EDITOR
Pierfrancesco Morganti
Dermatology Unit, University of Campania "Luigi Vanvitelli", Naples, Italy;
China Medical University, Shenyang, China;
Director of the R&D Nanoscience Centre MAVI, MAVI Sud Srl, Aprilia (Lt), Italy.

Editorial Office
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Cellulose Nanocrystals: Multifunctional Systems for Biomedical Applications

Elena Fortunati and José M. Kenny

Abstract: Nanocrystalline cellulose, micro- and nano-fibrillated cellulose and bacterial cellulose nanofibres, have become fascinating structures to design new biomaterials. Derived from the most abundant and renewable biopolymer, they are drawing an incredible level of attention. This growing interest is related to their important and useful physical and chemical properties. Their morphological and topographical likenesses allow the exploitation of cellulosic materials in the development of nanostructured composites targeting tissue engineering and regeneration. In this chapter, cellulosic materials, and in particular cellulose nanocrystals, are highlighted as promising alternatives in the production of nanocomposite dense and porous structures as well as hydrogels. Special focus will be given to recent developments of cellulose-based nanocomposite substrates that induce cell attachment and proliferation. Challenges and future opportunities of cellulose nanocrystal-based systems in the biomedical field will be discussed as well as obstacles remaining for their large use.

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1. Introduction

Cellulose is a sustainable and abundant raw material, that represents a key source on an industrial scale. For millennia, cellulose has been used in the form of wood fibers as an energy source, for building materials, and for clothing [1]. The production of nanoscaled cellulose fibers and their application in composite and nanocomposite approach have expanded the attention due to their high strength and stiffness combined with low weight, biodegradability, renewability and sustainability [2].

A number of possibilities have been considered to obtain and extract cellulose-based micro- and/or nano-metric fillers. In particular, microfibrillated cellulose (MFC) is formed by fibrous cellulose structures from wood or plant and it is characterized by several tens of microns in length and few tens of nanometers thick [3]. In other words, MFC is formed by long, flexible and entangled cellulose nanofibres, where both amorphous and crystalline phases are present [4]. Microfibrillated cellulose can be obtained from wood, agricultural by-products and waste, such as crops aimed at textile production (e.g., sisal) or aimed at food production (e.g., fruit crops, like pineapple, and cereal crops, such as wheat, sorghum, etc.). MFC can be extracted using different mechanical procedures as

high-pressure homogenization step, whilst in some cases the process has been optimized by applying chemical and enzymatic pre-treatments of the cellulose raw material [5].

Nanocelluloses are categorized into three groups: (1) Cellulose nanocrystals (CNC) or nanowhiskers (CNW), (2) Cellulose nanofibrils (CNF) and (3) Bacterial cellulose (BC). The yield of the extraction process (i.e., the quantity of nanocellulose obtained from a given weight of microfiber), depends on both the crystallinity of the specific plant fiber and the procedure adopted for the extraction [6,7]. Due to their unique features, cellulose and, in particular, cellulose nanocrystals have garnered a tremendous level of attention in the materials community, which can be confirmed by the increasing number of scientific publications in the field over the past decades and they found particular attention in the nanocomposite approach.

Nanocomposites are usually defined as a combination of two or more materials or phases in which one of the phases has at least one dimension in the nanometer range (1–100 nm) [8–10]. In biodegradable nanocomposites, also called bionanocomposites, matrices may be a biodegradable material derived from renewable resources while the reinforcement phase can include plant fibers and renewable resources or synthetic inorganic materials. Plant-based nanocellulose and bacterial cellulose are included in this definition [11]. Moreover, in a nanocomposite approach, the idea to combine different reinforcement phases with diverse specific and active properties to obtain multifunctional systems, gives the possibility to respond to practical needs for the final application [12–14]. The main reason to utilize cellulose at nanoscale in composite materials is because one can potentially exploit the high stiffness of the cellulose crystal for reinforcement [15]. Because of its unique structural properties combined with hydrophilic nature, biocompatibility, transparency and non-toxicity cellulose structures are attractive candidates for a wide range of applications related to biomedical and biotechnology applications [16]. The term biocompatibility is defined as the ability of a material to perform with an appropriated host response in a specific biomedical application [17]. These properties are the most relevant to consider these materials as good candidates for tissue engineering and regenerative medicine applications, both as tri-dimensional and porous scaffolds or as drug-delivery device. Nanotechnology can be applied across different application areas: the ability to control the material at the nanoscale and the evaluation of their influence in the micro- and macroscopic properties provides the opportunity to develop new bionanocomposite systems in a previously dimensions. Biodegradable nanocomposites are attractive materials for novel biomedical applications due to the matching of the length scales of their structure and the components of the extracellular matrix (ECM). Under these conditions, the development of nanostructured biomaterials for medical applications implies a multidisciplinary research approach. In this sense, much work has already focused on

designing ideal biomedical devices from cellulose and especially bacterial cellulose, such as artificial skin, artificial blood vessels, artificial cornea, heart valve prosthesis, artificial urethra, artificial bone, artificial cartilage, artificial porcine knee menisci, and deliveries of drug, hormone and protein [18–20]. As an introduction, the prospects for the various biomedical applications of cellulose-based materials are shown in Figure 1.

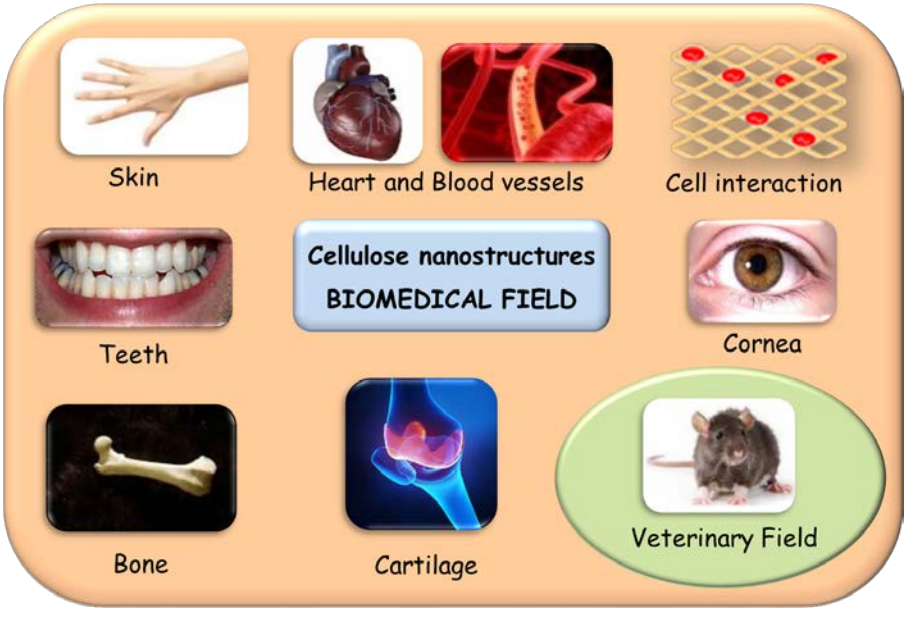

Figure 1. Perspective biomedical applications of cellulose based nanomaterials.

