous hybrid systems based on biopolyesters and Hap have been studied. In this context, it has been reported that these systems offer efficient support for the adhesion, growth and osteogenic differentiation of human mesenchymal stem cells (hMSCs) [65–67], osteoblasts (MG-63) [68], rat bone marrow cells (rBMSCs) [69], and mouse pre-osteoblasts (MC3T3-E1) [70]; properties that have been attributed mainly to the presence of Hap.

As described above, PLA and PHB have received special attention for the design of biomedical materials due their versatility to produce systems with different properties. In Table 1, hybrid fibrous scaffolds based on PLA and PHB with Hap obtained by different spinning techniques are described, with special emphasis on the fiber production technique and their biological performance.

It is interesting to observe the relationship between the fiber diameter and its production technique, even considering its dependence with precursory solution properties and operational conditions (see the Sect. 1.2.1). Among the techniques described in Table 1, the systems with the smallest diameters were obtained with the needle/needle-less electrospinning techniques, which in turn produced narrower fiber size distributions than those obtained with Forcespinning® (see standard deviations). These results have a correlation with the scaffold porosity (space between fibers) and pore sizes. High porosity and standardized pore sizes improve the 3D material performance to be used as scaffolds for tissue regeneration.

Based on the biological performance, it is difficult to make comparison between fiber production techniques, because the results are dependent on type of cells, assay, and experimental design. However, it is clear that the Hap incorporation through electrospraying improves cell adhesion. The Hap covering on the fibers provides a highly rough surface and enables direct and efficient interactions between cells and the bioceramic compound. Therefore, the electrospinning/electrospraying approach showed improved cell performance (Fig. 2) for the systems displayed in Table 1.

Derivatives of the PHA family, including PHB and poly(3-hydroxybutyric acid-*co*-3-hydrovaleric acid) (PHBV), and the three stereochemical forms of PLA, i.e. PLLA, PDLA and PDLLA, have shown favorable results in their interactions with different types of cells. Hybrid systems including Hap concentration between 15 and 50 wt% have been studied with mesenchymal stem and osteoblast cells. It has been demonstrated an inductive effect on the differentiation of h/BMSCs into osteoblasts and the osteogenesis. The differentiation has been confirmed by the increase in alkaline phosphatase (ALP) activity, indicating an early marker relevant in matrix mineralization [71]. Enhanced viability has been found at 20 wt% which was correlated with the high porosity (91%) that could facilitate the diffusive transport of nutrients improving cell viability [65]. On the other hand, filopodia formation from osteoblast cells, specifically MG63, have been promoted at 11 wt% of Hap combined with PHBV polymer matrix [72]. Osteoblast differentiation have been determined by the presence of osteocalcin, obtaining a concentration significantly higher in MG63 cells grown on PLA with 15 wt% Hap than in those grown on pristine PLA. Additionally, at this Hap concentration, it was found an increased performance in cell growth and viability. A combined effect between an adequate Hap concentration content in a 3D structure with wide distribution of fiber diameters allows for an efficient cell interaction with the scaffold at 15 wt% system [69].

Concerning the Forcespinning® technique, a couple of studies are described in Table 1. Padilla et al. [73] reported the design and characterization of PDLLA and PHB scaffolds and their performance interacting with human osteoblast-like cells (Saos-2) to evaluate their potential in bone tissue regeneration applications. The biological performance was evaluated for both polymers at 5 wt% of Hap. The results

Table 1 Main characteristics of hybrid fibrous scaffolds based on PLLA, PDLLA, PHB or PHBV with Hap nanoparticles: technique, Hap concentration ($\mathbf{C_{Hap}}$), average fiber diameter ($\overline{\boldsymbol{D_f}}$), pore size, porosity (**Ø**, inter-fiber space) and biological performance

System	Technique	C_{HAP} (wt%)	$\overline{D_f}$ (μm)	Pore size (μm)	Ø (%)	Biological performance	Reference
PHB/Hap (blend)	Electrospinning	0	950 ± 160	5–15[a]	61[a]	*MTS assay* Cell: hMSC – PHB/Hap (spray) shown a higher cell adhesion after 1 h cell-scaffold interaction than PHP/Hap (blend)	[56]
		15	640 ± 80	1–7	61		
PHB/Hap (spray)	Electrospinning/ electrospraying	15	950 ± 70	2–11[a]	77[a]		
PLLA/Hap	Electrospinning	0	770 ± 350	NR	80 ± 3[a]	*MTS assay and ALP activity* Cell: hMSCs – induce osteogenic and osteoclastogenic differentiation of human mesenchymal stem cells	[55]
		20	840 ± 330		91 ± 2		
		50	1030 ± 390		70 ± 4		
PHB/Hap	Gas-jet/ electrospinning	10	NR	NR	NR	*MTS assay* Cell: BMSC – good support for the attachment, proliferation and differentiation	[57]

(continued)

Table 1 (continued)

System	Technique	C_{HAP} (wt%)	$\overline{D_f}$ (µm)	Pore size (µm)	Ø (%)	Biological performance	Reference
PLLA/Hap	Needle-less electrospinning	0	160–340	NR	NR	*Cell viability (live/dead viability kit) and osteogenic cell differentiation* Cell: MG63 – no considerable cytotoxic effect in human osteoblast-likeMG63. 15 wt% of Hap presented a higher potential for tissue engineering application	[59]
		5	287–545				
		15	242–729				
PHBV/Hap	Electrospinning	0	2920 ± 280	NR	NR	*Resazurin assay* Cell: MG63 – Hap promoted the filopodia formation increasing the specific cell activities	[62]
		11	3760 ± 370				
PLLA/Hap	Forcespinning®	0	1030 ± 779	NR	NR	*Resazurin assay* Cell: Saos-2 – PDLLA systems promoted higher cellular response than those formulated with PHB	[63]
		5	1890 ± 1050		95.6 ± 0.1^b		
		10	–				
		15	–				
PHB/Hap		0	1720 ± 550	5 to 200^c	NR		
		5	1920 ± 710		92.9 ± 0.1^b		
		10	1820 ± 830		94.4 ± 0.4		
		15	1890 ± 680		96.2 ± 0.5		

(continued)

Table 1 (continued)

System	Technique	C_{HAP} (wt%)	$\overline{D_f}$ (μm)	Pore size (μm)	Ø (%)	Biological performance	Reference
PDLLA/Hap/ZnO	Forcespinning®	5	1840 ± 1030	2 to 2561.7[c]	92.7 ± 0.16[b]	*Resazurin assay, proliferation and migration study* Cell: MC3T3 – PDLLA scaffold promoted a favourable cell response, with viabilities above 80% – PDLLA-ZnO%-Hap 5% scaffold promoted favourable cell morphology – scaffold design promoted a sustained increase in cell proliferation and migration into the 3D structure	[64]

MTT assay, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide: it is based on reduction of the MTS tetrazolium by viable mammalian cells to generate a colored formazan dye that is insoluble in cell culture media

MTS assay, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium): it is based on reduction of the MTS tetrazolium by viable mammalian cells to generate a colored formazan dye that is soluble in cell culture media

Resazurin assay, is based on the reduction of oxidized non-fluorescent blue resazurin to a red fluorescent dye (resorufin) by the mitochondrial respiratory chain in live cells

NR: not reported

[a] Mercury intrusion porosimetry

[b] $\emptyset = \left(1 - \frac{m}{Z*A*H*\rho}\right) * 100$ where m, Z, A, H, and ρ stand for the mass, thickness, width, length of the scaffold and the density of the polymer

[c] Pore area in μm^2, as determined by the *Image J* software

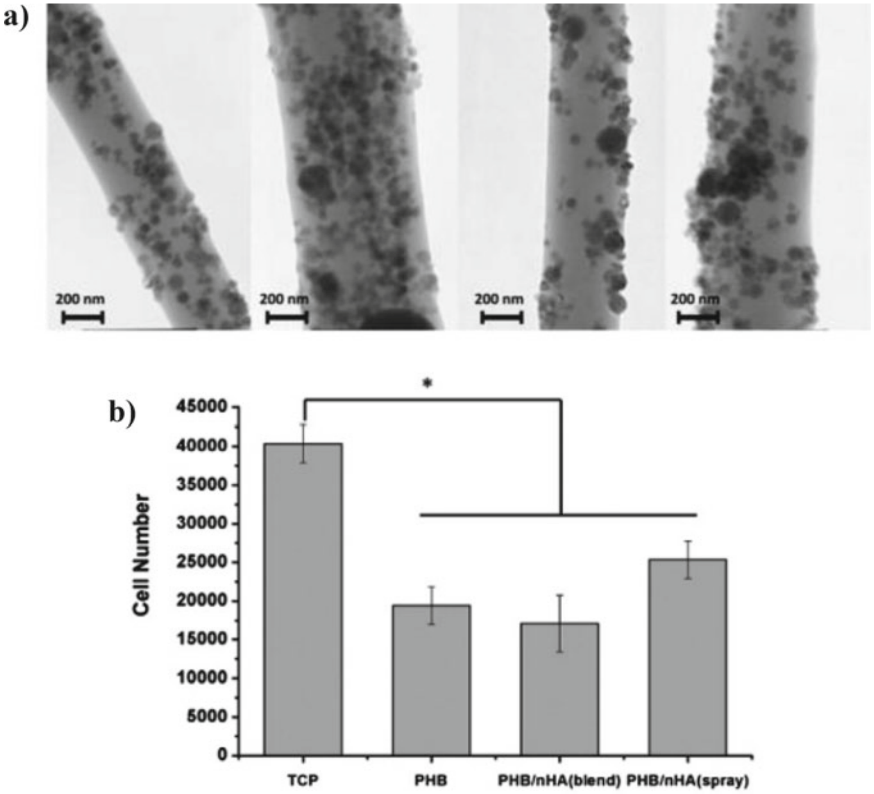

Fig. 2 PHB/Hap scaffolds at 15 wt% of Hap **a** TEM images of single fibers from blend (left side) and spray (right side) and **b** MTS assay of hMSC after 1 h of incubation on fibrous scaffolds. Bars represent mean values ± standard deviations (n = 4). Asterisks indicate significant difference obtained by t-test ($p \leq 0.05$). Reprinted from Ramier et al. [66] Copyright (2014), with permission from Elsevier

showed improved cellular response for PDLLA with a cell viability higher than 60%, where the PDLLA-Hap5% system promoted an increase of 10% in cell viability test by day 7 of cell interaction. This behavior could be attributed to the accessibility to the Hap nanoparticles (NPs) within the fibers because most of them were embedded in the PDLLA (Fig. 3f), avoiding the NPs-cells interactions for the first and third days of study. The fiber surface erosion (hydrolytic degradation) could produce the exposition of greater number of NPs, resulting in an increased cell metabolic activity by day 7. Additionally, the surface roughness of the final PDLLA fibers (Fig. 3b) could have played an important role in the different biological performance observed in the PHB fibers.

Padilla-Gainza et al. [74] reported an in-depth biological study of a bioactive (Hap) and antibacterial (ZnO) PDLLA scaffolds with potential applications for bone

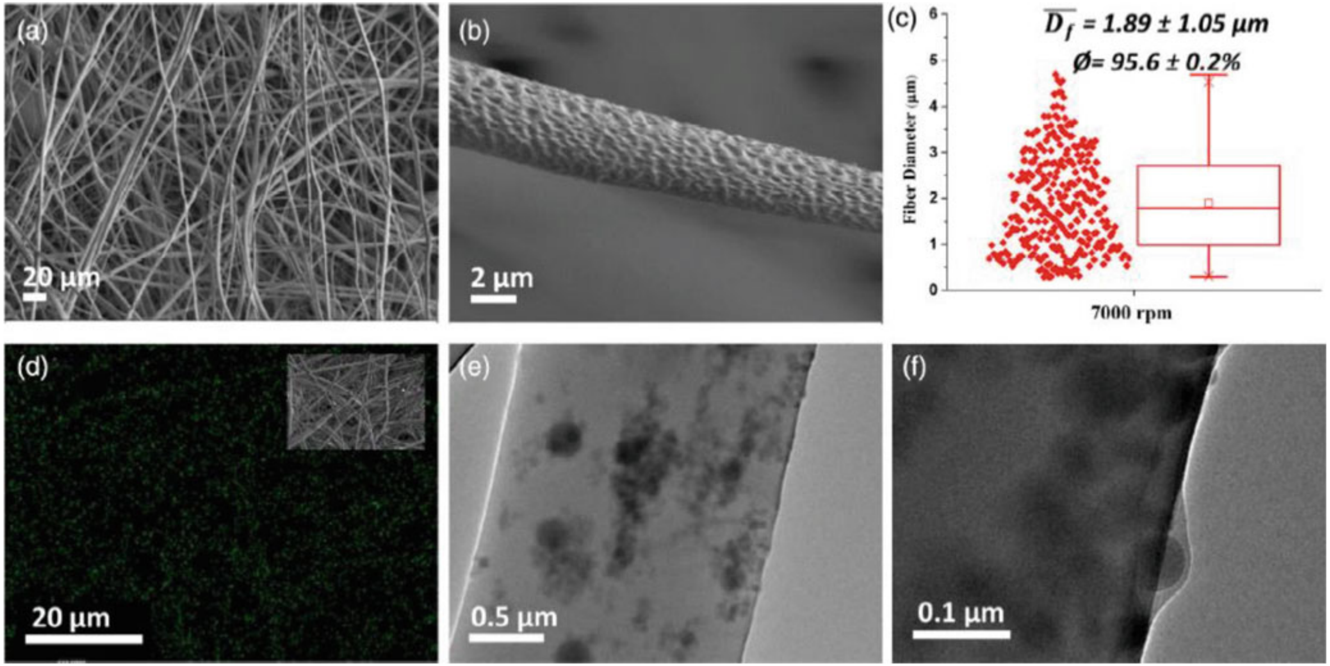

Fig. 3 SEM images (**a**, **b**, and **d**), TEM (**f**, **g**), box chart of fiber diameter (**c**) and mapping of phosphorous element obtained through EDS of PDLLA-Hap 5% system produced at ω 7000 rpm, $\overline{\boldsymbol{D}_f}$ and Ø are the average fiber diameter and porosity, respectively. Reprinted from Padilla et al. [74] Copyright (2021), with permission from Elsevier

