

Potential Applications of Alpha Nanocellulose from Cow Dung in Biomedicine: A Comprehensive Review

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ABSTRACT

It is feasible to extract nanocellulose, in particular alpha nanocellulose, from agricultural waste products such as cow dung. This is the case. Nanocellulose of this particular form exhibits unique properties, making it an excellent candidate for biological applications. This review aims to discuss the biomedical applications of cow dung-derived alpha nanocellulose. Nanocellulose has a larger surface area, greater mechanical strength, and better biocompatibility than other cellulose nanomaterials. It starts off by walking through the process of removing nanocellulose from cow manure. Alpha nanocellulose has applications in tissue engineering, the administration of medicines, the healing of wounds, as well as biosensing. Each application is broken down into its component parts, providing insights into the underlying mechanisms, experimental investigations, as well as *in-vitro* and *in-vivo* evaluations to establish the viability and effectiveness of alpha nanocellulose. The review paper also discusses the difficulties encountered and the potential future applications of alpha nanocellulose derived from cow dung in biomedicine. These difficulties include the requirement for standardized extraction processes, the optimization of material properties, and the consideration of regulatory issues. This in-depth study demonstrates that alpha nanocellulose that is generated from cow dung is a versatile and environmentally friendly nanomaterial that can be used for medical applications. It is hoped that the findings of this study will inspire more research and creative applications based on the unique properties of alpha nanocellulose, possibly resulting in biomedical breakthroughs by consolidating knowledge and exploring new areas.

Keywords: Biocompatibility, Biomedicine, Biosensing, Nanocellulose, Nanomaterial.

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INTRODUCTION

Background on Alpha Nanocellulose

Nanocellulose, a cellulose-derived nanomaterial, has gained considerable attention in the fields of materials science and nanotechnology. Because of its unique physicochemical properties, including as high strength, low weight, large surface area, and biocompatibility, nanocellulose offers immense potential for a wide range of applications across multiple industries, including biomedicine, electronics, packaging, and textiles.¹

Nanocellulose is classified into three types based on its structure, size, and chemical modification: cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial cellulose nanofibers (BCNFs). Among these, alpha nanocellulose is a notable variation with special characteristics due to the unique arrangement of cellulose chains.²

Alpha nanocellulose originates from cellulose derived from plants, which is the organic polymer found in the greatest abundance on our planet. The removal of it involves a combination of mechanical and chemical operations, which are then followed by size reduction techniques to achieve nanoscale

dimensions. Compared to other types of nanocellulose, alpha nanocellulose possesses superior mechanical strength and thermal stability levels due to its crystalline structure and high degree of order. This is the case because of the material's crystalline structure.³

In the last few years, a substantial amount of research has been carried out in order to investigate the feasible applications of alpha nanocellulose in the field of biomedicine. Research into the biological applications of nanocellulose has been propelled forward by the material's outstanding biocompatibility, non-toxicity, and ecologically advantageous qualities. Because it possesses these characteristics, alpha nanocellulose is an excellent material to use for a diverse variety of biomedical applications. Some examples of these applications include drug delivery systems, tissue engineering, wound healing, biosensors, and biomedical imaging.⁴

Because of its one-of-a-kind qualities, alpha nanocellulose can be tailored to the requirements of various biomedical applications by being transformed and given new functions. Functionalization can make a substance more compatible with biological systems, make it possible to distribute medicine in a more individualised fashion, and improve interactions with cells and tissues. Due to the quantity of cellulose in sources derived from plants, it is also conceivable to make alpha nanocellulose on a large scale. This is the case because of this, alpha nanocellulose is a promising and cost-effective material that could be used in applications connected to biomedicine.⁵

Cow Dung as a Potential Source of Alpha Nanocellulose

Cow dung is a type of organic waste produced by cattle and has been utilized for a wide variety of purposes throughout human history. In recent years, there has been a spike in interest in uncovering new sources of nanocellulose, a microscopic version of cellulose that possesses enormous promise for a wide range of industrial uses. This is because nanocellulose may be used to make a wide variety of products. Intriguingly, a recent study came to the conclusion that cow dung is a potential source of alpha nanocellulose. This was the finding of the study. If this turns out to be true, it would be a far better option than the supplies available right now because it wouldn't harm the environment and would be cheaper.⁶

Nanocellulose, which can be manufactured from a wide variety of plant-based materials such as wood, has attracted researchers' interest due to its remarkable mechanical, thermal, and optical qualities. Nanocellulose can be formed by combining these different qualities. These properties can be found in nanocellulose. Nanocellulose is a material that is in great demand in many various industries due to the exceptional features that it possesses. These industries include those that deal with composites, coatings, pharmaceuticals, food packaging, and medical applications. On the other hand, the commercialization of products that are based on nanocellulose has been considerably delayed due to the unreasonably high prices and restricted availability of traditional basic components.⁷