In this chapter we introduced an overview about the research executed with cellulosic materials in the biomedical field, with particular emphasis on tissue engineering and regenerative applications. The main focus will be on the development of nanocomposite systems and hydrogels based on cellulose components, in particular cellulose nanocrystal, within the previously described fields of application. The addition of plant-based and bacterial-based cellulose at nanoscale to enhance thermal and mechanical properties, as well as the biodegradability and sustainability of polymer matrices are also analyzed. This chapter intends to show that biomaterials from cellulosic sources have the potential to play an important role in human health and current research trends and a number of recent advances developed in our laboratories are reported and discussed.

2. Lignocellulosic Materials

Cellulose is one of the most important natural and abundant renewable polymers on earth and it has been used as a chemical raw material for about 150 years. Its annual production is estimated to be over 7.5×10^{10} tons [21]. Cellulose is widely distributed in higher plants; wood, consisting of up to 50% cellulose, is the most important raw material source for cellulose. Furthermore, it is also distributed in annual crops, and even in several marine animals (for example, tunicates), and to a lesser degree in algae, fungi, bacteria, invertebrates, and even amoeba [1]. Cellulose consists of a linear homopolysaccharide composed of β-D-glucopyranose units linked together by β-1-4-linkages. The repeat unit is a dimer of glucose, known as cellobiose. Each monomer has three hydroxyl groups, and it is, therefore, obvious that these hydroxyl groups and their ability to form hydrogen bonds play a major role in directing the crystalline packing and also governing the physical properties of cellulose [22]. The number of glucose units or the degree of polymerization is up to 20,000, but its value can vary among a wide range, and the value is around 10,000 in wood [22]. In nature, cellulose does not occur as an isolated individual molecule, but it is found as assemblies of individual cellulose chain-forming fibers. The morphological hierarchy is defined by elementary fibrils, which pack into larger units called microfibrils, which are in turn assembled into fibers [21,23]. Within the cellulose fibrils there are regions where the cellulose chains are arranged in a highly ordered structure (crystallites), and regions that are disordered (amorphous-like) [24].

Currently, studies on isolation, characterization and selection of prospective applications of novel forms of cellulose structures, variously termed crystallites, nanocrystals, whiskers, nanofibrils, and nanofibres, are generating interest. Novel methods for their production range from top-down methods involving enzymatic/chemical/physical methodologies for their isolation from wood and forest/agricultural residues, to the bottom-up approach of cellulose nanofibrils from glucose by bacteria [1].

2.1. Plant-Based Materials

Wood is a natural composite material with a hierarchical architecture in which cellulose, hemicellulose and lignin form a highly porous anisotropic microstructure [11]. Cellulose fibers can be classified according to their origin and grouped into leaf: abaca, cantala, curaua, date palm, henequen, pineapple, sisal, banana; seed: cotton; bast: flax, hemp, jute, ramie; fruit: coir, kapok, oil palm; grass: alfa, bagasse, bamboo; stalk: straw (cereal). The bast and leaf (the hard fibers) types are the most commonly used in composite applications [25]. Commonly used plant fibers are cotton, jute, hemp, flax, ramie, sisal, coir, henequen, and kapok [26]. Plant fibers are constitutes of cellulose fibers, consisting of helically wound cellulose microfibrils, bound together by an amorphous lignin matrix. Lignin keeps the water

in fibers, acts as a protection against biological attack and as a stiffener to give stem its resistance against gravity forces and wind. Hemicelluloses, that represent acompatibilizer between cellulose and lignin [26], are located mainly in the secondary cell walls, and together with cellulose and lignin, build up the structure of the plants.

Cellulose microfibrils extracted by a mechanical disintegration process from wood cell were first obtained by Herrick et al. [27] and Tubark et al. [28], in 1983 and named microfibrillated cellulose. MFC, composed of expanded high-volume cellulose, is produced by different mechanical treatment, consist of high-pressure homogenization and/or grinding [27,28]. However, this production route is normally associated with high energy consumption for fiber defibrillation [29] and some different alternative pre-treatments have been proposed, such as mechanical cutting, acid hydrolysis, enzymatic pre-treatment, and the introduction of charged groups [30–32]. Cellulose microfibrils are long and flexible nanoparticles presenting lateral dimensions in the order of 3–100 nm, and length generally in the micrometre scale depending on the source of cellulose, defibrillation process and pre-treatment. The Young's modulus of cellulose fibrils has been reported to be 138 GPa and the extremely good strength properties and good thermal stability of the fibrils make them suitable for use as reinforcement in nanocomposites and paper products [33].

CNC, characterized by their typical acicular structure and dimension ranged from 3 to 10 nm in width and 100–300 nm in length has been isolated from a wide variety of cellulosic sources, including plants, [34] microcrystalline cellulose, [13] animals, [35] bacteria, and algae [1] by hydrolysis with mineral acids. The isolation of CNC from cellulose source materials occurs in two stages: a pre-treatment of the raw precursor material and a second step that could be a controlled chemical or enzymatic treatment (generally hydrolysis) to remove the amorphous regions of the cellulose polymer [6]. Siqueira et al. [36] investigated various combinations of mechanical shearing, acid and enzymatic hydrolysis in order to produce nanoparticles from sisal pulp showing as CNC could be obtained by enzymatic hydrolysis.

Cellulose nanocrystals have better mechanical properties than the majority of the commonly used reinforcing materials, and for these reasons, they are perfect candidates to be used as reinforcements phase in thermoplastic and/or thermosetting matrices in a nanocomposite approach with new opportunities in biomedical applications.

2.2. Bacterial Cellulose

Bacterial cellulose (BC), also called bacterial nanocellulose (BNC), microbial cellulose, or biocellulose, is formed by aerobic bacteria, such as acetic acid bacteria of the genus Gluconacetobacterxylinum, as a pure component of their biofilms. These bacteria are wide-spread in nature where the fermentation of sugars and plant carbohydrates takes place. In contrast to MFC and CNC materials isolated

from plant based cellulose sources, BC is formed as a polymer and nanomaterial by biotechnological assembly processes from low-molecular weight carbon sources, such as d-glucose. The bacteria are cultivated in aqueous nutrient media, and the BNC is excreted as exopolysaccharide at the interface to the air. The resulting BNC hydrogel is composed of a nanofibre network (fibre diameter: 20–100 nm) enclosing up to 99% water. Cellulose derived from bacteria species has the advantage of being free from wax, lignin, hemicellulose and pectin, which are present in plant-based cellulosic materials. This BNC proved to be very pure cellulose with a high weight-average molecular weight (Mw), high crystallinity, and good mechanical stability. BC possesses a Young's modulus of about 114 GPa with theoretical values between 130 GPa and 145 GPa, depending on its crystallinity [37]. These values exceed that of synthetic glass fibres (about 70 GPa) and aramid fibres (about 67 GPa), considering that BC has a lower density (1.25 g cm^{-3}) than glass fibres (2.5 g cm^{-3}). BC exists naturally as a nanosized material (between 24 and 86 nm in diameter and several micrometres in length) [38] and it has a surface area of about 37 m^2 g^{-1} [39]. Such properties are highly advantageous for the production of composite materials as this implies that for the same amount of material, the interface between the matrix and the reinforcement will be larger for BC compared to micrometre-scale natural fibres. However, BC is extremely hydrophilic in nature and it will often have poor interfacial adhesion to hydrophobic polymer matrices.