regeneration. It is important to highlight that the antibacterial evaluation, optimization process, and cell viability study [73] previously reported by our research team defined the appropriate ZnO and Hap concentrations used for the design of this dual (bioactive/antibacterial) hybrid scaffold. The idea to combine ZnO in the final material was to diminish the risk to promote the adhesion and growth of pathogenic microorganisms. ZnO has demonstrated a selective toxicity for bacteria but exhibits minimal effect on human cells [75]. In addition, it has been proven that it exerts a positive osteoconductive and osteoinductive effect in mesenchymal cells [76]. The results showed an increased cell viability, above 80%, over the test course of days 1, 3, 5 and 7, which could be consistent with increased cell number. From day 5, the PDLLA scaffold showed viabilities above 100% with respect to the positive control, thus suggesting that cells in the positive control were near confluence. In the case of fibrous scaffolds, due to a higher surface area and adequate pore size, cells continuously proliferated during the tested period. For day 7, mitochondria morphology and proliferation studies were further addressed. All the scaffolds promoted the growth of MC3T3-E1 cells in clusters within the nanofiber matrix. Additionally, the mitochondria morphology showed an interconnected network, representing an efficient system to deliver energy or transfer calcium between different areas of cells (Fig. 4). Complementary osteoblast cells morphology was also studied by labeling actin cytoskeletal filaments (Phalloidin) and nucleus (DAPI). It is clearly observed in Fig. 5, that MC3T3-E1 cells lay on the fibers and surround them, while extending in the direction of the fiber. The systems revealed normal cell morphology and no signs of necrosis/apoptosis, showing widely spread and spindle-like morphology for blank and PDLLA-ZnO 5%-Hap 5%, respectively. As it could be observed in Fig. 5b, cells were completely in contact with the fibers and some filopodia-like extensions were evidenced coming up from the cells towards the fibers (see the arrows), indicating favorable cell-fiber interactions. Regarding cell migration (Fig. 6), cells were found at different depth levels of the scaffold, finding cells down to 37 μm depth from the surface. This behavior showed biocompatibility since cells were able to grow embedded within the scaffolds. These results suggest that the scaffold could promote the exchange of nutrients at different depths given the developed porosity, a feature that is desirable in biocompatible materials. This demonstrates a favorable design of the scaffold, as a result of its chemical nature, surface porosity, porosity (inter-fiber space) and fiber/pore size distribution.

3 Conclusion

In this chapter, important aspects about the design of polymeric fibrous composite materials for biological applications were addressed. Fiber size and their distribution, porosity (inter-fiber space), surface morphology, type of polymer matrix and the incorporation of agents in accurate concentration to prevent bacterial growth and improve cell interaction are crucial factors to design promising 3D fibrous structures for tissue engineering applications. Electrospinning and centrifugal spinning have

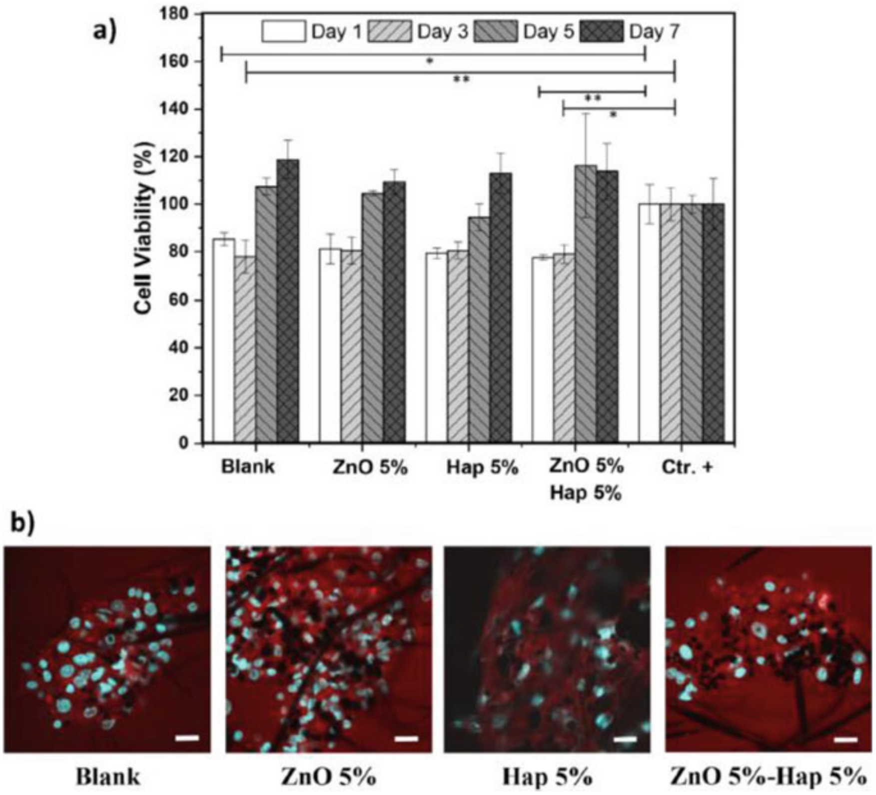

Fig. 4 **a** Resazurin-MC3T3-E1 cell viability test; Ctr.+: positive control (cells grown on glass coverslips) was taken as reference to determine the cell viability of the systems. Blank is defined by cell interacting with a PDLLA fiber without NPs. The results are presented as the mean of three experiments ± the standard deviation of the mean (*$p < 0.05$, **$p < 0.01$). **b** MC3T3-E1 Confocal micrographs (mitochondria are labeled with Mitotracker (red), while DAPI labels cell nuclei (blue)) obtained at day 7 of scaffold-cell interaction, after being subject to the cell viability test (scale bar: 20 µm). Reprinted from Padilla et al. [74] Copyright (2021), with permission from Elsevier

been the most extensive technologies used to produce fibrous scaffolds. Particularly, PHB and PLA based fibrous scaffolds have been widely used in medical applications, and their combination with ZnO and/or Hap have produced promising antibacterial and/or bioactive alternatives for wound healing, and bone tissue regeneration.

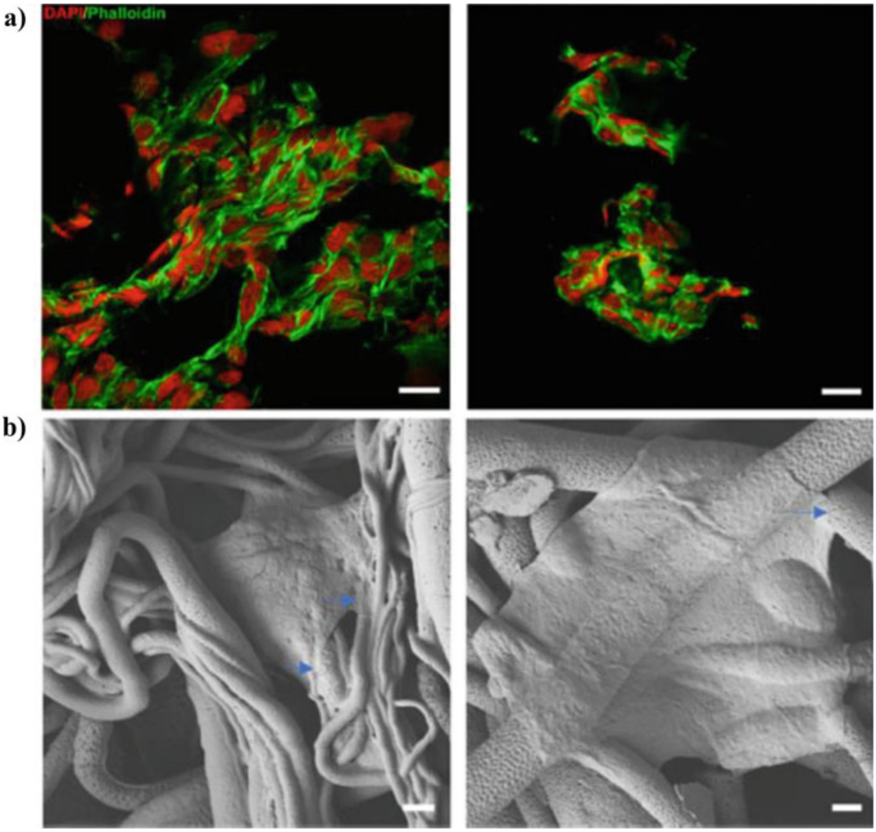

Fig. 5 Osteoblast cells (MC3T3-E1) morphology observed in PDLLA fiber scaffolds obtained through confocal microscopy, cytoskeletal filaments (green) and nucleus (blue): **a** Cell clusters found in day 5 (PDLLA blank) and 7 (PDLLA-ZnO 5%-Hap 5%) of the scaffold-cell interaction test (scale bar: 20 μm), and **b** SEM images of the osteoblast cells interacting with the fibers from PDLLA blank and PDLLA-ZnO 5%-Hap 5%, left and right side, respectively (day 7) (scale bar: 2 μm). Reprinted from Padilla et al. [74] Copyright (2021), with permission from Elsevier

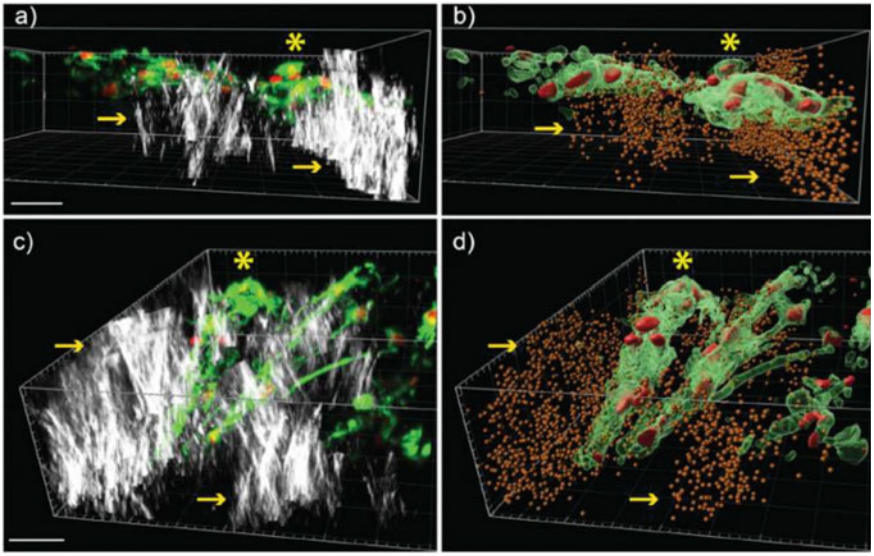

Fig. 6 MC3T3-E1 cell growth embedded in PDLLA-ZnO 5%-Hap 5% fiber scaffolds in three-dimensions. Lateral and upper view of fluorescence confocal Z-stacks (**a** and **c**, respectively) showing the cell cytoplasm (green) and nucleus (red) of MC3T3-E1 cells (yellow asterisks) into the PDLLA-ZnO 5%-Hap 5% fiber scaffolds (white), pointed by yellow arrows, for day 7 of cell-scaffold interaction. Imaris three-dimensional reconstruction of the fluorescent signal for cytoplasm, nucleus, and fiber scaffolds (represented as orange dots for better viewing) are shown in (**b**) and (**d**). Scale bar for all figures is 30 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article). Reprinted from Padilla et al. [74] Copyright (2021), with permission from Elsevier

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Nanotechnology Applied to the Management of Fungal Diseases in Tropical Crops

C. Pabón-Mora, E. Quevedo, and R. Ortega-Toro

Abstract Nanotechnology offers solutions in the agricultural area through the application of biocontrollers. The application of biocontrol agents has been considered as an option for the sustainability of agriculture and the control of fungal diseases. Thus, the main objective of the study is to present the recent advances in research and development of nanotechnology in the management of fungal diseases that are applied in tropical crops. In this context, the main related fungal diseases in crops, control mechanisms, as well as Nanobiocontrollers and their future trends have been investigated. The deterioration and greater losses of the fruits in tropical climates are of microbial origin and phytopathogenic fungi, bacteria, insects, parasites, among others, cause sensory deterioration such as dark spots, decreasing the commercial value. Among the traditional methods, the use of insecticides, fungicides, nematicides, herbicides, plant growth regulators, good agricultural and manufacturing practices, but cause harm to both human health and the environment. Therefore, natural enemies have been used by means of biomolecules extracted from plants, fungi, bacteria that have a prolonged action and/or desirable properties for cultivation. The nanoscale bio controller has huge prospects for increased agricultural productivity and disease control in tropical crops.