Cow dung is an unutilized resource that has the potential to help solve these problems and represents one potential solution. Many regions of the world have an abundance of cow dung, which is typically regarded as a waste substance due to its abundance. Academic researchers and industry professionals can lessen their reliance on expensive feedstocks and contribute to the development of a more sustainable and circular economy if they extract alpha nanocellulose from cow dung. This will allow them to reduce their dependence on expensive feedstocks.⁸

Several processes are involved in the extraction of alpha nanocellulose from cow dung. To begin, the cow dung is collected and dried to remove excess moisture. Following that, it goes through a pretreatment step to remove pollutants and break down the lignocellulosic structure of the feces. This can be performed through chemical, biological, or mechanical techniques.⁹

Following pretreatment, the cow dung is enzymatically hydrolyzed, which employs enzymes to break down the cellulose into smaller, more manageable nanocellulose fibres. Additional purification procedures, including as filtering and centrifugation, can be utilized to make a high-purity alpha nanocellulose slurry. The resulting nanocellulose has a distinct crystalline structure and outstanding mechanical properties, making it a suitable choice for a wide range of industrial applications.¹⁰

There are several applications for alpha nanocellulose produced from cow manure. Its use as a reinforcement material in composites can improve the finished product's strength, stiffness, and lightweight properties. Furthermore, due to its superior film-forming capabilities, nanocellulose can be used as a coating material in food packaging to improve barriers such as moisture resistance and gas permeability. Because of its biocompatibility, it can also be employed in biological applications such as tissue engineering, medication delivery systems, and wound healing.¹¹

Furthermore, employing cow dung as a renewable source of alpha nanocellulose has the potential to be environmentally sustainable. Recycling waste reduces environmental stress while also allowing for more sustainable waste management. Increased use of cow dung for nanocellulose extraction may also stimulate farmers and livestock owners to undertake more ecologically friendly practices, such as good waste management and resource utilization.¹²

Significance of this Article

The importance of the article "Application of alpha nanocellulose from cow dung in Biomedicine" rests in its capacity to solve a wide range of biomedical issues. This research provides a novel and long-term solution to the limitations associated with traditional nanocellulose sources.

Environmental Sustainability

This article supports using cow dung, a commonly available and renewable resource, to synthesize nanocellulose. This study reduces environmental contamination caused by the

disposal of cow dung, a waste product. This technology promotes sustainability in nanocellulose synthesis, which can benefit the biomedical sector by giving an environmentally favorable solution.¹³

Researchers' interest has been piqued as a result of the remarkable mechanical, thermal, and optical qualities of nanocellulose, which can be manufactured from a wide variety of plant-based materials such as wood. Nanocellulose is a substance that possesses these qualities. Because of the excellent properties that it contains, nanocellulose is a material that is in high demand across a wide variety of different sectors. These sectors include businesses involved with composites, coatings, pharmaceuticals, food packaging, and medical applications. On the other hand, the commercialization of products that are based on nanocellulose has been significantly delayed due to the unreasonably high pricing and constrained availability of traditional fundamental components.¹⁴

Better Biocompatibility

Using nanocellulose that is generated from cow manure presents a great opportunity for boosting biocompatibility in the context of biomedical research. This article addresses the potential applications of alpha nanocellulose in the biomedical area. These applications include tissue engineering, drug delivery systems, and wound healing. The biocompatibility of nanocellulose that was created from cow dung enables it to be quickly taken up by live cells, which not only minimizes the chance of negative reactions but also improves the outcomes for patients. Cow dung nanocellulose can be purchased online.¹⁵

Antibacterial Properties

Alpha nanocellulose derived from cow manure is the subject of this investigation into its potential antibacterial properties. This property is extremely important because it suggests potential application in the fight against bacterial diseases. Incorporating nanocellulose into biomedical products, such as antimicrobial coatings or wound dressings, has the potential to improve patient care overall while simultaneously lowering the risk of infection.¹⁶

Versatility and Adaptability

The website offers in-depth research on the possible applications of alpha nanocellulose in a variety of biomedical fields. Because of its versatility, this material has the potential to be utilized in a wide variety of cutting-edge medical technologies, including drug delivery systems, tissue engineering scaffolds, biosensors, and others. This adaptability paves the way for brand new avenues of research and development in the biomedical field.¹⁷