The potential applications of BC are quite vast; it can be used in the preparation of materials targeting decellularised devices for wound healing or as scaffold for skin engineering after seeded with epithelial cells or more recently in vascular grafts [40,41]. Bodin et al. [42] have also proposed BC as meniscus implant since BC can be produced in a meniscus shape. The nanoscaled structured and topographical similarities with collagen make BC very attractive for cell immobilization, cell migration, and the production of ECMs. Finally, Klemm et al. [43], proved that celluloses with different functionalities open novel fields of application, specifically as nanoporous hydrophilic membranes, nanoscaffold-based composite materials, and medical implants for repair of hard and soft tissue, as well as cardiovascular substitutes.

3. Tissue Regeneration

Tissue engineering (TE) is a multidisciplinary field concentrated on the development and application of knowledge in chemistry, physics, engineering, life and clinical sciences to the solution of critical medical problems, such as tissue loss and organ failure [8].

Biomaterials play important roles in regenerative medicine and tissue engineering controlling cellular activities and functions [44]. Although remarkable advances have been made, much work remains in the development of biomaterials

that integrate biological identification and signaling properties of the extracellular environment for practical applications in tissue engineering and regenerative medicine. For the new therapeutic strategies, it is indispensable to provide cells with a local environment that enhances and regulates their proliferation and differentiation for cell-based tissue regeneration [45].

Recently, nanostructured biomaterials having physical nano-features such as nanocrystals, nanofibres, nanosurfaces, nanocomposites, etc., have gained much interest in regenerative medicine [46]. Nanotechnology, and in particular the use of nanomaterials, can mimic surface properties of natural tissues while nanomaterials have been highlighted as promising candidates for improving traditional tissue engineering materials [47]. Different kinds of nanostructured materials (carbon nanostructures, metal, ceramic and polymeric nanoparticles, cellulose micro- and nano-crystals, etc.) are currently used to modulate the properties of biodegradable polymers in order to develop new dense films or scaffolds with multifunctional characteristics. Nanoscale materials can have chemical, physical and biological properties that differ from their larger counterparts and these properties can be exploited to influence cell attachment, proliferation and differentiation [48]. D'Angelo et al. recently demonstrated that nanocomposite fibrous mats of poly(L-lactic acid) (PLLA) loaded with different content of calcium-deficient nanohydroxyapatite (d-HAp) have an active role in inducing human multipotent (hBM-MSCs) and murine pluripotent (iPSCs and ESCs) stem cell differentiation toward the osteogenic lineage in the absence of exogenous soluble differentiating agents, proving the possibility to modulate the polymer matrix structural and functional properties due to the introduction of specific nanostructures.

Although research on cellulose, and in particular cellulose nanocrystals, has experienced a tremendous increase in a variety of fields over the last two decades, it has only recently attracted the interest of researchers in the field of tissue engineering and regenerative medicine, both as scaffolds or as drug-delivery systems. In fact, most of the studies addressing this topic have been published during the last five years, indicating that within the TE field, CNC have a large unexploited potential despite their many well-known attractive properties and possible applications in this context, as will be discussed later in this review.

Recently, Dugan et al. [49] reviewed the literature on bacterial cellulose scaffolds and discussed in detail the studies on the biocompatibility of CNC, addressing its potential in this field. Moreover, Fang et al. [50] demonstrated that bacterial cellulose/hydroxyapatite (HA) nanocomposite scaffolds were biocompatible and could promote cell proliferation and differentiation in vitro. More recently, Saska et al. [51] also described the preparation of membranes composed by BC reinforced with HA and evaluated the biological behavior of these membranes for the regeneration of noncritical bone defects in rat tibiae, over a time period of

16 weeks. The work revealed that the membranes are effective for bone regeneration since they accelerated new bone formation. Furthermore, bionanocomposites porous architectures based on BC were prepared in a collagen solution demonstrating the biocompatibility of the proposed formulations [52,53].

These recent results suggest that the cellulose-based materials have important potential in bone TE applications. For these reasons, the literature on nanocomposites based on both natural and synthetic origin polymer matrices relevant for TE field is reviewed here. Particular emphasis is given to functional materials obtained through different fabrication strategies usually employed to obtain scaffolds with various formats, namely, dense films and membranes (mostly considered as a prequel for three-dimensional (3D) scaffolds development), porous constructs (micro/nanofibres mats, foams and sponges), and hydrogels (Figure 2).

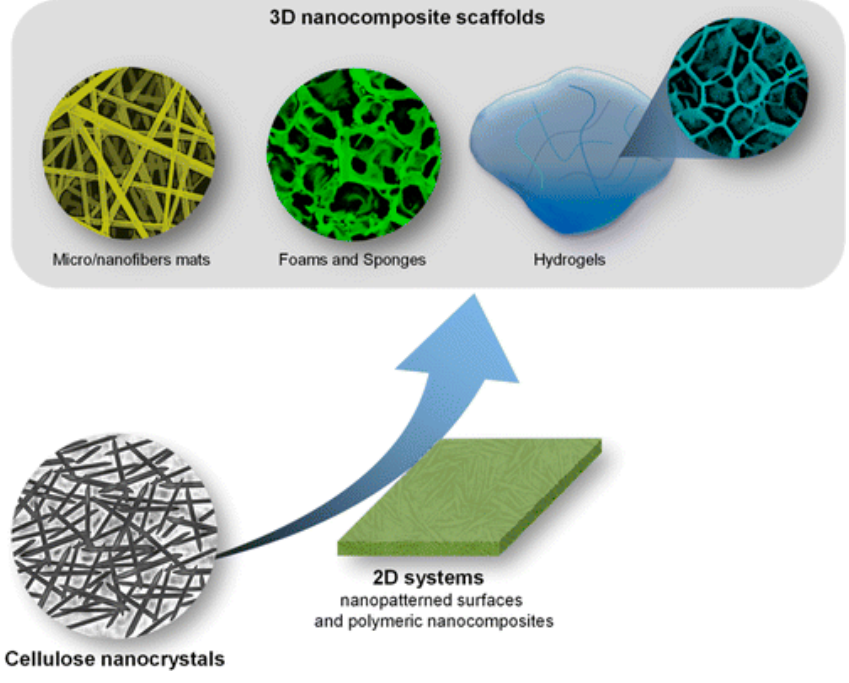

Figure 2. Summary of the current utilization of CNC on nanocomposite processing for tissue engineering applications. Reprinted with permission from Rui M. A. Domingues, Manuela E. Gomes, and Rui L. Reis [54].