Keywords Agricultural · Bio-controller · Nanoscale · Anthracnose · Fungicides

1 Introduction

Currently, the agricultural sector faces challenges related to climate change, low productivity, decreased soil organic matter, low nutrient use efficiency, macro and

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micronutrient deficiency, reduction of agricultural land and water availability, pollution environment due to the increase in pesticide residues and the shortage of skilled labour. These factors are affected by the growing demand for food at a rate of 4%, which is needed to support a world population of 9 billion by 2050 [1].

The agricultural sector has received importance for the control and management of crops [2, 3]. Thus, nanotechnology in the agricultural sector can range from nanocide-pesticide delivery, stabilization of green pesticides and biopesticides using nanomaterials (NM), slow and controlled release of micronutrients assisted by NM, fertilizers and biofertilizers, nanomaterials in transport of genetic materials for the development of crops, nano-biosensors for the rapid and selective detection of plant pathogens, fungal diseases and pesticides, etc. [1, 4, 5].

Therefore, there is an urgent need to explore the use of nanotechnology to improve the food and agricultural system [6, 7]. Consequently, the nanobiotechnology-enforced farming system offers minimal agricultural inputs for optimal food production and minimizes agricultural waste before reaching the consumer level [8]. Similarly, the scope of nanotechnology in agriculture ranges from intelligent supply systems, providing nutrients to the soil through nano-fertilizers that strengthen the growth and quality of the fruits. Other aspects of implementing nanotechnology in agriculture is to strengthen the same system of plants that have been subjected to adverse conditions, that is, the response to stress is positive, not only does it improve growth, but it also increases the bioavailability of some elements. On the other hand, the inclusion of nanotechnology to manage and optimize the amount of pesticides is a good solution, to control the amount in controlled doses generating nano-pesticides. Another implementation of nanotechnology in agriculture [9].

In particular, nanotechnology allows the development of fungicides and agricultural practices for fungal diseases, taking in to account that from nanoparticles in the form of spraying and soaking from soil to plants, it can be used to control a series of fungal diseases. In this way, nanoparticles may be a future solution for the control of fungal diseases, due to their large surface/volume ratio and their bactericidal, fungicidal and anticancer properties [10]. Below are shown the recent advances in research and development of nanotechnology in the management of fungal diseases that are applied in tropical crops.

2 Main Fungal Diseases of Tropical Crops

Studies reveal that there are more than 19,000 fungi that cause diseases in crop plants worldwide, which can persist in both living and dead plant tissues, depending on the conditions for their proliferation. Table 1 summarizes the main fungal diseases present in tropical crops. It is important to mention that fungal leaf diseases can be controlled if they are detected and correctly identified in a timely manner [11]. Crops are susceptible to climatic changes that depend on factors such as temperature, increases in CO_2 and changes in rainfall. In this way, the conditions and environments that surround them can favor the proliferation of a large number of microorganisms, among the most important are the phytopathogens that cause pests, including viruses, parasites, bacteria, among others, and in tropical climates, are responsible for significant economic losses [12].

As can be seen in Table 1, there are multiple variations of fungal diseases caused that depend specifically on the type of crop, region, climatic conditions, etc.

Other studies determined that the fruits of guarana (*Paullinia cupana* var. *Sorbilis*), which belong to a tropical crop from the Amazonian forest of economic importance in Brazil as it is used in the production of soft drinks (up to 70% of national production), ice cream, juices, syrups, stimulants and medications, are highly affected by *Colletotrichum* spp. causing anthracnose, so further research is required as it is little studied [29]. Likewise, the economic, social and environmental impact caused by fungal diseases in cocoa have estimated losses of around 20–30% of the global yield, due to the *Phytophthora* spp., research focuses on effective measures to counteract the attack caused by phytopathogenic fungi [28].

3 Traditional Mechanisms to Control Fungal Diseases of Tropical Crops

Mainly, preventive measures have been used in crops that consist of the application of intensive cleaning during cultivation and at the end of the crop cycle, together with the application of selective active ingredients with proven fungicidal action [30]. Figure 1 shows the traditional methods for mitigating fungal diseases applied to tropical crops, both the use of chemical and natural substances.

In this sense, integrated pest management (IPM) has been used in field crops since the mid-twentieth century, but prophylactic management strategies have recently been progressively adopted, including the systemic neonicotinoid insecticides imidacloprid, clothianidin or thiamethoxam, generally in combination with fungicides such as mefenoxam and fludioxonil [31]. On the other hand, the control of fungal diseases is based on the use of fungicides, taking in to account that pesticides can be classified as insecticides, fungicides, nematicides, herbicides and plant growth regulators [32]. However, farmers in the tropics face situations of care in the application of pesticides and management strategies as it can cause food insecurity and loss of income, adverse effects on human and environmental health, export restrictions due to pests, quarantine diseases and maximum residue limits for pesticides [33].

As is the case, the use of fungicides is the most widely used method of controlling maize foliar diseases as active ingredient the group of carboxamides, triazoles, dithiocarbamates, benzimidazoles and strobilurins [34]. However, it has dangerous effects on humans as well as animals by precipitating into plant tissues and then transferring them to humans and animals causing many health complications [35]. In particular, in natural tropical ecosystems, termites play a fundamental role in citrus, since around 10% have the potential to become pests in agricultural systems. Therefore, among the control strategies is the excessive use of chemical products initially

Table 1 Main fungal diseases associated with various crops

Associated fungal diseases	Tropical crops (scientific name)	Authors
Southern corn blight (*Puccinia sorghi; P. polysora*) Northern leaf blight (*Exserohilum turcicum*) Gray leaf spot (*Cercospora zeae-maydis*) Corn white spot (*Pantoea ananatis*) Diplodia leaf spot (*Stenocarpella macrospora; S. maydis*) Anthracnose (*Colletotrichum graminicola*) Corn ear rot (*Fusarium verticillioides*) *Fusarium* head blight (*Gibberella zeae*)	Corn cultivation (*Zea mays*)	[13]
Alternaria spp. or *Aspergillus* spp.	Pomegranate (*Punica Granatum*)	[14]
Fusariosis (*Fusarium guttiforme*)	Pineapple (*Pineapple comosus*)	[15]
Alternaria alternata, Colletotrichum gloeosporioides, Colletotrichum capsici, Phyllosticta zingiberi, Cercospora curcumae, Rhizoctonia solani, Pythium aphanidermatum, Fusarium solani	The leaves and stems of mango ginger plants (*Curcuma amada*)	[16]
Anthracnose caused by *Colletotrichum gloeosporioides* *Aureobasidium melanogenum, F. concentricum, F. hainanense, F. mangiferae, F. pernambucanum, F. proliferatum, F. sulawesiense and F. verticillioides are capable of causing leaf spot symptoms on artificially injured leaves*	Mango (*Mangifera indica* L.)	[17–20]
Cordana leaf spot caused by the fungus *Cordana musae*, *Deightoneilla* leaf spot caused by the fungus *Deightoneilla torulosa.* Sigatoka leaf spot caused by the fungus *Mycosphaerella musicola* Banana wilt disease, caused by *Fusarium oxysporum* f. sp. cubense Tropical Race 4 (Foc TR4)	Banana (*Musa paradisiaca*)	[21–25]

(continued)

Table 1 (continued)

Associated fungal diseases	Tropical crops (scientific name)	Authors
Green mold (GM), blue mold (BM) and acid rot (SR), caused by *Penicillium digitatum* (Pers Fr.) Sacc, *Penicillium italicum* Wehmer and *Geotrichum citri-aurantii* (Ferraris) Postharvest decay caused by the fungi *Penicillium digitatum, Penicillium italicum, Geotrichum citri-aurantii, Aspergillus niger, and Aspergillus flavus*	Citrus species (*Citrus* spp.)	[26, 27]
Black ear disease, caused by various species of *Phytophthora* spp.	Cocoa (*Theobroma cacao* L.)	[28]

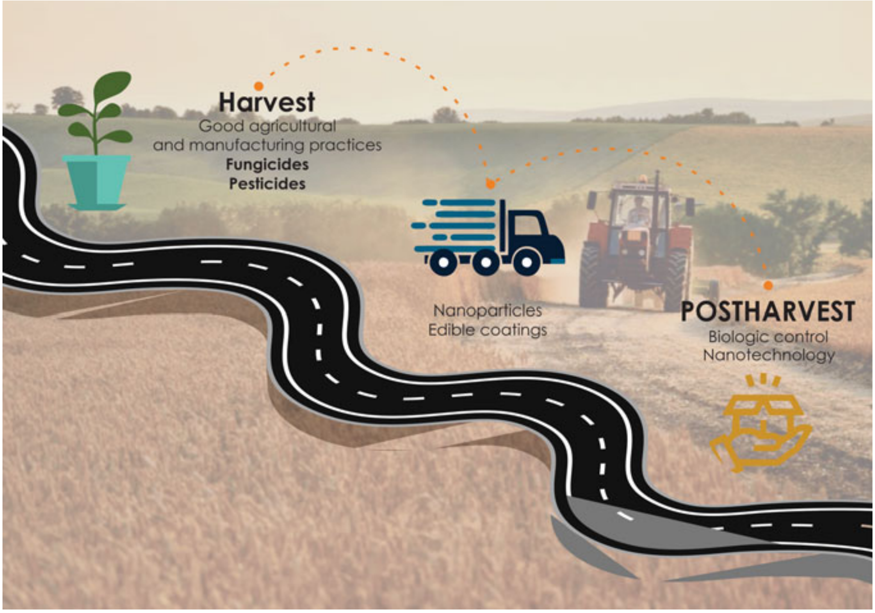

Fig. 1 Control mechanisms against pathogenic fungi in the harvest and post-harvest stage

of powerful and persistent organochlorine insecticides such as dieldrin and aldrin. Later, with some success, carbamates (aldicarb, carbosulfan), chlorinated phenols (pentachlorophenol) and organophosphates (malathion, chlorpyrifos, dichlorvos) were used to replace them. Commonly, these techniques consist of repeated spraying of pesticides on the ground, causing massive destruction of species and gradual deterioration of the environment. Among the cultural practices without chemical means, agricultural practices are suggested, especially those related to the care of plants to prevent damage related to termites [36].

The *Zingiber officinale Rosc* is a tropical plant, known as ginger, it is cultivated in different parts of the world from China to Hawaii, it has great economic importance for its medicinal and culinary properties. This crop is attacked by rhizome rot, an important disease of ginger, generally caused by *Pythium* spp. Studies demonstrate that frequent handling of chemical fungicides resulted in resistance in pathogenic fungi to fungicides and also leads to reduced soil fertility. Therefore, the use of biological control agents such as Trichoderma harzianum and plant extracts is suggested as an alternative to chemical fungicides [37–39]. Another study by Rodrigues et al. [40] in which they evaluated current technologies for the control of postharvest fungal diseases of papaya (*Carica papaya* L.), a climacteric fruit with a rapid, year-round growth cycle that grows in tropical and subtropical climates, and losses due to fungal diseases are estimated at 50% of production volumes. For the control of the disease, they determined that the best treatment is the combined method (thermal and fungicide) in stages of storage and transport under refrigeration, it helps to delay the maturation and growth of phytopathogens.

However, papayas kept at room temperature, the method is not enough. In this way, although fungicides are efficient, the possible effects for the environment and human health when using fungicides are negative, therefore, Mbarga et al. [28], improved the biological management for the black cocoa pod disease caused by the phytopathogen *Phytophthora megakarya*, using *Trichoderma asperellum* as a control agent, which was emulsified with oil, obtaining a product that remains for more time on the cob; the tests were carried out under laboratory conditions and in experimental plots. With the tests, they obtained a new bioformulation that overcomes the environmental barriers and prolongs the application of the biocontrol for a longer time, reflected in much healthier ears compared to those exempts from the biocontroller.

4 Nanobiocontrollers

Given that nanotechnology can offer promising applications in the agricultural area, providing a solution to the indiscriminate and cumulative use in the environment of agrochemicals such as: herbicides, fungicides, insecticides and pesticides, which not only affect human health, but also the development of resistance in pathogens, requiring greater quantity for their control [41–43].

In this way, biocontrollers have been used, also called biopesticides, which refers to the reduction of insects, parasites and/or pathogens to a desired level using natural enemies, including some pathogens that stimulate plant growth [42]. That is why products of natural or synthetic origin (biocontrollers) are currently available on the market, from a nanoscale called nanobiocontrollers and resistance inducers (synthetics) that stop or delay the entry of the pathogen into the crop plant, limiting its activity in the infected plant tissue or organ, without having a direct effect or specific activity on phytopathogens [44].