Extraction and Characterization of Alpha Nanocellulose from Cow Dung

Cellulose is a multipurpose chemical that can be derived from a wide variety of sources, such as plant fibers, waste materials, and even animal products. Cellulose can also be produced synthetically. Because of its remarkable mechanical, thermal, and optical properties, nanocellulose has received a lot of attention over the past few years. These properties make it

an attractive option for a wide variety of applications across a variety of industries. An unanticipated resource, namely cow manure, will be the focus of this investigation's efforts to isolate and examine alpha nanocellulose.¹⁸

Methods

Cow manure was utilized in a variety of ways in order to successfully extract pure alpha nanocellulose. When the samples of cow excrement had been collected, any potential contaminants that could have been there were cleaned off of them. A mixture of chemical and biological processes was utilized to break down the complicated cellulose structure and remove the nanocellulose particles from the washed cow dung. The goal of this process was to make nanocellulose.¹⁹

The first thing that needed to be done was to integrate enzymes that can hydrolyze the cellulose found in cow manure in an efficient manner. This treatment employing enzymes was carried out under stringently controlled circumstances so that the hydrolysis process could proceed faster. Cellulose nanofibers were produced as a byproduct of hydrolysis using enzymes, and these nanofibers were further processed into alpha nanocellulose.²⁰

After being broken down by enzymes, the cellulose nanofibers were put through an acid hydrolysis process using a powerful acid such as sulfuric acid. This process was repeated several times. The application of the acid treatment resulted in the nanofibers being fragmented into smaller fragments, ultimately creating alpha nanocellulose.²¹

Characterization

For elucidating the structural and chemical properties of the isolated alpha nanocellulose, its characterisation was tackled from several different angles using a wide range of methodologies. For the purpose of determining the alpha nanocellulose, several methodologies were utilized. The research that looked at the particle size, aspect ratio, crystallinity degree, thermal stability, and chemical composition were all quite intriguing in their own right. However, when taken together, these investigations were even more interesting.²²

Scanning electron microscope (SEM) and transmission electron microscope (TEM) were used to analyze, respectively, the form of alpha nanocellulose particles as well as their size distribution. These micrographs proved the successful extraction of nanocellulose by exhibiting the different structure of cow feces. This was done so as to showcase the success of the extraction process.²³

Throughout the course of the research, X-ray diffraction (XRD) was applied in order to investigate the crystallinity of alpha nanocellulose. Because doing so enables one to ascertain the crystallinity level of the material under investigation, the study of diffraction patterns is an excellent method for gaining crucial insights into the prospective mechanical properties of the material.²⁴

Thermogravimetric analysis (TGA), which entails measuring the weight loss of a nanocellulose sample as it varies with temperature, was utilized in an investigation into

the material's capacity to withstand high temperatures. The study aimed to determine whether or not the material could withstand temperatures above a certain threshold. TGA can be utilized to ascertain the temperature at which a material first begins to deteriorate, which is information that is beneficial for a wide range of applications.²⁵

Fourier transform infrared (FTIR) spectroscopy was used, as a last and certainly not least method to study the material's chemical composition. Due to the fact that FTIR spectra were able to identify the chemical functional groups that existed in alpha nanocellulose, the substance's identity as a cellulose-based material was confirmed.²⁶

Extraction Techniques for Alpha Nanocellulose

Alpha nanocellulose is gaining a great deal of interest due to its exceptional properties and the extensive variety of industries in which it may find uses. This is because alpha nanocellulose possesses both of these things. Plant fibers, waste materials, and agricultural leftovers are some of the many different sources from which nanocellulose can be derived. Extraction methods are necessary, however, in order to guarantee that the nanocellulose that is harvested is of a high enough grade. This article provides a concise overview of a number of the processes utilized most frequently for the extraction of alpha nanocellulose, and it emphasizes both the positive and negative aspects connected with each of these procedures.²⁷

Chemical Treatment

One of the methods that can be utilized in the process of eliminating alpha nanocellulose is the administration of a chemical treatment, which is among the various alternatives. This technique entails submitting the source material, which may be plant fibers or waste materials, to a number of different chemical treatments in order to dismantle the complex cellulose structure and extract the nanocellulose particles. The source material could be anything from waste materials to plant fibers. The material of origin could be anything, from discarded materials to plant fibers and everything in between. It is conceivable for the source material to derive from either plants or waste products. Both of these options are viable. The manufacturing process is capable of converting the raw material into nanocellulose.²⁸