4. Cellulose Nanocrystals Based Systems

4.1. Cellulose Nanocrystal Based Nanocomposites: Dense Films and Porous Structures

Considering their safety and efficacy, cellulose structures and in particular, cellulose nanocrystals (CNC), have attracted increasing attention in biomedical applications as reinforcements in composite materials. Dense bi-dimensional (2D) films and tri-dimensional (3D) porous scaffolds with different properties, architectures and pore sizes can be produced from synthetic or naturally occurring materials and cellulose nanofibres, cellulose nanocrystals and bacterial nanocellulose are widely used as bio-based nanoreinforcements in several polymeric matrices owing to their superior mechanical properties [11]. The high surface-area-to-volume ratio of the nanofibers favor cell adhesion, proliferation, migration and differentiation, which are useful properties for tissue engineering applications [55]. The development of nanofibres can be used to design nanocomposites able to mimic the architecture of natural human tissue. Moreover, toxicity tests conducted on cellulose nanostructures indicate that they are non-toxic to cells [56], and does not give serious environmental concerns [57,58]. Cellulose fiber reinforced polymer composites have received much attention because of their low density, nonabrasive, combustible, nontoxic, low cost and biodegradable properties. A lot of research studies have been performed on the use of cellulose fibers as a reinforcing materials although certain drawbacks, such as incompatibility with the hydrophobic polymer matrix, the tendency to aggregate and water-swellable nature of cellulose, reduce the potential of natural fibers as reinforcements in polymers. Moreover, considering, in particular, CNC as reinforcements, their interfacial adhesion with the selected polymer matrix is one of the most important factors affecting nanocomposite final properties. In order to enhance the dispersion of CNC and increase the interfacial strength between the two phases, various methods have been used, such as magnetic field alignment within the polymer [59], use of plasticizers [60] and surfactants [61], or specific surface modifications [62].

A quite extensive range of polymer matrices, both synthetic and natural, has been used to produce nanocomposite dense films and porous membranes containing CNC. Recently, Pooyan et al. have developed a cellulose-based nanohybrid material in which CNC were dispersed within a cellulose acetate propionate matrix to form a 3D rigid percolating network, aimed at fabricating a potential scaffold candidate in TE of small diameter vascular grafts [63]. CNC extracted from microcrystalline cellulose were suspended in acetone prior to the composite fabrication in order to ensure a uniform distribution. Thin films were produced by solvent casting and, according to the authors, the obtained bio-nanocomposites exhibited excellent mechanical performance at body temperature, claiming that CNC impart significant strength and directional rigidity even at very low concentrations.

In a multifunctional nanocomposite approach, Rescignano et al. [64] described the production of a new generation of hybrid bio-nanocomposites dense films based on a poly(vinyl alcohol) (PVA) matrix reinforced with a combination of cellulose nanocrystals and poly (D,L lactide-co-glycolide) (PLGA) nanoparticles (NPs) loaded with bovine serum albumin fluoresceinisothiocynate conjugate (FITC-BSA). The authors demonstrated that the studied bio-nanocomposite films are suitable to vehiculate biopolymeric nanoparticles to adult bone marrow mesenchymal stem cells successfully, representing a new tool for drug delivery strategies. In a different ternary nanocomposite system based on PVA matrix, George et al. combined bacterial CNC and silver (Ag) nanoparticles (1 wt %) as nanofillers [65]. In addition to the antimicrobial properties imparted by the Ag nanoparticles, a synergistic effect on the improvement of the mechanical properties of the PVA films was obtained by combining these two nanomaterials; while CNC increased the modulus and tensile strength of the films, the Ag nanoparticles decreased its brittleness. These effects could be useful in the development of multifunctional hybrid materials for TE applications. Taking into account the use of natural polymer, Li et al. have developed collagen-based nanocomposite films reinforced with CNC for potential applications in skin TE. The films were prepared by a solvent-casting method. The authors proved that the addition of CNC to collagen-based nanocomposite films improved their bulk ordering and stability and led to an increase in the swelling capacity, which is highly desirable for the proposed application, as it enables the absorption of a large amount of exudates in the early stages of wound healing. The mechanical properties of the composite films were also significantly improved when compared with those of the pure collagen film while the composite formulations showed no obvious cytotoxicity facilitating cell adhesion and displaying an excellent biocompatibility [66].

The fabrication of ternary fibrous mats based on poly(lactic) acid (PLA), cellulose nanocrystals (CNC), both pristine (p-CNC) and modified with a commercial surfactant (s-CNC)) and silver nanoparticles by electrospinning is reported by Cacciotti et al. [67]. The addition of Ag and/or pristine p-CNC fillers did not remarkably affect the fiber morphology and the average size dimension. On the other hand, more interestingly, the authors proved that the presence of surfactant-modified s-CNC promoted the self-assembling of submicrometric fibers in bundles randomly organized in polygonal structures, such as pentagons and hexagons, resulting in a honeycomb-like network (Figure 3). The study demonstrated that it is possible to modulate the thermal, mechanical properties and especially morphological properties of PLA fibers, properly selecting the cellulose-based reinforcements able to induce specific topography useful for the final applications.

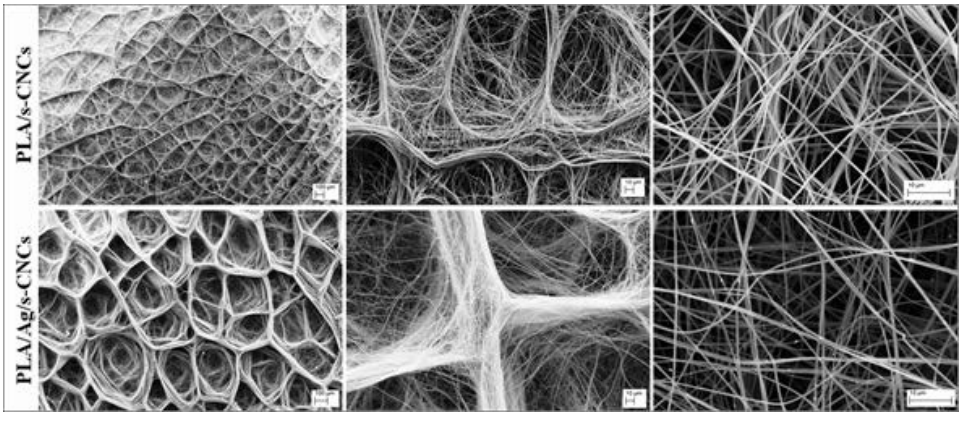

Figure 3. SEM micrographs of electrospun PLA/s-CNC and PLA/Ag/s-CNC mats. Reprinted with permission from Ilaria Cacciotti, Elena Fortunati, Debora Puglia, Josè Maria Kenny and Francesca Nanni [67].

Natural silk fibroin/CNC-based porous nanocomposites have also been prepared by electrospinning. Huang et al. [68] produced electrospun silk fibroin nanofiber mats reinforced with CNC extracted from Morus alba L. branch bark (20–40 nm in diameter and 400–500 nm in length). The authors reported that CNC were homogeneously dispersed and aligned along the fiber axis in the silk fibroin matrix while an increase in mechanical response was detected. Similar enhanced mechanical properties were achieved with bacterial cellulose nanocrystal as nanofillers in silk fibroin nanofibers [69].

The increase of knowledge on the cellulose-based components acted as a leverage regarding its practical use in the biomedical, pharmaceutical and/or cosmetic markets. Wound dressing systems based on pure oxidized regenerated cellulose (ORC) have been developed such as Tabotamp® and as mixtures from collagen and ORC named Promogran®. Tabotamp® is a thin gauze layer used in acute wounds like trauma, surgical injuries and burns while Promogran® is a commercial spongy collagen matrix and cellulose introduced in the US and European markets for the treatment of exuding diabetic and ulcer wounds [11]. Moreover, bacterial cellulose-based porous membranes have been applied for guided bone regeneration in bone defects, periodontal lesions and as a resorbable barrier membranes for preventing the invasion of fibroblast cells and fibrous connective tissue into bone defects [11].