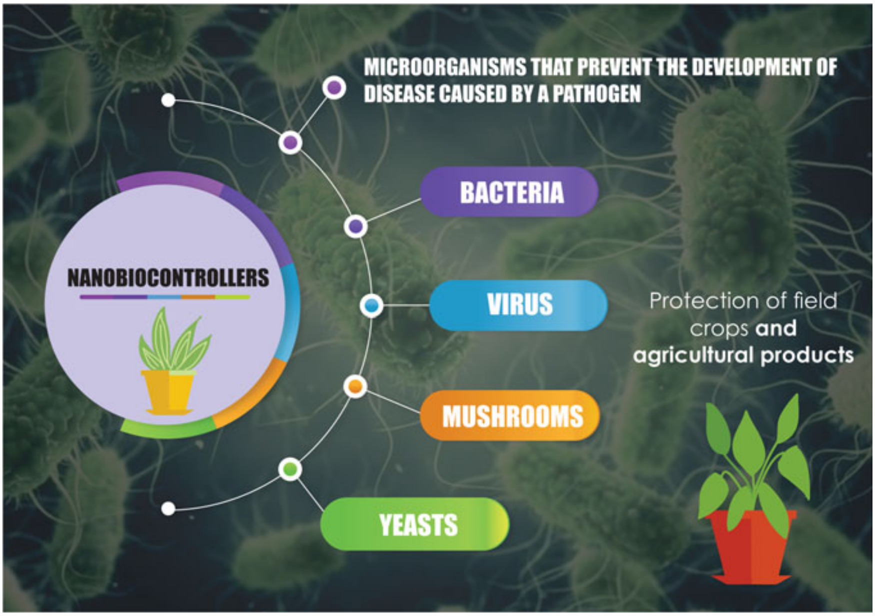

Fig. 2 Application of nanotechnology in the development of nanobiocontrollers for crop protection

Thus, Fig. 2 shows that the Biocontrollers prevent phytopathogens (bacteria, fungi, nematodes or viruses) from causing damage to the host plant through the production of acylases or lactonases, antibiotics, toxins and other molecules, such as hydrocyanic acid [45–47].

Biological control mechanisms act as systemic induced responses. Thus, protection is produced by molecular signals induced by microorganisms that activate the basal plant defense system, culminating in the comprehensive protection of the plant against the action of pathogens [48]. Due to the above, it is necessary to deepen the research on biopesticides since studies affirm that its market grows annually at a rate of 45% in North America, 25% in Europe and Oceania, 15% in Latin American countries Latin America and the South and 7% in Asia in fungal and bacterial biopesticides [49].

Likewise, nanotechnology has been chosen to improve the stability and efficacy of biopesticides through the encapsulation of biomolecules extracted from different sources (plants, fungi and/or bacteria) with a prolonged action or biomaterials synthesized by biogenic processes, generating nanobiocontrollers, biocompatible and more efficient [50–52].

4.1 Nano-Biocontrollers Most Used in Fungal Diseases

Nanocomposites-biocontrollers moderate soil productivity, nitrogen fixation and phosphorus solubilization by containing living beings and certain strains in their formulation, necessary for the synthesis of substances that regulate plant growth [53].

There are multiple applications of nano-biocontrollers, but they can mainly be found for biological control method of conservation and increase of useful life. For the first case, studies carried out by Wang et al. [54] indicate that gray mold in strawberry storage causes considerable post-harvest losses, reducing the texture and quality of this unique fruit. Therefore, an antagonistic agent, Bacillus halotolerant, was used as a control, not only reduced gray mold, but also induced defense enzymes in strawberries inoculated with the control agent compared to control strawberries, exempt from the biocontrol without altering their quality. Among the most frequent nano-biocontrollers are the rhizobacteria, responsible in produce metabolites that induce systemic resistance in plants and, in turn, inhibit the growth of pathogens [55]. The rhizobacteria fulfill different ecological functions in their natural environment due to forming symbiotic relationships with other plants. Some are detrimental to plant growth and health, while others are beneficial, acting as biofertilizers and phytostimulators that promote plant growth, as well as controllers of plant pathogens (biocontrollers). The control mechanism of biocontrollers depends on the production and excretion of secondary metabolites, which are numerous and diverse and include, among others, antibiotics, siderophores, semiochemical inhibitors and/or inducers of the induced systemic resistance of a plant. The ability of rhizobacteria in the agricultural area to develop efficient biopesticides is highlighted [56]. Likewise, authors state that a nano-biofertilizer or nano-biocontroller is a conglomerate of plant growth-promoting rhizobacteria together with coatings of silver (Au) nanomaterials [57].

Bacillus subtilis belongs to one of the most studied plant growths promoting rhizobacteria as it has the ability to promote plant growth and control plant pathogens by several mechanisms, reflecting improved nutrient availability and altered homeostasis of phytohormones, as well as the production of antimicrobials and the activation of induced systemic resistance, respectively. It is important to mention that it has been extensively studied under laboratory conditions and can form resistant spores, drawing attention to a promising target for agricultural application, but it still needs to be studied in field applications with the difficulty that the bacteria cannot persist in the rhizosphere [58, 59]. Table 2 reviews some biocontrol agents used against fungal diseases.

In Table 2, it is found that the mentioned applications improve the useful life of the crop. Ram et al. [65] mention that biocontrollers also known as biofertilizers, biopesticides and plant enhancers not only control plant diseases, but also support plant growth, improve plant tolerance against stress, develop resistance in plants and contribute to the acquisition of nutrients. This study suggests that they can be used to suppress pathogens that infect various cereals, fruits, flowers, legumes

Table 2 Review of nano-biocontrol agents used as resistance to fungal diseases

Nano-Biocontrollers	Application in fungal diseases	Results	Authors
Native strains of *Trichoderma harzianum*	Control tomato wilt disease (*Solanum lycopersicum* L.) caused *by F. oxysporum* wilt	Tomato wilting was reduced between 10 to 30%. Among the 12 strains that reduced the growth of phytopathogens, concentrations between 13.38 and 21.14 μg/ml of the strains: FCCT 16, FCCT 58, FCCT 199–2 and FCCT 363–2, exhibited the highest auxin production indole 3-acetic acid	[60]
13 novel strains of *Trichoderma*	Biological control against Fusarium strains	Ten strains of *T. asperellum* (10), 2 strains of *T. harzianum* (2) and one as *T. longibrachiatum* were identified *Trichoderma*presented an average of 0.1207 cm h^{-1} in growth rate compared to *Fusarium* spp., which was lower (0.031 cm h^{-1}). The antibiosis tests with the best level of antagonistic efficacy were for *T. asperellum* UCP 0149 versus *F. solani* UCP 1395 (82.2%) and *F. solani* UCP 1075 (70.0%)	[61]
M. pulcherrima yeast and chitosan	Biocontrol of postharvest Alternaria rot in table grapes	The best biocontrol agent in in vivo postharvest trials against *A. alternata* was chitosan, it produced incidence rates in prolonged cold storage than those obtained with the traditional control (SO_2). And in the marketing stage, the yeast *M. pulcherrima* (RCM2) caused a reduction in the incidence of the disease	[62]

(continued)

Table 2 (continued)

Nano-Biocontrollers	Application in fungal diseases	Results	Authors
Active compounds of medicinal plants	Control of phytopathogenic fungi of postharvest fruits	*Solidago chilensis Meyen., Asteraceae, Drimys winteri JR Forst. & G.Forst., Winteraceae and Polygonum stelligerum Cham., Polygonaceae*s howed high inhibitory antifungal properties	[63]
Antagonists *Chaetomium globosum*and *Trichoderma harzanium*	Biocontrol of fungal endophytes	Endophytic fungi were isolated from the inflorescence of A. vera., which demonstrated functional properties such as growth promoter and antifungal activity	[64]

and ornamental plants. Pérez et al. [66] reported some killer yeasts as biological control agents of postharvest fungal diseases of lemons, especially for “green mold” and “blue mold”, which are caused by *Penicillium digitatum* and *P. italicum*. The results showed that the inhibition in the development of in lemons was due to the *Kazachstania exigua* and *Saccharomyces cerevisiae* strains. In turn, the Kazachstania exigua strain also demonstrated antimicrobial effects against *Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa.* On the other hand, they suggest that *Candida guilliermondii* and *Debaryomyces hansenii* were active against *P. digitatum* on grapefruit.

4.2 Synthesis of Nano-Biocontrollers

The biological control of fungal diseases in crops ensures the use of nanotechnology through the use of nano-biocontrol agents for the functioning of ecosystem services and productive agroecosystems. Figure 3 shows the method of foliar spraying with natural compounds as protection against pathogenic diseases. Unlike the spraying of synthetic chemical pesticides that are sprayed a few days before harvest or after harvest and therefore could be a source of danger to consumers [67]. In fruits such as the medlar, [68] evaluated the efficacy of foliar applications before harvest by spraying with acetylsalicylic acid, spermidine and calcium, with the aim of exploring the potential beneficial effect on the qualitative attributes of the medlar fruit. In the results, it was found that the quality parameters of the medlar fruit were affected by the spray treatments, the day of harvest and the periods of useful life applied. In addition, acetylsalicylic acid is suggested as an effective and profitable treatment for

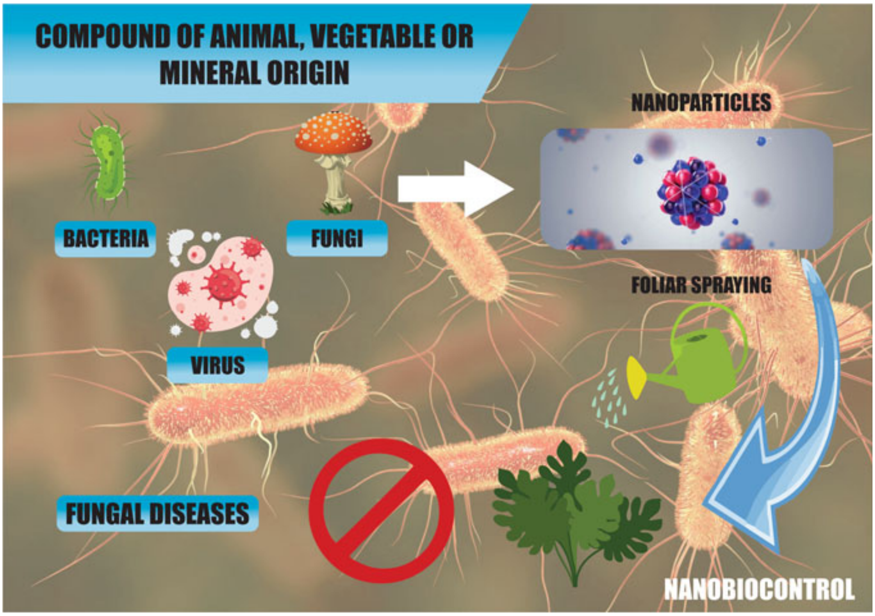

Fig. 3 Application of nanobiocontrollers by foliar spraying through the synthesis of nanoparticles extracted from living organisms

the production of medlar fruits with improved quality properties that requires further study.

Similar studies indicate that individual strains considered particularly predestined for biocontrol activities are from the bacterial genus: *Pseudomonas, Serratia, Streptomyces, Burkholderia, Bacillus* and the fungal genus *Trichoderma* [69, 70]. In particular, the strains of the phylogenetic subgroup Pseudomonas fluorescens are used as biocontrol bacteria and/or plant growth promoters, taking in to account the close relationship between biocontrol and the promotion of plant growth, being an alternative for sustainable agriculture, as was evident in a recent study by Agaras et al. [71] who inoculated *Pseudomonas* in wheat and corn seeds.

4.3 Physical and Chemical Properties of Nano-Biocontrollers

A biological control agent or also known as a biocontroller has the ability to protect against pests and pathogens that harm crops. In this way, the properties of biocontrollers vary in relation to the type of microorganism, interaction with the environment, among multiple factors. Given the importance of applying biological control agents (biocontrol), bacteria have been used as biological control agents for plant pathogens, taking in to account among their properties the ability to manifest antagonistic activity against phytopathogens by producing secondary metabolites such as antimicrobial compounds by through nutrient competition and by protecting the host plant by activating defense-related enzymes and inducing systemic resistance.

Studies establish that among its beneficial properties offered by bacteria used as biocontrols, it stands out to boost the host defense mechanism by directly or indirectly controlling pathogens, through ecological interactions. There are three possible routes that bacteria could act directly on a pathogen: competition for micro or macronutrients [72, 73], hyperparasitism, and antimicrobial compounds. In the first case, an example is the competition for iron [74, 75], in the second, when they produce enzymes that digest the cell wall of fungi such as Beta-glucanase [76], chitinase [77, 78] or protease [54, 77] and in the latter case, includes antibiotic antibiotics [67, 79] and other antimicrobial compounds, such as volatile compounds [80, 81]. Regarding indirect control, they can influence or strengthen host defense through different mechanisms such as detoxification [67].

Vrublevskaya et al. [82] studied the biocontrol properties of *Vitis vinifera* endophytic bacteria, they not only contribute to plant growth, but also have the ability to protect them against phytopathogens. Among the properties they showed was the production of siderophores and antifungal substances using endophytic bacteria. They isolated four different varieties of Vitis vinifera from 2 vineyards, conventional and organic. The results indicated that the endophytic bacteria of *V. vinifera* can produce antifungal substances against the phytopathogens tested (*Botrytis cinerea* DBM 4111, *Fusarium solani* CCF 2967 and *Mucor plumbeus* CCF 2626). The antifungal activity was observed in a greater number of bacteria isolated from vineyards with conventional management with 56% of the total isolates, compared to isolates from organic vineyards (30%). On the other hand, the production of siderophores was higher compared to isolates from organic vines (80% of total isolates) than isolates from conventional vines (61%), which could be used in microbial pesticides to protect plants without an environmental load.