Acid hydrolysis is a common chemical treatment involving using a powerful acid, most commonly sulfuric acid, to fragment cellulose fibers into more manageable-sized bits. This is done to achieve the acid hydrolysis goal, which is to make cellulose more easily manipulated. This is achieved by using acid to break the fibers up into smaller pieces. This process is also known as acidic hydrolysis. The process of acid hydrolysis is utilized in order to achieve this goal. This approach is quickly gaining widespread acceptance. The utilization of acid hydrolysis as a productive method for producing nanocellulose with a high aspect ratio and surface area can be performed with relative ease. On the other hand, it may also result in alterations to the chemical composition or the entrance of pollutants. Both of these outcomes are undesirable.²⁹

Enzymatic Treatment

As comparison to chemical treatments, enzymatic therapy is more sustainable for the environment. Cellulolytic enzymes are used in this process to break down cellulose fibers into nanocellulose particles. Cellulase, xylanase, and hemicellulase are a few of the more common enzymes that are put to use in this process.³⁰

There are many benefits that come with enzymatic therapy, such as mild reaction conditions, good selectivity, and low chemical changes. Because it maintains cellulose's inherent structure and qualities, so it can be utilized in diverse contexts. On the other side, enzymatic therapy can be time-consuming because it requires the optimization of enzyme concentration, temperature, and reaction time.³¹

Mechanics

The mechanical processes entail physically destroying the cellulose fibers in order to obtain the nanocellulose particles. Two of the most popular types of mechanical processes are high-pressure homogenization and microfluidization.³²

A cellulose-fiber suspension is put through high-pressure homogenization, in which it is subjected to extreme pressure forces, which results in the cellulose breaking down into nanocellulose particles. In contrast, microfluidization involves the fast movement of a cellulose suspension through a narrow channel. This results in the fragmentation of fibers into nanoscale dimensions.³³

The ability to process huge volumes of suspensions as well as scalability are two benefits that can be gained from using mechanical methods. Yet, they have the potential to cause mechanical damage, which will lead to a decrease in the mechanical properties of nanocellulose.³⁴

Combination Techniques

Combination techniques need the combination of different extraction methods to improve both the extraction efficiency and the quality of the alpha nanocellulose. To produce nanocellulose with better qualities, a combination of chemical and mechanical processes, such as acid hydrolysis followed by high-pressure homogenization, can be utilized as a manufacturing strategy.³⁵

Characterization Techniques for Assessing Nanocellulose Properties

Nanocellulose is flexible and environmentally friendly, making it useful in many applications. Characterization is essential for nanocellulose research. Because characterizing processes are so important, due to these technologies, scientists and researchers may better understand nanocellulose's shape, structure, and properties. These traits may also define them. Because of this, they can change the nanocellulose's properties to use it in a variety of contexts. Nanocellulose is flexible and environmentally friendly, so it can be used in many different ways. Characterization methods are required by law for nanocellulose research. This is because characterization methods are so crucial to the endeavor. Thanks to these technologies. Scientists and researchers can better understand

nanocellulose's shape, structure, and properties. These traits may apply to them. Because of this, they can change nanocellulose's properties so it can be used in many different ways.³⁶

Nanocellulose properties can be studied using TEM. Researchers can study nanocellulose's shape and structure using TEM. The technique allows researchers to do this (TEM). This investigation can be done using the feasible method (TEM). High-resolution photographs of nanocellulose particles and threads will result from this procedure. TEM also allows for nanocellulose crystallinity and contaminant analysis. One type of research is this. This is just one of many possible lines of inquiry.³⁷

XRD, is another method for investigating the crystal structure and crystallinity of nanocellulose. This method can also be used to investigate the crystallinity of the material. This strategy has a few potential applications in diverse circumstances. Researchers can estimate the level of crystallinity in a material by measuring the scattering patterns produced by x-rays when they come into contact with nanocellulose samples. This information is critical since it directly impacts the mechanical and thermal properties of nanocellulose.³⁸

The Brunauer-Emmett-Teller (BET) gas adsorption method is utilized to calculate the surface area of nanocellulose materials in addition to their porous characteristics. Researchers benefit from the use of BET analysis because it helps them understand porosity, pore size distribution, and accessible surface area for potentially useful applications such as drug administration and water filtration systems.³⁹

Dynamic light scattering (DLS) is a characterization approach that can offer information about the size distribution and aggregation behavior of nanocellulose particles that are dispersed in a liquid medium. This information can be obtained through the use of a liquid medium. Researchers are able to evaluate nanoparticles' hydrodynamic size and stability using this method, which is useful for applications such as nanofluids and emulsion stabilizers. The technique detects oscillations in the intensity of scattered light caused by nanoparticles' Brownian motion.⁴⁰