The summarized recent research studies concerning cellulose-based materials and their uses as products in biomedical or pharmaceutical fields will contribute to sustain these market developments.

4.2. Cellulose Nanocrystals Based Hydrogels

Hydrogels are polymeric materials, chemically or physically crosslinked, characterized by a three-dimensional (3D) and elastic network capable to swell or de-swell when immersed in aqueous solutions. In particular, chemically crosslinked hydrogels are prepared either through water-soluble polymer crosslinking or by converting hydrophobic into hydrophilic polymers, which in turn are then crosslinked to form a network [70]. Lately, a particular class of polymer hydrogels has gained more interest in the scientific community; the so-called smart or stimuli-responsive hydrogels, which are able to change their size and shape in response to an external stimulus, such as temperature, pH, ionic force, pressure, electric and magnetic field. The change in solubility or the degree of swelling are due to a fine balance among competing interactions such as electrostatic forces and hydrophobic dehydration [71]. Moreover, some systems have been developed to combine two or more stimuli-responsive mechanisms into one polymer system. Recently, dual stimuli-responsive or ternary stimuli-responsive polymer hydrogel microspheres were prepared and applied in various fields, especially in controlled release drug delivery systems [72]. Hydrogels were the first biomaterials to be rationally designed for use in humans. They are biocompatible, their aqueous environment can protect cells and fragile drugs (such as peptides, proteins, etc.), they present good transport of nutrients to cells and metabolic products from the cells, they may be easily modified with cell adhesion ligands, or they can be injected in vivo [73]. All these properties make it an interesting candidate for biomedical and pharmaceutical applications [74]. The main areas in which hydrogels are used as biomaterials are in contact lenses, synthetic wound coverings, drug-delivery systems, permselective membranes, and in organ and tissue replacements, such as skin, tendon, cartilage, heart valve stents and bone [75]. However, because of the random nature of the crosslinking reactions produced by a large number of organic crosslinker polymer, hydrogels exhibit poor mechanical properties, which strongly limit their use in structural applications. For such a reason, different nanofillers, such as silicates [76], ceramics [77], metals [78], magnetic particles [79] and graphene [80] have been introduced into the hydrogel matrices, thus obtaining the corresponding nanocomposites. The incorporation of nanoparticles into the hydrogel 3D matrix, producing a class of materials known as nanocomposite hydrogels (NHPs), has been a widely investigated strategy to improve some existing physical properties or to provide them with new physical or chemical features. Considering the excellent dispersion of cellulose nanocrystals in water, they are obvious candidates to prepare NHPs, having many advantages compared to other polymeric or metal nanoparticles [54]. Cellulose-based hydrogels represent an important material class in biomedical fields due to their biocompatibility and biodegradability. Several water-soluble cellulose derivatives can be used

as mono-components of multi-component systems and they can be used also as reinforcement phases in some other biodegradable polymers to form hydrogel networks with specific properties in terms of swelling capability and sensitivity to external stimuli [81]. However, the current trend in the design of cellulose-based hydrogels is associated with the use of non-toxic crosslinking agents or crosslinking chemical treatments, to further improve the safety of both the final product and the manufacturing process [11], and few recent studies have investigated the incorporation of CNC with particular surface modifications in the preparation of NPHs, thus acting as both filler and cross-linker to reinforce hydrogel systems. Zhou et al. [82] demonstrated that the CNC acted as reinforcing agents, and also as multifunctional cross-linkers, accelerating the formation of hydrogels and increasing their effective cross-linking density. Moreover, chemically cross-linked gelatin/CNC hydrogels were prepared using oxidized CNC as cross-linkers [83]. The authors proved that the dialdehyde groups of oxidized CNC reacted with the free amine groups of gelatin to cross-link the hydrogel framework and the final properties of these materials were dependent on the amounts of CNC aldehyde groups.

Recently, Sanna et al. [71] reported on the synthesis and characterization of thermoresponsive poly(N-vinylcaprolactam), PNVCL, nanocomposite hydrogels containing nanocrystalline cellulose produced by a frontal polymerization technique, which is a convenient, easy and low-energy-consuming method of macromolecular synthesis. The authors proved that the presence of CNC resulted in a significant increase of the mechanical properties even at very low CNC concentrations, as confirmed by rheological tests indicating that the nanocellulose has a great potential to reinforce PNVCL polymer hydrogels. Finally, Yang et al. arguably reported the first injectable hydrogels reinforced both physically and covalently with CNC, based on a carboxymethyl cellulose (CMC)/dextran system [84]. Their approach was based on coextruding aldehyde functionalized CNC with dihydrazide-modified CMC and aldehyde-modified dextran solutions through a double-barrel syringe. The produced NPHs and their components revealed no evident cytotoxicity to NIH 3T3 fibroblast cells. The authors hypothesize that these CNC reinforced injectable polysaccharide hydrogels are of potential interest for TE applications where longer term dimensional stability and enhanced mechanical strength are desirable [84].

5. Concluding Remarks

Over the past few years, a growing interest has emerged in applying cellulose structures, and in particular cellulose nanocrystals, as biomaterials for the development of advanced functional bionanocomposites which could find a wide range of potential applications in TE. This Chapter has attempted to provide a general overview of the potential of CNC in the design of these functional

nanomaterials, through various examples involving different approaches and processes. Recent studies demonstrated that different types of nanomaterials presenting desired properties and functions could be produced from CNC. Research on nanocomposite films, membranes and hydrogels with potential application in TE strategies was summarized.

The use of lignocellulosic materials in drug-delivery devises has been studied for several years while the use of cellulose derivatives as excipients in drug formulations is already a standard in the pharmaceutical industry. In the future, the appearance of commercial formulations that are able to control the release rate and timeframe of different drugs, is expected. However, it is necessary to attract the industry to this growing market opportunity capable of creating sustainable technological improvements in bionanocomposites.

The positive results obtained by the research activity concerning increased performance of the bionanocomposites, will contribute to sustain these market developments although it is important to validate the most sustainable supplies of lignocellulosic materials. In addition, it is also relevant to create mechanisms to contribute to the certification of these nanostructured biomaterials according to common methods and standards.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Klemm, D.; Kramer, F.; Moritz, S.; Lindström, T.; Ankerfors, M.; Gray, D.; Dorris, A. Nanocelluloses: A new family of nature-based materials. *Angew. Chem. Int. Ed.* **2011**, *50*, 5438–5466. [CrossRef] [PubMed]
2. Siro, I.; Plackett, D. Microfibrillated cellulose and new nanocomposite materials: A review. *Cellulose* **2010**, *17*, 459–494. [CrossRef]
3. Krassig, H.A. *Cellulose: Structure, Accessibility and Reactivity*; Polymer Monographs 11; Huglin, M.B., Ed.; Gordon and Breach Science Publishers: Amsterdam, The Netherlands, 1993; p. 376.
4. Tingaut, P.; Zimmermann, T.; Sèbe, G. Functionalization of Microfibrillated Cellulose (MFC) and cellulose nanowhiskers fort the elaboration of functional materials. *J. Mater. Chem.* **2012**, *22*, 20105–20111. [CrossRef]
5. Habibi, Y.; Chanzy, H.; Vignon, M.R. TEMPO-mediated surface oxidation of cellulose whiskers. *Cellulose* **2006**, *13*, 679–687. [CrossRef]
6. Brinchi, L.; Cotana, F.; Fortunati, E.; Kenny, J.M. Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications. *Carboydr. Polym.* **2013**, *94*, 154–169. [CrossRef] [PubMed]
7. Lin, S.P.; Calvar, I.L.; Catchmark, J.M.; Liu, J.R.; Demirci, A.; Cheng, K.C. Biosynthesis, production and applications of bacterial cellulose. *Cellulose* **2013**, *20*, 2191–2219. [CrossRef]