4.4 In Vitro Applications of Nano-Biocontrollers

An alternative for the control of diseases caused by fungi in crops is the use of rhizobacteria that act as plant growth promoters represented in Fig. 4. Similar studies by Blanco et al. [55] evaluated the capacity of ten rhizobacteria of the genera *Rhizobium, Bradyrhizobium, Sinorhizobium, Ochrobactrum* and *Pseudomonas*, carried out physiological, biochemical and in vitro antagonism tests against phytopathogens: *Fusarium oxysporum, Colletotrichum gloeosporioides* and *Rhizoctonia solani.*

Of all the strains evaluated, five produced a greater amount of indole acetic acid compared to the remaining strains in the presence of tryptophan, the strain encoded as ES1 (*Ochrobactrum* sp.) produced hydrogen cyanide, while 50% of the strains released siderophores and 60% solubilized phosphorus. All strains evaluated were positive for nitrogen fixation. Nine strains inhibited between 40 and 65% the growth

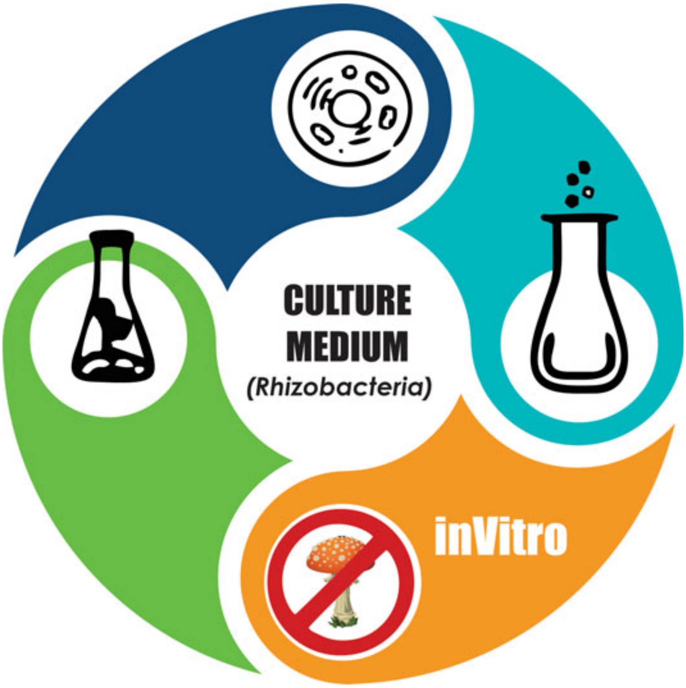

Fig. 4 Stages of in vitro culture for the control of fungal diseases using rhizobacteria

of *F. oxysporum* and the *Pseudomonas fluorescens* strain inhibited the growth of *C. gloeosporioides* by 22%, while neither inhibited the growth of *R. solani*. In this way, the tested *rhizobia* and the *Pseudomonas fluorescens* strain can have beneficial effects on plants by direct and/or indirect mechanisms.

4.5 In Vivo Applications of Nano-Biocontrollers

Figure 5 refers to in vivo plant cultures of nano-biocontrollers for the control of post-harvest diseases [83, 84].

Tomato (*Lycopersicum esculentum*) production is affected by several diseases, one of them is wetting with incidence between 25 to 40%, anthracnose and rotting is frequent between 30 and 40% caused by *Colletotrichum capsici*, while wilting bacterial, caused by *Ralstonia solanacearum* represents between 30 and 45%, causing serious economic losses.

Studies carried out by Amaresan et al. [85] evaluated both in vitro and in vivo six bacteria associated with tomato plants, showed characteristics of plant growth promotion and biological control against phytopathogens. The averaged results of two field trials revealed that tomato plants inoculated with strains BETS11 (11.73

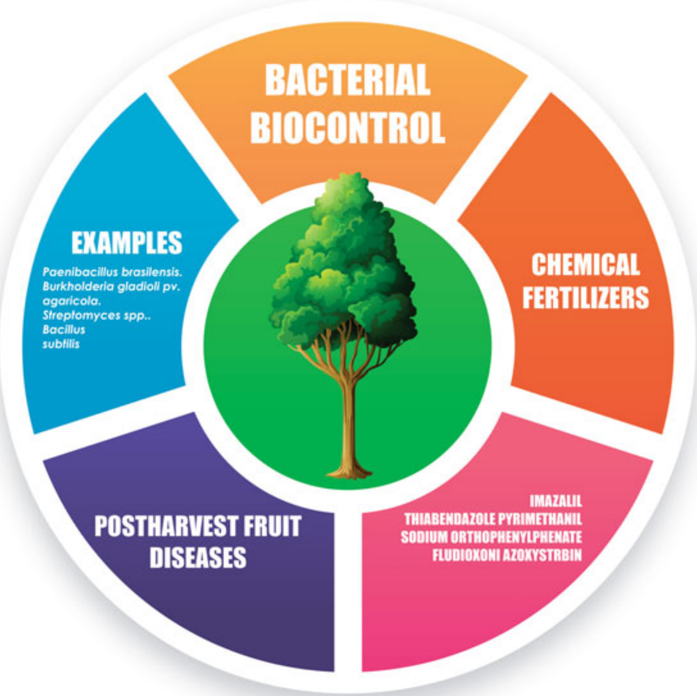

Fig. 5 In vivo applications of chemical fertilizers compared to the use of biocontrollers

t/ha) and BETR11 (11, 24 t/ha) showed significantly higher yield and disease reduction on par with the chemical control (11.81 t/ha). Therefore, the isolates BETS11 (*B. subtilis*) and BETR11 (*Bacillus* sp.) are considered possible biocontrol agents and efficient fertilizer in tomato production in the agricultural field.

Han et al. [86] evaluated in vitro and in vivo tests of marine yeasts *Sporidiobolus pararoseus* ZMY-1 for its application in the biocontrol of fungal infections in fruits. The results show that it can antagonize *A. alternata, P. expansum* and *B. cinerea*, three common postharvest pathogenic fungi. The optimal compound protectant containing 1.5% skimmed milk powder, 0.1875% polyethylene glycol and 5.25% trehalose increases the survival of the freeze-dried marine yeast *S. pararoseus* ZMY-1 by up to 93.9%. Lyophilized *S. pararoseus* ZMY-1 showed a good biocontrol effect on postharvest diseases of apple fruits stored at 4 °C.

Table 3 Differences between the use of chemical fertilizers and bio controllers on soil and water

Chemical fertilizers	Bio-nanocontrollers
Groundwater contamination	Favors decomposing microorganisms, both fungi and bacteria
Loss of nutrients in the soil	Greater availability of micronutrients to the crop
Soil infertility	Improves natural fertilization

4.6 Impact of Bio-nano Controllers on Soil and Water

Science and nanotechnology have made it possible to show evidence about the application of biofertilizers or bio controllers from rich organic matter and/or bioactive microorganisms that can improve soil quality while suppressing soil-borne diseases. However, to carry it out, massive amounts of biofertilizers must be applied to the field per unit area. Therefore, farmers use chemical fertilizers that weigh less than biofertilizers, which facilitates transportation and cost. Table 3 shows the difference between the use of fertilizers and the impact on the soil. Due to the above, the great abuse of chemical fertilizers on the soil and water has caused loss of organic matter in the soil, the degradation of the communities of soil microorganisms and environmental contamination. Thus, studies suggest that the most promising way forward is to use biological control microorganism agents for root rot control, depending on ecological or economic concerns [87, 88].

Similar studies indicate that biofertilizer use increases the soil's ability to retain moisture, improves the availability of soil nutrients (nitrogen and phosphorus) for plants, and maintains relatively healthier soil through enrichment of soil microbial status and helps soil aeration and natural fertilization. But, it is not found suitable for long term due to the scarcity of beneficial bacterial strains.

Therefore, the impact of nanobiocontrollers is focused considering that in the formulation of nanobiofertilizers, the biofertilizer (containing nutrients and plant growth promoting bacteria) is coated with nanoscale polymers (nanoencapsulation). In this way, nanoencapsulation allows to protect the components of biofertilizers, improve their useful life and dispersion in the fertilizer formulation and allow the controlled release of biocontrol. In addition, studies affirm that it can accelerate the efficiency of the use of nutrients (Nitrogen, Phosphorus and Potassium), contribute to the beneficial microbial population for the soil, improve the activity of the related enzyme system, comprehensively improve soil fertility, facilitate the improvement of quality of agricultural products and improve the resistance of crops to diseases [57, 89, 90].

Studies by Ram et al. [65] evaluated the efficacy of biocontrol What *Trichoderma* sp., *Pseudomonas fluorescens, Beauveria bassiana* and, *Bacillus* sp., for sustainable crop production in the rhizosphere region. The study revealed an antagonistic impact against a number of plant pathogens through the secretion of various secondary metabolites antimicrobial and hydrolytic enzymes (protease, pectinase, chitinase, lipase, etc.). Therefore, a study that includes functional genomics is suggested, transcriptomics, proteomics, metabolomics and secretomics for a multiomics approach.

Ghosh et al. [91] studied the molecular mechanism of nanofertilizers in crops. The findings indicated that nanofertilizers improve crops by improving seed germination, shoot and root growth, chlorophyll content, photosynthesis, tolerance to abiotic stress, and ultimately increasing crop yield and quality. In addition, they regulate the activity of some enzymes involved in nitrogen metabolism such as nitrate reductase, glutamate dehydrogenase, etc.

5 Future Trends

Figure 6 highlights future research aspects and methods of fungal biopesticides as an integral part of sustainable agriculture for fungal diseases. Studies by Luo et al. [92] reported that biological control strains have a strong antagonistic capacity and are harmless to plants, which has already led to the search for new biological control strains to be combined with high-quality organic fertilizers for prepare fertilizer bioorganic. Bio-organic fertilizers provide a high-quality carrier for antagonistic strains as well as high-quality nutrients for plants.

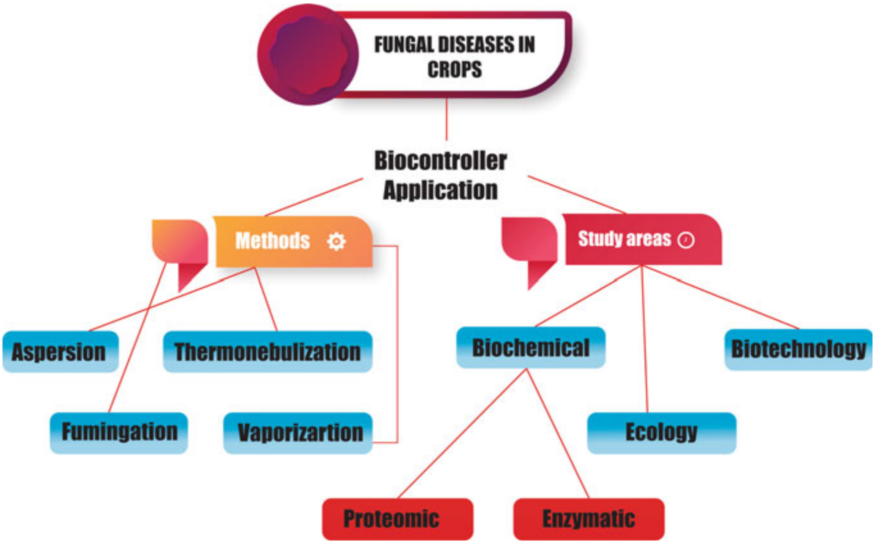

Fig. 6 Trends of studies to expand and deepen for the application of biocontrollers in fungal diseases of crops

On the other hand, Bramlett et al. [93] carried out a biochemical review on RNA-based biocontrols as a new paradigm in crop protection. To do this, they studied the process of RNA interference (RNAi) complement the arsenal of treatments of foliar spray, soil or seed applied as chemical and biological pest control agents and PIPs. In this way, RNA-based active ingredients (AIs) have a unique mechanism of action and can be used by both genetic modification (GM) and biological control approaches. Therefore, RNA-based AIs promise to offer these electivity and the sustainability desired in the futures phytosanitary agents.

Gour et al. [94] investigated the nanomaterials as a therapeutic and diagnostic tool for disease control of plants through the use of nanoscience and nanotechnology. The design included nanoformulations in nanomaterials carbon-based and nanoparticles magnetic for the delivery of nutrients and fractions biofunctional to plant cells. In addition, the bactericidal and fungicides conventional nanoparticles are increasingly being replaced by nanoparticles such as nanosilver nanoparticles, nano-copper and metal oxide nanoparticles such as zinc oxide nanoparticles, copper oxide, magnesium oxide and titanium dioxide. For its anti-infective properties. Another area of interest to investigate diseases in plants is the use of nanosensors/nanobiosensors that transmit responses associated with diseases through biomarkers.

A recent study by Marrone [95] looked at the status and potential use of biocontrols for crop protection, and research in recent years has grown due to demands for safer foods and restrictions on these synthetic products. In this study, the growth of biocontrol solutions was established to continue at compound annual growth rates of between 15 and 20% with additional benefits such as biodegradability, lower carbon footprint, and low risk to non-target organisms, including pollinators. However, other studies Singh et al. [96] reported the use of biocontrollers as a tool for soil fertility management in a changing climate in which they indicate that the response of crops to biological fertilizers depends largely on the crop species/strains of microbes and the method of application, as well as climatic conditions. Considering that the application of beneficial microbes reduces the nitrogen requirement by 50–70% and increases the yield by up to 20%. Additionally, bacterial inoculation not only provides nitrogen, phosphorus and growth hormones, but also contributes to a healthy plant and less susceptible to pathogens.