8. Armentano, I.; Dottori, M.; Fortunati, E.; Mattioli, S.; Kenny, J.M. Biodegradable polymer matrix nanocomposites for tissue engineering: A review. *Polym. Degrad. Stab.* **2010**, *95*, 2126–2146. [CrossRef]
9. Kamel, S. Nanotechnology and its applications in lignocellulosic composites, a mini review. *Express Polym. Lett.* **2007**, *1*, 546–575. [CrossRef]
10. Darder, M.; Aranda, P.; Ruiz-Hitzky, E. Bionanocomposites: A new concept of ecological, bioinspired, and functional hybrid materials. *Adv. Mater.* **2007**, *19*, 1309–1319. [CrossRef]
11. Fernandes, E.M.; Pires, R.A.; Mano, J.F.; Reis, R.L. Bionanocomposites from lignocellulosic resources: Properties, applications and future trends for their use in the biomedical field. *Prog. Polym. Sci.* **2013**, *38*, 1415–1441. [CrossRef]
12. Armentano, I.; Bitinis, N.; Fortunati, E.; Mattioli, S.; Rescignano, N.; Verdejo, R.; Lopez-Manchado, M.A.; Kenny, J.M. Multifunctional nanostructured PLA materials for packaging and tissue engineering. *Prog. Polym. Sci.* **2013**, *38*, 1720–1747. [CrossRef]
13. Fortunati, E.; Armentano, I.; Zhou, Q.; Iannoni, A.; Saino, E.; Visai, L.; Berglund, L.A.; Kenny, J.M. Multifunctional bionanocomposite films of poly(lactic acid), cellulose nanocrystals and silver nanoparticles. *Carboydr. Polym.* **2012**, *87*, 1596–1605. [CrossRef]
14. Fortunati, E.; Peltzer, M.; Armentano, I.; Jiménez, A.; Kenny, J.M. Combined effects of cellulose nanocrystals and silver nanoparticles on the barrier and migration properties of PLA nano-biocomposites. *J. Food Eng.* **2013**, *18*, 117–124. [CrossRef]
15. Eichhorn, S.J.; Dufresne, A.; Aranguren, M.; Capadona, J.R.; Rowan, S.J.; Weder, C.; Thielemans, W.; Roman, M.; Renneckar, S.; Gindl, W.; et al. Review: Current international research into cellulose nanofibres and nanocomposite. *J. Mater. Sci.* **2010**, *45*, 1–33. [CrossRef]
16. Dahman, Y. Nanostructured biomaterials and biocomposites from bacterial cellulose nanofibres. *J. Nanosci. Nanotechnol.* **2009**, *9*, 5105–5122. [CrossRef] [PubMed]
17. Williams, D.F. On the mechanisms of biocompatibility. *Biomaterials* **2008**, *29*, 2941–2953. [CrossRef] [PubMed]
18. Nadia, H.; Mohd, C.I.M.A.; Ishak, A.; Zulkifli, M.H.; Noriah, J. Swelling of bacterial cellulose–acrylic acid hydrogels: Sensitivity towards external stimuli. *Sains Malays.* **2009**, *38*, 785–791.
19. Oshima, T.; Taguchi, S.; Ohe, K.; Baba, Y. Phosphorylated bacterial cellulose for adsorption of proteins. *Carbohydr. Polym.* **2011**, *83*, 953–958. [CrossRef]
20. Wang, J.H.; Gao, C.; Zhang, Y.S.; Wan, Y.Z. Preparation and in vitro characterization of BC/PVA hydrogel composite for its potential use as artificial cornea biomaterial. *Mater. Sci. Eng. C* **2010**, *3*, 214–218. [CrossRef]
21. Habibi, Y.; Lucia, L.A.; Rojas, O.J. Cellulose nanocrystals: Chemistry, self-assembling, and applications. *Chem. Rev.* **2012**, *110*, 3479–3500. [CrossRef] [PubMed]
22. John, M.J.; Sabu, T. Biofibres and biocomposites. *Carbohydr. Polym.* **2008**, *71*, 343–364. [CrossRef]
23. Fengel, D.; Wegener, G. *Wood Chemistry, Ultra-Structure, Reactions*; Walter de Gruyter: Berlin, Germany; New York, NY, USA, 1984.

24. Moon, R.J.; Martini, A.; Nairn, J.; Simonsen, J.; Younblood, J. Cellulose nanomaterials review: Structure, properties and nanocomposites. *Chem. Soc. Rev.* **2011**, *40*, 3941–3994. [CrossRef] [PubMed]
25. Kalia, S.; Dufresne, A.; Cherian, B.M.; Kaith, B.S.; Avérous, L.; Njuguna, J.; Nassiopoulos, E. Cellulose-based bio- and nanocomposites: A review. *Int. J. Polym. Sci.* **2011**, *2011*, 1–35. [CrossRef]
26. Kalia, S.; Kaith, B.S.; Kaur, I. Pretreatments of natural fibres and their application as reinforcing material in polymer composites: A review. *Polym. Eng. Sci.* **2009**, *49*, 1253–1272. [CrossRef]
27. Herrick, F.W.; Casebier, R.L.; Hamilton, J.K.; Sandberg, K.R. Microfibrillated cellulose: Morphology and accessibility. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1983**, *37*, 797–813.
28. Turbak, A.F.; Snyder, F.W.; Sandberg, K.R. Microfibrillated cellulose, a new cellulose product: Properties, uses, and commercial potential. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1983**, *37*, 815–827.
29. Zimmermann, T.; Bordeanu, N.; Strub, E. Properties of nanofibrillated cellulose from different raw materials and its reinforcement potential. *Carbohydr. Polym.* **2010**, *79*, 1086–1093. [CrossRef]
30. Boldizar, A.; Klason, C.; Kubat, J.; Näslund, P.; Saha, P. Prehydrolyzed cellulose as reinforcing filler for thermoplastics. *Int. J. Polym. Mater.* **1987**, *11*, 229–262. [CrossRef]
31. Henriksson, M.; Henriksson, G.; Berglund, L.A.; Lindstrom, T. An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers. *Eur. Polym. J.* **2007**, *43*, 3434–3441. [CrossRef]
32. Pääkkö, M.; Ankefors, M.; Kosonen, H.; Nykänen, A.; Ahola, S.; Österberg, M.; Ruokolainen, J.; Laine, J.; Larsson, P.T.; Ikkala, O.; et al. Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels. *Biomacromolecules* **2007**, *8*, 1934–1941. [CrossRef] [PubMed]
33. Berglund, L. Cellulose-based nanocomposites. In *Natural Fibers, Biopolymers, and Biocomposites*; Mohanty, A., Misra, M., Drzal, L., Eds.; CRC Press: Boca Raton, FL, USA, 2005; pp. 807–832.
34. Fortunati, E.; Puglia, D.; Monti, M.; Peponi, L.; Santulli, C.; Kenny, J.M.; Torre, L. Extraction of cellulose nanocrystals from Phormiumtenax fibres. *J. Polym. Environ.* **2012**, *21*, 319–328. [CrossRef]
35. Favier, V.; Canova, G.R.; Cavaille, J.Y.; Chanzy, H.; Dufresne, A.; Gauthier, C. Nanocomposite materials from latex and cellulose whiskers. *Polym. Adv. Technol.* **1995**, *6*, 351–355. [CrossRef]
36. Siqueira, G.; Tapin-Lingua, S.; Bras, J.; da Silva Perez, D.; Dufresne, A. Morphological investigation of nanoparticles obtained from combined mechanical shearing, and enzymatic and acid hydrolysis of sisal fibers. *Cellulose* **2010**, *17*, 1147–1158. [CrossRef]
37. Hsieh, Y.C.; Yano, H.; Nogi, M.; Eichhorn, S.J. An estimation of the Young's modulus of bacterial cellulose filaments. *Cellulose* **2008**, *15*, 507–513. [CrossRef]
38. Chanliaud, E.; Burrows, K.M.; Jeronimidis, G.; Gidley, M.J. Mechanical properties of primary plant cell wall analogue. *Planta* **2002**, *215*, 989–996. [CrossRef] [PubMed]

39. Kim, D.Y.; Nishiyama, Y.; Kuga, S. Surface acetylation of bacterial cellulose. *Cellulose* **2002**, *9*, 361–367. [CrossRef]
40. Petersen, N.; Gatenholm, P. Bacterial cellulose-based materials and medical devices: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 1277–1286. [CrossRef] [PubMed]
41. Backdahl, H.; Risberg, B.; Gatenholm, P. Observations on bacterial cellulose tube formation for application as vascular graft. *Mater. Sci. Eng. C* **2011**, *31*, 14–21. [CrossRef]
42. Bodin, A.; Concaro, S.; Brittberg, M.; Gatenholm, P. Bacterial cellulose as a potential meniscus implant. *J. Tissue Eng. Regen. Med.* **2007**, *1*, 406–408. [CrossRef] [PubMed]
43. Klemm, D.; Schumann, D.; Kramer, F.; Heßler, N.; Hornung, M.; Schmauder, H.P.; Marsch, S. Nanocelluloses as innovative polymers in research and application polysaccharides II. *Adv. Polym. Sci.* **2006**, *2015*, 49–96.
44. Jagur-Grodzinski, J. Polymers for tissue engineering, medical devices, and regenerative medicine. Concise general review of recentstudies. *Polym. Adv. Technol.* **2006**, *17*, 395–418. [CrossRef]
45. Armentano, I.; Fortunati, E.; Mattioli, S.; Rescignano, N.; Kenny, J.M. Biodegradable Composite scaffolds: A strategy to modulate Stem cell behaviour. *Recent Pat. Drug Deliv. Formul.* **2013**, *7*, 9–17. [CrossRef] [PubMed]
46. Dvir, T.; Timko, B.P.; Kohane, D.S. Nanotechnological strategies for engineering complex tissues. *Nat. Nanotechnol.* **2011**, *13*, 13–22. [CrossRef] [PubMed]
47. Zhang, L.; Webster, T.J. Nanotechnology and nanomaterials: Promises for improved tissue regeneration. *Nano Today* **2009**, *4*, 66–80. [CrossRef]
48. D'Angelo, F.; Armentano, I.; Cicciotti, I.; Tiribuzi, R.; Tiribuzi, R.; Quattrocelli, M.; Del Gaudio, C.; Fortunati, E.; Saino, E.; Caraffa, A.; Cerulli, G.G.; et al. Tuning multi-/pluri-potent stem cell fate by electrospun poly(L-lactic acid)-calcium-deficient hydroxyapatite nanocomposite mats. *Biomacromolecules* **2012**, *13*, 1350–1360.
49. Dugan, J.M.; Gough, J.E.; Eichhorn, S.J. Bacterial cellulose scaffolds and cellulose nanowhiskers for tissue engineering. *Nanomedicine* **2013**, *8*, 287–298. [CrossRef] [PubMed]
50. Fang, B.; Wan, Y.Z.; Tang, T.T.; Tang, T.T.; Gao, C.; Dai, K.R. Proliferation and osteoblastic differentiation of human bone marrow stromal cells on hydroxyapatite/bacterial cellulose nanocomposite scaffolds. *Tissue Eng. Part A* **2009**, *15*, 1091–1098. [CrossRef] [PubMed]
51. Saska, S.; Barud, H.S.; Gaspar, A.M.M.; Marchetto, R.; Ribeiro, S.J.L.; Messaddeq, Y. Bacterial cellulose-hydroxyapatite nanocomposites for bone regeneration. *Int. J. Biomater.* **2011**, *2011*, 1–8. [CrossRef] [PubMed]
52. Zhijiang, C.; Guang, Y. Bacterial cellulose/collagen composite: Characterization and first evaluation of cytocompatibility. *J. Appl. Polym. Sci.* **2011**, *120*, 2938–2944. [CrossRef]
53. Mathew, A.P.; Oksman, K.; Sain, M. Mechanical properties of biodegradable composites from poly lactic acid (PLA) and microcrystalline cellulose (MCC). *J. Appl. Polym. Sci.* **2005**, *97*, 2014–2025. [CrossRef]
54. Domingues, R.M.A.; Gomes, M.E.; Reis, R.L. The potential of cellulose nanocrystals in tissue engineering strategies. *Biomacromolecules* **2014**, *15*, 2327–2346. [CrossRef] [PubMed]