Nanotechnology allows the control and manipulation of matter at the nano-scale through the application of intelligent systems of supply of nutrients, pesticides and genetic materials to improve soil fertility and offer protection against fungal diseases. There are multiple applications of nano-bio controllers, but they can mainly be found for biological control, conservation method and increase of useful life. Considering their impact on the environment, the application of nanobiocontrols at multiple stages of development, alone or in combination with a nutrient supplement, constitutes a future prospect for future research.

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The Role of Polymer-Based Materials in Sustainable, Safe, and Efficient Metal Batteries

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Abstract This chapter deals with the increasing importance of polymer-based materials in batteries, along with their contribution to make them safer, more efficient and sustainable. Polymers possess unique mechanical properties, being tough, flexible and light, and they can be processed in scalable and sustainable ways. They are also easy to reshape and reprocess, improving recyclability. Because of all that, their incorporation in electrodes and electrolytes in energy storage systems offers many advantages. At the same time, polymers can be ionic and electronic conductors, as to act as functional materials for electrodes and electrolytes. Composite and multiphasic polymer materials, and more recently nanocomposites and nanostructured ones, improve performance in terms of thermal, mechanical and transport properties. This chapter will go through the state of the art of polymer-based materials to be used as functional electrolytes, electrodes and binders, and how their incorporation enables improved performance. A detailed review on current synthesis, preparation and processing strategies of new polymer-based battery components is carried out, with the focus put on the progress towards making them cleaner and more sustainable including the potential application of new green chemistry and engineering approaches.

Keywords Polymer-based electrolytes · Polymer-based electrodes · Batteries · Safety · Sustainability

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1 The Green Revolution in Energy Storage

The transition towards a greener and more sustainable society requires a drastic reduction of the fossil fuels consumption, especially in the automotive sector and an increase on the use of renewable energies. Both require the use of energy storage systems as batteries.

The global market is going to need large amounts of batteries (>2600 GWh/year by 2030, 14 times the amount of 2018), and the current state-of-the-art lithium ion batteries (LIBs) are to become a bottleneck, since they strongly rely on geo-localized critical elements. Moreover, LIBs are subject to fires and explosions, being safety an issue that must be addressed.

Thus, next-generation of batteries are to be based on abundant raw materials, avoiding the use of cobalt (Co), nickel (Ni) and lithium (Li). Besides, they need to avoid liquid leaks, be non-flammable and able to decrease the risk of short-circuiting and other safety hazards. Tendencies of the R&D in new metal-ion battery technologies can be classified in alkalines other than Li (Na, K) and multivalent metals (Ca, Mg or Al). The increased efforts of R&D in these metals over the last decades are presented in Fig. 1a.

Na-ion batteries

For its similarities with Li, its high redox potential (−2.71 V vs. SHE for Na/Na^+), highly natural abundance and low price, sodium (Na) is one of the potential game-changers to take over the role of Li. The theoretical properties of sodium ion batteries (NIBs) make them good candidates for stationary applications, ($E_{\text{Na-ion}}$ ~100–130 Wh/kg). Moreover, anode-free Na batteries could be competitive to LIBs in terms of specific energy (E_{Na} anode-free ~400–450 Wh/kg vs. $E_{\text{Li-ion}}$ ~481 Wh/kg). However, NIBs currently suffer from shorter life-time than LIBs and potentially the same safety issues. Performance is limited by electrode materials and electrolyte solutions. Some

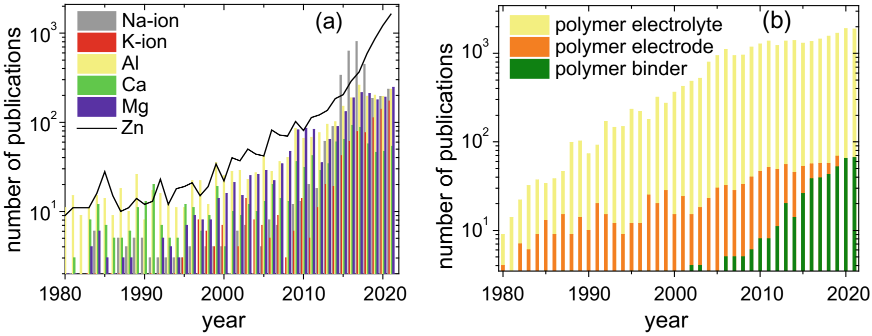

Fig. 1 **a** Evolution on the number of published papers from 1980 to 2021 containing. Black bars: "Sodium ion secondary batteries"; Red bars: "Potassium ion secondary batteries"; Green bars "Aluminium secondary batteries"; Blue bars: "Calcium secondary batteries"; Magenta bars: "Magnesium secondary batteries". **b** Polymers in electrolytes, electrodes and as cathode binders. Source Scopus

NIBs devoted start-ups like Faradion Ld. (U.K.) or Novasis Energies Inc. (Brazil) have achieved promising results but their cells, based on using hard-carbon as anode, do not reach energy density above 100–130 Wh/kg [1]. Therefore, there is a need for development of materials in order to reach higher specific energies [2].

Potassium based batteries

Potassium is highly available and cheap with interestingly high redox potential (−2.93 V vs. SHE for K/K^+). However, its low melting point (64 °C) and the slow kinetics due to the large size of the K^+ ion remain its main drawbacks [3]. Research on electrodes, electrolytes and solid interphases are still in its infancy [4].

Calcium based batteries

Calcium is affordable and highly available, with interesting theoretical energy density (2073 mAh/cm^3) and a reduction potential of −2.84 vs. SHE. However, Ca plating is challenging and was left aside for many years. Nevertheless, strong efforts have been made since 2016 in order to demonstrate the feasibility of the reversible plating and stripping of Ca. Despite its potential low cost and theoretical good performance, the reality is that many bottlenecks are yet to overcome including finding suitable electrodes and electrolytes [5]. Currently, research work is focused on developing inorganic intercalation host materials with transition metal ions as cathode, in combination with electrolytes with enough stability towards the plating and stripping of Ca and Ca metallic anode.

Magnesium based batteries

Mg based batteries are environmentally friendly and benefit from a high volumetric capacity (3833 mAh/cm^3) and a reduction potential of −2.36 vs. SHE. Moreover, the electrodeposition of Mg is less prone to dendrite growth. However, the sluggish kinetics of the insertion/extraction of Mg^{2+} requires the development of suitable cathode materials able to accommodate the complex reaction mechanisms of Mg^{2+}. In addition, Mg^0 anodes have a great tendency to form a passivation layer in a wide variety of electrolytes, blocking electrochemical reactions [6]. Therefore, the compatibility of electrodes and electrolytes is key and current alternatives are either highly corrosive or flammable [7, 8]. Most recent advances have been made combined with computational methods with flexible VS_4/rGO cathode, with high capacity >300 $mAhg^{-1}$ [9].

Aluminium based batteries

Aluminium is abundant, safe and low cost. Although with a more moderated reduction potential (Al/Al^{3+} −1.68 V vs. SHE), Al based batteries have very high theoretical volumetric capacity (8040 mAh/cm^{-3}), specific capacity (2980 mAh/g) and promising demonstrated performance at lab-scale [10]. Aprotic electrolytes, such as imidazolium, pyridinium or ammonium based ionic liquids, saturated with aluminium chloride ($AlCl_3$) is the most advanced approach to date to achieve reversible electrodeposition/stripping of Al [11]. However, these liquids are still quite expensive, although more affordable electrolytes, i.e. urea-based, are being explored [12]. Nevertheless, challenges arise from the complex mechanisms of the Al species in the electrolyte and from passivation and dendritic growth in the anode. So far, an ultimate solution is still lacking for long-life Al secondary batteries being the chloroaluminates and the development of suitable cathode the main bottlenecks.

Given its expected omnipresence, for the development of new battery technologies in addition to using highly available metals, safety must be considered. Liquid electrolytes are very often toxic or corrosive, and leakages are a safety issue because many liquid electrolytes are flammable. Thus, research is necessary to (i) find non-toxic, non-corrosive and non-flammable liquid electrolytes, (ii) use solid ones (for the so-called Solid or Quasi Solid State Batteries, SSB or QSSB) in which leakages, explosions and fires are eliminated or strongly minimized. Solid or quasi-solid electrolytes can be inorganic (ceramic), organic (polymeric) or organo-inorganic hybrids or composites. Polymer-based electrolytes, including composites and hybrids, are the most advanced ones, for they can combine solid and liquid properties, including excellent wetting of the electrodes. Interest in them has not stopped increasing since 1980 to date, as shown in Fig. 1b, where the number of articles on polymer electrolytes is represented (in yellow). Moreover, the various functions that polymeric binders of electrode particles may play is progressively being better understood, not only their contribution to the mechanical integrity of the cathodes but also to their electrochemical performance. As a matter of fact, research on electrode binders, represented in green in Fig. 1b, is growing very quickly in the last decade. Furthermore, wherever they are used, polymer-based materials provide many additional advantages: flexibility, lightness, processability and scalability, and they can also contribute greatly to the sustainability of processes and products. The presence and role of polymer-based materials in battery components is reviewed in the next section.

2 Polymers in Batteries

In batteries, polymers are present as electrolytes, casings and separators, and also at the electrodes, either as binders or as conductive polymers. This is illustrated in Fig. 2, which lists the features and properties they provide to each individual component and in general to the device as a whole.

2.1 Polymers as Electrolytes

2.1.1 Overview of Polymer Electrolytes for Li-Ion Transport

In the 70s-decade, Wright [13] discovered the ionic conductivity of polyethylene oxide (PEO) with alkali salts (Na and K) and few years after, Armand recognized the value of that discovery to produce polymer electrolytes [14]. Basic research followed, focused on studying the transport mechanisms of different salts in PEO [15, 16].

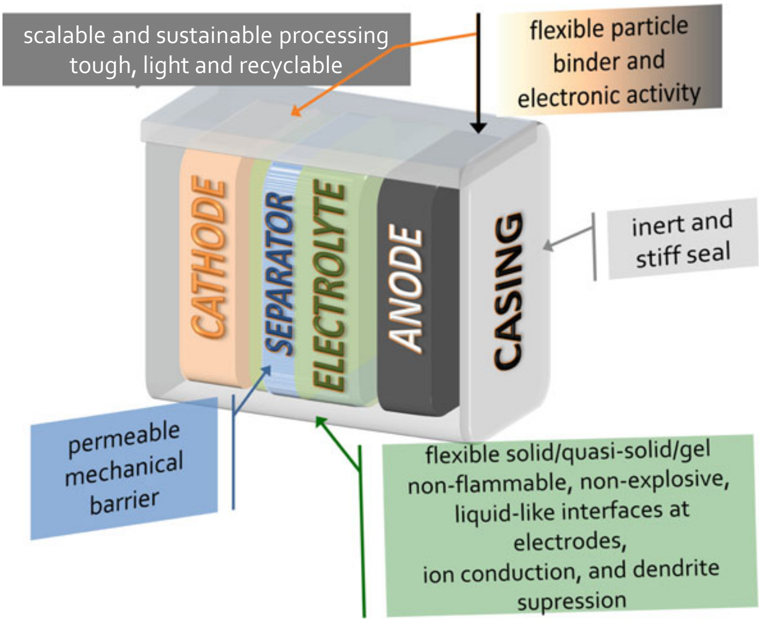

Fig. 2 Schematic view of the role and features provided by polymers in the battery as a whole and in the individual components

PEO is a solid because of its crystalline phase and though its elimination increases ion conductivity, it also turns the polymer into a viscous liquid. Different strategies were explored to improve ion conductivity in PEO while preserving mechanical strength. They included blending with other polymers, block copolymerization, branching [17] and the design of polymer composites (micro or nanocomposites). Angell et al. [18] proposed the "polymer in salt" (superionic rubber) strategy, where very high concentration of low glass transition salts and a small fraction of high-molecular weight polymer (5–10 mol %) are combined. This approach cleverly tackles the coupling of rheology and ion conductivity. Composite electrolytes were introduced by the pioneer work by Weston and Steele [19], where the incorporation of inorganic particles (i.e., α-alumina) to PEO enhanced its mechanical properties very substantially. Some years later, Scrosati et al. [20] showed how nano-sized fillers improved both the ionic conductivities and the interfacial stabilities of polymer electrolytes.

More recently, phase-separated systems [21–23] or covalent organic frameworks (COF) [24], are smartly designed to build fast ion-conductive pathways in an otherwise rigid matrix, aiming at decoupling mechanical properties and ion conductivity in Solid Polymer Electrolytes (SPE). Unfortunately, processability and scalability are usually compromised. Mackanic et al. reported a polymer system with ion-conducting domains and orthogonally functional H-bonding which provides high mechanical properties [25]. However, to reach workable levels of both features (σ

$= 1.2 \times 10^{-4}$ S cm^{-1} at 25 °C), it was necessary to add a low molecular weight plasticizer (20 wt% of diethylene glycol dimethyl ether) and an inorganic filler (2 wt% of SiO_2).

Besides the rheology/ion conductivity trade-off issue, the strong interactions between EO groups and Li+, links the ion conductivity in PEO electrolytes to the segmental motions of the chain. To increase ion mobility, it is necessary to decrease the EO/Li+ interaction and crosslinked polytetrahydrofuran (PTHF) [26] and polyethylene carbonate (PEC) [27] have been tested, proving a decrease in Li coordination whereas slightly increasing σ. Sun et al. [28] report a polysulfur copolymerized with an ionic liquid (1-allyl-3-vinylimidazolium bis((trifluoropropyl)-sulfonyl)imide) in the 10^{-4} S cm^{-1} range that relies on the similar ability of sulfur and oxygen atoms to coordinate Li salts.