55. Vasita, R.; Katti, D.S. Nanofibres and their applications in tissue engineering. *Int. J. Nanomed.* **2006**, *1*, 15–30. [CrossRef]
56. Roman, M.; Dong, S.P.; Anjali, H.; Lee, Y.W. Cellulose Nanocrystals for Drug Delivery. In *Polysaccharides Materials: Performance by Design*; ACS Symposium Series; Edgar, K.J., Heinze, T., Buchanan, C., Eds.; American Chemical Society: Washington, DC, USA, 2010; Volume 1017, pp. 81–91.
57. Kovacs, T.; Naish, V.; O'Conner, B.; Blaise, C.; Gagne, F.; Hall, L.; Trudeau, V.; Martel, P. An eco-toxicologial characterization of nanocrystalline cellulose (NCC). *Nanotoxicology* **2010**, *4*, 255–270. [CrossRef] [PubMed]
58. Kümmerer, K.; Menz, J.; Schubert, J.; Thielmens, W. Biodegradability of organic nanoparticles in the aqueous environment. *Chemosphere* **2011**, *82*, 1387–1392. [CrossRef] [PubMed]
59. Pooyan, P.; Kim, I.T.; Jacob, K.I.; Tannenbaum, R.; Garmestani, H. Design of a cellulose-based nanocomposite as a potential polymeric scaffold in tissue engineering. *Polymer* **2013**, *54*, 2105–2114. [CrossRef]
60. Li, R.; Zhang, Y.; Zhu, L.; Yao, J. Fabrication and characterization of silk fibroin/poly(ethylene glycol)/cellulose nanowhisker composite films. *J. Appl. Polym. Sci.* **2012**, *124*, 2080–2086. [CrossRef]
61. Fortunati, E.; Peltzer, M.; Armentano, I.; Torre, L.; Jiménez, A.; Kenny, J.M. Effects of modified cellulose nanocrystals on the barrier and migration properties of PLA nano-biocomposites. *Carbohydr. Polym.* **2012**, *90*, 948–956. [CrossRef] [PubMed]
62. Rueda, L.; Saralegi, A.; Fernandez-d'Arlas, B.; Zhou, Q.; Alonso-Varona, A.; Berglund, L.A.; Mondragon, I.; Corcuera, M.A.; Eceiza, A. In situ polymerization and characterization of elastomeric polyurethane-cellulose nanocrystal nanocomposites. Cell response evaluation A. *Cellulose* **2013**, *20*, 1819–1828. [CrossRef]
63. Pooyan, P.; Tannenbaum, R.; Garmestani, H.J. Mechanical behavior of a cellulose-reinforced scaffold in vascular tissue engineering. *Mech. Behav. Biomed. Mater.* **2012**, *7*, 50–59. [CrossRef] [PubMed]
64. Rescignano, N.; Fortunati, E.; Montesano, S.; Emiliani, C.; Kenny, J.M.; Martino, S.; Armentano, I. PVA bio-nanocomposites: A new take-off using cellulose nanocrystals and PLGA nanoparticles. *Carbohydr. Polym.* **2014**, *99*, 47–58. [CrossRef] [PubMed]
65. George, J.; Sajeevkumar, V.A.; Ramana, K.V.; Sabapathy, S.N. Augmented properties of PVA hybrid nanocomposites containing cellulose nanocrystals and silver nanoparticles. *J. Mater. Chem.* **2012**, *22*, 22433–22439. [CrossRef]
66. Li, W.; Guo, R.; Lan, Y.; Zhang, Y.; Xue, W.; Zhang, Y. Preparation and properties of cellulose nanocrystals reinforced collagen composite films. Biomed. *Mater. Res. Part A* **2014**, *102*, 1131–1139. [CrossRef] [PubMed]
67. Cacciotti, I.; Fortunati, E.; Puglia, D.; Kenny, J.M.; Nanni, F. Effect of silver nanoparticles and cellulose nanocrystals on electrospun poly(lactic) acid mats: Morphology, thermal properties and mechanical behavior. *Carbohydr. Polym.* **2014**, *103*, 22–31. [CrossRef] [PubMed]
68. Huang, J.; Liu, L.; Yao, J. Electrospinning of *Bombyxmori* silk fibroin nanofiber mats reinforced by cellulose nanowhiskers. *J. Fibers Polym.* **2011**, *12*, 1002–1006. [CrossRef]

69. Park, D.J.; Choi, Y.; Heo, S.; Cho, S.Y.; Jin, H.J. Bacterial cellulose nanocrystals-embedded silk nanofibers. *J. Nanosci. Nanotechnol.* **2012**, *12*, 6139–6144. [CrossRef] [PubMed]
70. Slaughter, B.V.; Khurshid, S.S.; Fisher, O.Z.; Khademhosseini, A.; Peppas, N.A. Hydrogels in regenerative medicine. *Adv. Mater.* **2009**, *21*, 3307–3329. [CrossRef] [PubMed]
71. Sanna, R.; Fortunati, E.; Alzari, V.; Nuvoli, D.; Terenzi, A.; Casula, M.F.; Kenny, J.M.; Mariani, A. Poly(*N*-vinylcaprolactam) nanocomposites containing nanocrystalline cellulose: A green approach to thermoresponsive hydrogels. *Cellulose* **2013**, *20*, 2393–2402. [CrossRef]
72. Garbern, J.C.; Hoffman, A.S.; Stayton, P.S. Injectable pH- and temperature-responsive poly(*N*-isopropylacrylamide-co-propylacrylic acid) copolymers for delivery of angiogenic growth factors. *Biomacromolecules* **2010**, *11*, 1833–1839. [CrossRef] [PubMed]
73. Allan, S.H. Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **2002**, *54*, 3–12.
74. Liang, X.; Kozlovskaya, V.; Chen, Y.; Zavgorodnya, O.; Kharlampieva, E. Thermosensitive multilayer hydrogelsof poly(*N*-vinylcaprolactam) as nanothin films and shapedcapsules. *Chem. Mater.* **2012**, *24*, 3707–3719. [CrossRef] [PubMed]
75. Millon, L.E.; Wan, W.K. The polyvinyl alcohol-bacterial cellulose systemas a new nanocomposite for biomedical applications. *J. Biomed. Mater. Res. Part B* **2006**, *79*, 245–253. [CrossRef] [PubMed]
76. Loizou, E.; Butler, P.; Porcar, L.; Kesselman, E.; Talmon, Y.; Dundigalla, A.; Schmidt, G. Large scale structures in nanocomposite hydrogels. *Macromolecules* **2005**, *38*, 2047–2049. [CrossRef]
77. Liang, L.; Liu, J.; Gong, X. Thermosensitive poly(*N*-isopropylacrylamide)-clay nanocomposites with enhancedtemperature response. *Langmuir* **2000**, *16*, 9895–9899. [CrossRef]
78. Stuart, C.M.A. Supramolecular perspectives in colloidscience. *Colloid Polym. Sci.* **2008**, *286*, 855–864. [CrossRef]
79. Liu, T.Y.; Hu, S.H.; Liu, K.H.; Shaiu, R.S.; Liu, D.M.; Chen, S.Y. Instantaneousdrug delivery of magnetic/thermally sensitive nanospheresby a high-frequency magnetic field. *J. Controlled Release* **2008**, *126*, 228–236. [CrossRef] [PubMed]
80. Sanna, R.; Alzari, V.; Nuvoli, D.; Scognamillo, S.; Marceddu, S.; Mariani, A. Polymer hydrogels of 2-hydroxyethylacrylate and acrylic acid obtained by frontal polymerization. *J. Polym. Sci. Part A Polym. Chem.* **2012**, *50*, 1515–1520. [CrossRef]
81. Sannino, A.; Demitri, C.; Madaghiele, M. Biodegradable cellulose-basedhydrogels: Design and applications. *Materials* **2009**, *2*, 353–373. [CrossRef]
82. Zhou, C.; Wu, Q.; Yue, Y.; Zhang, Q.J. Application of rod-shaped cellulose nanocrystals in polyacrylamide hydrogels. *Colloid Interface Sci.* **2011**, *353*, 116–123. [CrossRef] [PubMed]
83. Wang, Y.; Chen, L. Impacts of nanowhisker on formation kinetics and properties of all-cellulose composite gels. *Carbohydr. Polym.* **2011**, *83*, 1937–1946. [CrossRef]
84. Yang, X.; Bakaic, E.; Hoare, T.; Cranston, E.D. Injectable polysaccharide hydrogels reinforced with cellulose nanocrystals: Morphology, rheology, degradation, and cytotoxicity. *Biomacromolecules* **2013**, *14*, 4447–4455. [CrossRef] [PubMed]