Newly described ion transport mechanisms different from the conventional viscosity-dependent ion diffusion, such as anion-exchange Li transport [29] have been found in polymer electrolytes with very high LiFSI concentration [30]. For instance, in gels of polycationic polymerized ionic liquids (polyimidazoliums and polypyrrolidiniums) [31, 32], anion-exchange Li transport occurs in microphases with high local Li concentration. Another option are single-ion conducting polyanions. These were first reported by Bannister et al. [33], and research on them has been very prolific since. Polyanions, among the best studied sulfonamide, sulfonate or sulfonamide, increase the cation transference number [34] to virtually 1, hindering polarization of the electrolyte and enlarging electrochemical stability window beyond that of PEO [35]. A special class of polyanions are those polymerized ionic liquids (PILs) with (trifluoromethylsulfonyl)imide (TFSI) as anion, such as poly(lithium 1-(3-(methacryloyloxy)-propylsulfonyl)-1-(trifluoromethylsulfonyl)imide) (PMTFSI), with high Tg [36]. However, they present some drawbacks: low conductivity, high price and more importantly, poor mechanical properties. Thus, these polyanions are frequently copolymerized with other flexible monomers such as PEO or polymethacrylates like poly(ethylene glycol) methacrylate [35, 36]. In this connection, the copolymers of different length glycols and mono-diacyl-capped orthoborate moieties proposed by Angell et al. [37] are particularly interesting because of their mechanical properties and their affinity to many different liquid electrolytes.

As for now, it seems that the mobility imparted solely by polymer segmental motions is too low for practical application, therefore most of the systems described above required "modest" amounts of platicizers. Very successful strategies are those incorporating large concentrations of *ad-hoc* liquid electrolytes, producing either gels or highly plasticized polymers and their composites with inorganic fillers [38–41]. Depositing these gel electrolytes onto spun matts [42] or infiltrating them into porous membranes, where micro phase segregation is possible [43–45] is a way to add mechanical resistance to the otherwise soft electrolytes.

2.1.2 Polymer Electrolytes in Post-Li Batteries

The quicker way to progress for post-Li batteries is to blueprint Lithium technologies. On the one hand, PEO is an outstanding polymer for turning liquid electrolytes into solid or pseudo-solid electrolytes. Li, Na, K [13, 14], Zn [46], Mg [47], Ca [48] and Al [49] ions are found to be complexed by the PEO chains. Gels can be formed with these electrolytes and polymers such as PTHF or PEGylated acrylates. Polyvinylidene fluoride (PVDF) polymer gel electrolytes (PGE) of K electrolytes have been reported, reinforced with spun fibers of polyacrylonitrile (PAN) [50]. Other polymers which have been used as gel or membrane formers with K electrolytes are crosslinked polymethyl methacrylate (PMMA) [51] and PEO [52]. A Mg PGE has been prepared by crosslinking PTHF [27], and other Mg gels have been synthesized by THF in-situ polymerization [53] in a novel Cl-free Mg $(B(hfip)_4)_2$ (MgBOR). These Mg PGEs seem capable of improving the electrochemical performance of the neat liquid electrolyte. A composite Zn electrolyte comprising PEO and inorganic oxides such as TiO_2 or SiO_2 shows enhanced ion conductivity and improved interfacial contact with the electrodes. Single-ion conducting polymers are also being studied as Na, Ca and K electrolytes [54], finding that many of the lessons learnt for Li can be translated to these other metal cations.

In the case of Al electrolytes, blueprinting from Li is not straightforward, at least for chloro-aluminates. These electrolytes suffer chemical transformations in the presence of polymers containing Lewis acids [55], such as PEO, PVDF or PAN so new approaches are needed to obtain PGEs. A way to circumvent this issue is to use ultra-high molecular weight (UHMW) polymers, where the polymer concentration needed to produce a gel is very low and thus the chemical transformation of the electrolyte is better controlled [49, 56].

The strong interaction of these metal cations with the EO units slows down the cation diffusivity and is a drawback. Finding polymers where cation complexing is weaker than in PEO can facilitate ion transport through other mechanisms, which would be a breakthrough in the area [57].

2.1.3 Avoiding Metal Dendrite Growth: Solid or Quasi-Solid Electrolytes

Metal dendrites are formed by non-homogeneous deposition of the metal at the anode during the charging of the battery. They occur in all metal-battery technologies and because of the high elastic modulus of these dendrites, they can pierce the separator, ultimately producing a short-circuit. The first strategy to avoid dendrites was the use of solid electrolytes, composite electrolytes comprising polymer-containing electrolytes and inorganic particles [58], or porous hosts [59]. Polymer-in-ceramic (garnet) electrolytes [60, 61] and porous polymer membranes [59, 62] have shown good conductivity and electrochemical properties for Li metal batteries. MXenes are also being employed in combination with polymers (PMMA and PVDF) and Zn liquid electrolytes to obtain solid dendrite-free Zn electrolytes [63].

Very recently, a completely different strategy to avoid dendrite growth in gels or even liquid electrolytes has been proposed. It was found that the presence of low concentration of polymers (polyacrylamide, PEG adsorbed at the anode, polyacrylic acid (PAA) forming a hydrogel, PEO) can largely reduce dendritic growth in gelled Zn electrolytes because their presence increases viscosity, promoting a homogeneous Zn deposition-dissolution process. In 2014 Archer and coworkers reported for the first time a low-modulus crosslinked PGE where dendrite suppression was observed [64]. In the next years, the dendrite mitigation/elimination in Li, Na, Zn, and Cu salts [65–67] gels prepared with UHMW PMMA or organic liquid electrolytes was reported. Other authors have reported on this phenomenon in a K electrolyte consisting of PAN fibers and a PVDF gel, where mitigation of K dendrite growth has been observed [50]. In all these gels, the ionic conductivity of the liquid electrolyte is preserved. This is a major breakthrough over the general understanding that for reducing dendrites it was necessary a mechanical resistance to their growth. This would require of polymer electrolytes with comparatively higher elastic modulus and consequently low ion mobility.

2.2 Polymers in Electrodes and Binders

Intercalation electrodes are made of an active material (AM) able to host the metallic cation, a conductive additive, usually conductive carbon particles and a polymer binder, which acts as the agglutinant [68, 69]. In fact, cathodes can be viewed as composite materials comprising active particles and polymer binders.

In the case of LIBs, many electrode materials have been developed thought the last 30 years in order to maximize their power density and specific energy [70]. Polymers are widely used in their formulation, both as binders of the AM formulation or as conductive additives (electroactive conducting polymers).

2.2.1 Polymeric Binders in Cathodes

The cohesion imparted by the polymer binder to the cathode is essential to improve the cycle life of batteries [71]. Until very recently, the binder material was considered as a mere adhesive of no research interest. However, nowadays its decisive role on electrode performance is being recognized, and research has boosted (Fig. 1b). Some common polymers used as binders are PEO [72, 73], poly(vinylpyrrolidone) (PVP) [73, 74] and PVDF, which is the most used for LIBs and SIBs [71]. However, PVDF has strong environmental issues, as it requires the use of toxic solvents during processing leading to the generation of hazardous compounds during the degradation processes [75]. Therefore, more benign binders are required [76] that can be dissolved in water, are F-free and/or come from natural and renewable sources. Some examples are carboxymethyl cellulose (CMC) [77, 78] chitosan or alginate and polyacrilyc acid (PAA) [78, 79]. Alginate is a natural polymer obtained from brown algae. This material showed an improved performance in terms of its reversible Li-extraction cycling compared to PVDF in Li-ion batteries [69]. Sun et al. reported the use or polydopamine (a natural polymer) as a polymer binder for LIBs and SIBs [80], acting as both redox-active organic material and binder. This type of binders have been explored for carbon, silicon-based electrodes, transition metal oxides and cathode materials like $LiCoO_2$ (LCO), $LiFePO_4$ (LFP) or $LiNiMnCoO_2$ (NMC) [71]. Other water-soluble binders, such as the biodegradable polysaccharide pullulan have been tested for sustainable and cheap production of NMC532 cathodes, as well as for easy recovering of the active and carbon materials in wasted batteries [81].

2.2.2 Electrically Conducting Polymers (ECPs) in Electrodes

Polymer binders are electrically insulating. Therefore, capacity or cycling stability are usually decreased if high amounts of binder are added [68]. Binder-free methods are in consequence preferred to improve electrochemical performance, but they require of more complex, expensive, and time-consuming preparations [80].

An alternative to the insulating issue can be using conductive polymers. Conducting polymers present conjugated π bonds, where electron transport is possible. Some of the best-known conjugated polymers are poly(3,4-ethylenedioxythiophene) (PEDOT), polyaniline (PANI) and polypyrrole (PPy). ECPs have been proposed as a conductive binder to serve both as a conductive network and to improve adhesion. One interesting strategy is the use of a commercial ECP in Li–S batteries [82]. The composite electrode showed a superior performance in comparison with a conventional PVDF binder due to a better affinity of PEDOT chemical structure with sulfur particles. Another interesting study of the effect of some of the most employed ECPs was made by Li et al. [83]. They reported the preparation of composites materials based on hollow sulfur nanospheres coated by PEDOT, PANI and PPy observing superior capacity and higher cycling stability.

In addition to the use of conducting binders based on ECPs, there is a growing interest in the development of polymers which serve as a redox AM for pure organic-based batteries [84–86]. Among them, two types of redox polymers can be found, the first ones with π conjugated bonds on its chemical structure, described in the previous paragraph and the second one those with redox active sites [85, 86]. Some of the most studied redox active polymers derive from (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO). These redox AM are usually present as pendant groups on a polymer structure. Probably the most studied material for batteries is (poly(2,2,6,6-tetramethylpiperidinyloxy-4-ylmethacrylate)) (PTMA), which was first reported by Nakahara et al. [87]. In addition to PTMA, other interesting structures like the polyantraquinones like poly(5-amino-1,4-dyhydroxy anthraquinone) (PADAQ) or poly(anthraquinonyl sulfide) (PAQS) have emerged as promising materials for these organic batteries. Table 1 collects some of the most studied polymer AM and polymer electrolytes for its application in batteries.

Table 1 Polymers with electronic activity employed at the cathode and ion conducting polymers employed at the electrolyte

Polymer	Function	Processing	Refs
Cathodes: polymer with electronic activity			
PEDOT	Conductive binder	Slurry/doctor blade	[82]
PEDOT	Conductive binder	Slurry/doctor blade	[88]
PEDOT	Conductive additive	Slurry/doctor blade	[83]
PEDOT	Electrode material	Solvent casting	[89]
PANI	Conductive additive	Roll-press	[90]
PANI	Conductive binder	Slurry	[91]
PANI	Coating layer	Compression	[92]
PANI	Electrode material	Compression	[93]
PPy	Coating layer	Slurry/casting	[94, 95]
PTMA	Electrode material	Compression	[87]
PTMA	Electrode material	Slurry/casting	[96, 97]
PADAQ	Electrode material	Mixture of components	[98]
PAQS	Electrode material	Blade casting	[99]
Electrolytes: polymers with ionic conductivity			
PEO	Metal ion conductor (Li, Na, K, Zn, Mg)	Casting/extrusion	[13, 14, 46–49]
PTHF	Metal ion conductor	In-situ polymerization/casting	[26, 54]
PEC	Li	Casting	[27]
Polymeric sulfur with an ionic liquid	Li	In situ polymerization	[28]
Electrolytes: polyanions			
Sulphonic and perfluoroalkyl carboxylic acid	Li	Polymerization/casting	[33]

(continued)

Table 1 (continued)

Polymer	Function	Processing	Refs
Sulfonamide, sulfonate or sulfonamide	Li Na, K, Ca	Polymerization/swelling	[34, 35, 54]
PILs containing TFSI PMTFSI	Li, Zn, Na	Polymerization/swelling/casting	[36]
Copolymers of glycols and borates	Li	Polymerization	[37]

3 Battery Component Preparation: Learning Lessons from Polymer Processing

The transformation of polymers at industrial or laboratory scale is a well-known and well-developed field, and nowadays polymers can be processed in simple, solvent-free, scalable and sustainable ways compared to other materials. In this section it is described how adapting the polymer processing strategies to SSB or QSSB can be a huge step forward for the energy storage industry.

As a general rule, both in electrode and electrolyte processing, it is desirable to avoid the use of auxiliary solvents [100] (typically N-Methyl-2-Pyrrolidone (NMP) in cathode processing, or acetonitrile in film casting), because the drying step is slow, expensive and environmentally harmful. Auxiliary solvents are those needed for the material processing but which do not form part of the final material formulation. When avoiding their use is not possible, the amount employed should be reduced, or solvent recovery procedures should be implemented, although this requires expensive, polluting and complicated industrial processes [100, 101]. Obviously, using water as solvent is preferable in most cases [75].

A summary of the processing methods currently applied in the preparation of polymer-containing electrodes and electrolytes appears in Fig. 3. Some of the methods there depicted, such as extrusion, are very conventional and have been used for decades in the polymer industry; others, such as the numerous 3D printing methodologies, are more recent and their scope is still being explored. In the figure, methods are classified into solvent-free and solvent-based.

3.1 Electrode Processing

3.1.1 Inks (Slurry) and Other Methods Employing Auxiliary Solvents

Porous electrodes are processed as polymer membranes, although the binder content is usually lower than 10 wt%. The polymer binder is dissolved in the chosen solvent, and then the AM and carbon particles are dispersed in the solution, making a slurry

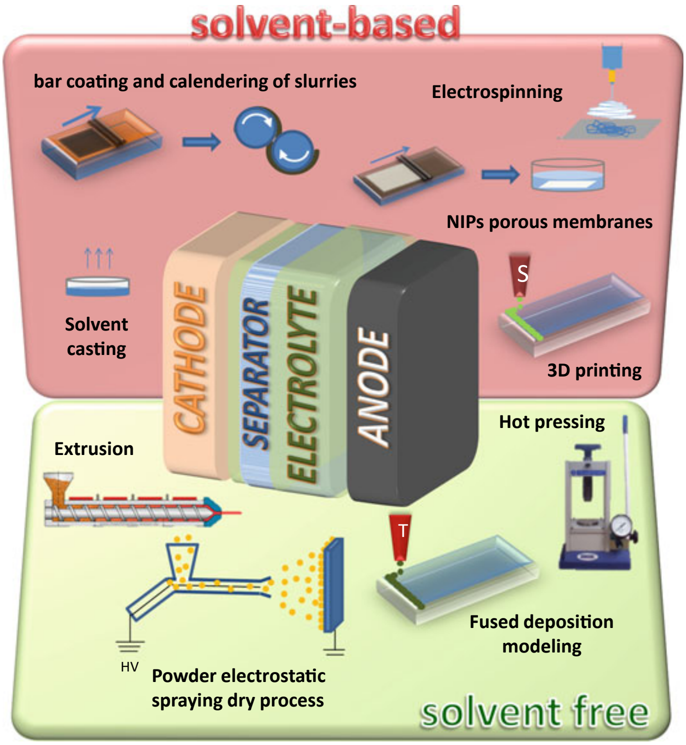

Fig. 3 Processing methodologies for the preparation of electrodes and electrolytes containing polymers, classified into solvent-free and solvent-based

or "ink". This ink is then coated, dried, calendared and cut. In all the processing steps, the polymer plays a role. In the slurry, the polymer controls viscosity, de-agglomeration and dispersion of the inorganic particles. In the drying process, the solvent evaporation rate is responsible for the polymer morphology, which controls the final mechanical properties i.e., the fragility of the electrode. Finally, the polymer occupies the interstices between electrode particles, controlling the ionic mobility and the percolation of the AM [102].

Water can be used as solvent [75] in the slurry preparation. It is safe, reduces the time and energy of the drying step compared to NMP, and water-soluble polymers (PEO, PVP, PAA, cellulose derivatives) are usually cheaper than PVDF. However, it requires additives and surfactants to disaggregate the inorganic AM and overcome its hydrophobicity, and limits polymer binders to water-soluble ones [103, 104].

More recently, 3D printing has begun to be used for electrode processing. In this method, a composite filament is made by dissolving polylactic acid (PLA) in dichloromethane (DCM) and dispersing the AM and carbon conductive particles, sometimes with a plasticizer, and extruding the mixture in the filament geometry that can be printed. So far, a non-solvent electrode 3D printing procedure does not exist [105–107].

3.1.2 Processing of Electrodes Without Auxiliary Solvents

A Powder Electrostatic Spraying dry process has been developed [108, 109], in which the powdery mixture comprising the AM, the carbon conductor and the polymer binder is sprayed, and then the formed film is calendared.

Hot-rolled processing has also been used to prepare a carbon–sulfur cathode for Li–S cells. The preparation procedure consists on the mixing of the carbon nanotubes, sulfur and poly(tetrafluoroethylene) as binder. Then the powdery mixture is ground in a mortar and rolled out at 150 °C forming the continuous cathode material [110].

Extrusion, a well-known processing technique in polymers, cost-effective and easy to scale up, starts to be explored for electrode processing [111, 112]. The high viscosity of the formulation, due to the low content of polymer binder, can be reduced by adding a solvent to the mixture [111], or by introducing an aid-binder that is removed after the electrode processing, increasing the wt. % of AM and the porosity of the cathode [113–115].

3.2 Polymer Electrolyte Processing

3.2.1 Casting and Other Methods Employing Auxiliary Solvents

So far, the most extended procedure to prepare SPE or GPE has been solvent casting [116–118], very frequently using acetonitrile as solvent. It is also common to swell a porous membrane in the liquid electrolyte. Separators are prepared by the non-solvent induced phase separation (NIPs) process [119, 120], from fluorinated polymers. In a more sophisticated version, NIPs is used to prepare solid electrolytes by combining insoluble/soluble polymers [45, 62].

Electrospinning [121] is another alternative to obtain porous separators and electrolytes [122–125] where the pore size and tortuosity in the membrane are controlled by the diameter of the fibers and their separation.

Direct ink writing is a 3D printing technique where an ink is deposited on a substrate by direct extrusion through a nozzle. In the case of polymer electrolytes, the ink consists of a dissolution in a volatile solvent of the polymer and the metallic salt [126–128]. In stereolithography, another 3D printing technique, a building platform is immersed in a liquid resin that can be polymerized. This technique is more expensive, but allows obtaining complicated geometries [129].

3.2.2 Processing of Electrolytes Without Auxiliary Solvents

The simplest solvent-free electrolyte processing method is hot pressing [130–133]. Seeking scalability, melt-compounding is the best option, making it possible to prepare the electrolyte in a continuous process. This procedure has been used to obtain a wide variety of SPEs [61, 134–138], demonstrating its viability.

Fused deposition modeling is a 3D printing technique that can fabricate low-cost free form geometries without auxiliary solvents. For the moment, only a few examples of PEO [139] or PLA-PEO [140] mixtures as polymer matrix have been reported as electrolytes processed by this method.

4 Heading to a Greener Future

To become sustainable, the entire life cycle of batteries must be considered. In coming years, the wasted batteries will represent a huge environmental issue due to the amount of critical and toxic materials they contain.

4.1 Role of Polymer-Based Materials in the Recycling of Batteries

In lead-acid batteries, recovery of the constituents is very high. Casings (mostly PP) are recycled [141–143] and research on elastomeric polymer binders which allow the recovery of cathode alloy powders by dissolution [144] was carried out in the 90s.

In Li-ion batteries, the first and most important battery recycling strategy is their reuse in less demanding applications (second-life), for instance vehicle batteries can be reused as stationary state energy storage. Nevertheless, at the end of this cascade of battery reuse, the components must be separated and reused/recycled. Future batteries will be designed assuring a complete dismantling and recovery of all its components for specific recycling of each [145]. Dismantling hazards linked to volatile flammable electrolytes will be greatly decreased in SSB or QSSB, where the physical separation of components will be possible. As for now, state-of-the art battery recycling in Li-ion batteries comprises the separation of casings and wiring and then the crushing of the cells. From the crushed cells, some valuable metals can be recovered by hydrometallurgical or pyrometallurgical processes, the rest of components are mostly lost.

Currently used fluorinated binders are a concern in the recycling industry for they produce aggressive chemical reagents such as HF which damage industrial facilities [146]. Therefore, prior to any cathode treatment, the elimination of these binders is needed. Environmentally friendly dimethyl isosorbide (DMI) or supercritical CO_2 [146] have been proposed [147] to recover cathode AM by dissolving the PVDF binder.

An effort to develop a fully biodegradable SIB was reported by Lee et al. [148]. They used a $Na_4Fe_3(PO_4)_2(P_2O_7)$ cathode and a pyroprotein-based carbon as anode with cellulose acetate and carboxymethyl cellulose as their respective polymer binders, an electrolyte of $NaClO_4$ in propylene carbonate and a cellulose acetate mesh as separator encapsulating all with a biodegradable pouch.

4.2 Heading to More Sustainable Batteries: The Future

It is possible to push forward the sustainability of batteries at a fast pace starting with the lessons learnt in other fields such as Green Chemistry and Engineering or advanced polymer-based materials in the packaging industry, where scalability, sustainability and recycling of materials has been a hot-topic for many years now.

Cellulose based materials, largely used in the packaging industry for their mechanical properties, renewable source and recyclability, have been recently introduced as battery components [149]. New catalytic routes are being developed for the production of bio-based compounds derived from vegetables oils or lignocellulosic biomass [150–153]. Catalysis is also key for the valorisation of CO_2 into monomers or polymers, as for example polycarbonates [154–157]. The use of biotechnological processes for monomer/polymer biosynthesis is another hot-topic in the substitution of petroleum-based chemistry [158].

Sustainable processes must also be developed during battery processing in terms of environmental and health preservation. In the preparation of separators or electrolytes based on porous membranes, solvents cannot always be avoided, but relevant advances have recently been made to replace polar aprotic solvents (e.g., NMP, DMAc or DMF) in the manufacture of polymeric membranes by NIPs using non-toxic bio-based solvents such as γ-Valerolactone, dihydrolevoglucosenone (Cyrene) or methyl-5-(dimethylamino)-2-methyl-5-oxopentanoate (Polarclean) [159, 160].

Likewise, the solvents for metal salt dissolutions are being revised bearing in mind safety and sustainability. Flammable and highly volatile cyclic carbonates have been traditionally used in LIBs, because they are cheap and produce highly conducting salt dissolutions, but they are progressively being replaced [39]. Metal salts dissolutions in ionic liquids and the cheaper Deep Eutectic Solvents (DES) are explored as non-flammable, mostly non-toxic alternatives. Also, glymes have aroused interest [161] in the last years as a green electrolyte, in particular with Li salts for LiS and Li metal batteries.

An important impulse is given to chemical recycling of polymers which allows recovering monomers and/or oligomers producing new virgin quality polymers. Chemical recycling makes it possible to separate monomers from other substances in multicomponent materials, such as battery electrodes or electrolytes.

Finally, another potential solution for battery recycling and recovery is dynamic chemistry. Dynamic bonds have the ability to break/reform and reorganize either autonomously or under a suitable stimulus (heat, pH or light), thus endowing polymer-based materials with recyclability, self-healing or weldability, among others [162–164]. Battery industry could take advantage of reversibly crosslinking adhesives [165, 166] to produce delaminable cells and make easier the dismantling as learnt by multilayer packaging manufacturing [167].

These are only some hints of how research on different areas of chemistry and polymer science and technology may impact future battery technologies. Every day, new findings and approaches appear in this very dynamic field. Figure 4 classifies all of them into four pillars: the selection of adequate raw materials, the use of sustainable processes and products, pushing forward battery safety, and designing and fabricating fully recyclable batteries. Producing technologies which fulfill these requirements is a challenge which encompasses necessary actions from many different viewpoints, from research to legislation, from engineering to organic chemistry.

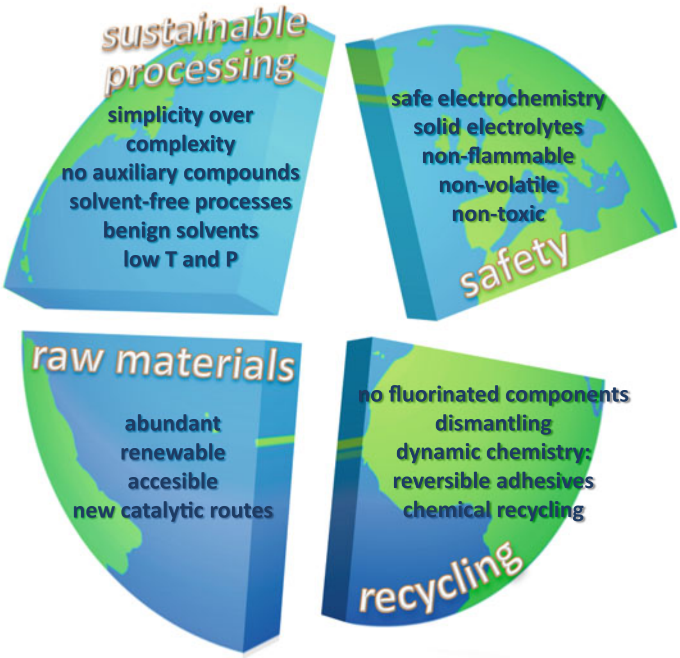

Fig. 4 Four pillars to build the fully sustainable batteries of the future

5 Conclusions

In summary, in this chapter we have seen how polymers are present in batteries at many levels and how they are playing an important role in making them more sustainable and safer. Particularly interesting is the translation of processing technologies and know-how from the polymer field to the design and fabrication of electrolytes and electrodes. The main disadvantage of polymer-based materials is their lower thermal and electrochemical stability in comparison with inorganic ones, which will limit their applications in some devices. However, polymer-based materials including micro and nanocomposites, can display very appealing ion transport and electrochemical properties what, in combination with their well-known and unique mechanical properties, makes them very versatile materials. Moreover, as it happens in packaging, infrastructures, building or aerospace, weight is crucial in batteries, and the intrinsic lightness of polymers is going to be an extraordinary advantage. In the future, the intense research activity in this field will yield materials with improved performance, cleaner synthetic and processing methodologies and new recycling strategies which will allow a higher recovery of the raw materials. In consequence, polymer-based materials are expected to progressively take more and more part as components in cells and batteries.

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