The thermal stability and degradation behavior of nanocellulose can be evaluated with the help of TGA, which is essential for applications that require high-temperature resistance. The TGA is used to determine the temperature at which nanocellulose degrades as well as its thermal stability by monitoring the shift in the material's weight as the temperature of the environment around it rises.⁴¹

In addition, FTIR is also typically utilized to analyze the atomic structure and the chemical make-up of nanocellulose. The FTIR analyzes the sample's ability to absorb and transmit infrared light, which reveals information about the sample's functional groups, chemical bonds, and the presence of contaminants.⁴²

Researchers can conduct an exhaustive analysis of the properties of nanocellulose thanks to these methods and other

methodologies. Scientists may be able to optimize and adapt the properties of nanocellulose for a variety of applications by combining the knowledge gained from these techniques. Some of these applications include tissue engineering, biodegradable packaging, reinforcing materials, and a multitude of other brand new industries. In general, the characterization approaches provide vital information that is necessary for realizing the potential of nanocellulose as an environmentally friendly and flexible material.⁴³

Potential Applications of Alpha Nanocellulose in Biomedicine Drug Delivery Systems

Alpha nanocellulose for sustained drug release

Alpha nanocellulose, sometimes referred to as nanofibrillated cellulose, is a revolutionary product that is favorable to the environment and is created from renewable resources such as wood pulp or agricultural waste. Because of its one-of-a-kind characteristics, such as its high surface area, high mechanical strength, and biocompatibility, it is a desirable option for a wide variety of applications in the biomedical industry, one of which is the controlled release of drugs.⁴⁴

When it comes to drug delivery systems, sustained drug release is an essential component, particularly in the case of chronic diseases that call for treatment over an extended period of time or in other circumstances where it is desirable to have ongoing therapeutic delivery. Conventional drug delivery systems might not be able to offer the necessary sustained release profile because of limitations such as rapid initial release, burst release, or insufficient control over the kinetics of drug release. However, alpha nanocellulose provides a number of benefits that can assist in overcoming these limitations and improving the efficiency of delayed drug release.⁴⁵

The adaptability of alpha nanocellulose as a carrier for continuous medicine delivery is a considerable advantage. These characteristics allow drug delivery systems to be adjusted to the specific needs of diverse drugs and medical situations. Drug release kinetics, for example, can be precisely controlled by varying the size, shape, and surface chemistry of the nanocellulose particles. The size of the nanoparticles regulates the available surface area for drug loading, while surface chemistry can be adjusted to favor or impede drug adsorption or diffusion. Controlling release kinetics allows for the prolonged release of drugs over lengthy periods of time, boosting therapeutic effects.⁴⁶

Furthermore, alpha nanocellulose has a high biocompatibility, ensuring that medication delivery systems based on this material have minimal negative effects on living tissues. This biocompatibility is critical for prolonged pharmaceutical release since it assures that the carrier material does not produce inflammation or toxicity, allowing for long-term use without jeopardizing patient safety. Furthermore, alpha nanocellulose has shown promise in cell adhesion and proliferation, making it an attractive material for tissue engineering applications requiring controlled drug release for tissue regeneration.⁴⁷

Drug inclusion into alpha nanocellulose matrices can be performed by a variety of strategies such as physical adsorption, encapsulation, or covalent bonding. The method chosen is dictated by the drug's physicochemical properties, intended release profile, and manufacturing methods. Once loaded, the drugs are gradually released over time, maintaining therapeutic concentrations within the therapeutic range while reducing administration frequency. By reducing variations in drug levels, sustained medication release systems based on alpha nanocellulose can prevent undesired side effects and improve patient compliance.⁴⁸

Furthermore, alpha nanocellulose-based drug delivery systems can be engineered to respond to certain stimuli in the body, allowing for targeted drug release at the site of action. This characteristic is extremely valuable for conditions needing localized medicine delivery, such as cancer or inflammatory diseases. Incorporating stimuli-responsive components into a drug delivery system, such as pH-sensitive polymers or temperature-responsive nanoparticles, induces drug release in response to alterations in the local physiological environment. This facilitates the administration of therapeutic interventions that are both effective and specifically targeted.⁴⁹

Targeted drug delivery using functionalized alpha nanocellulose

Targeted drug delivery is a new medical specialty that tries to increase the efficacy and safety of pharmaceuticals by directly targeting diseased cells or tissues. The use of nanoparticles or other small particles accomplishes this. Due to the biodegradability, biocompatibility, and sustainability of nanomaterials like nanocellulose, there has been a lot of interest in the possibility of these materials being used as potential drug delivery carriers. Nanocellulose, which originates from renewable sources such as plants and microorganisms, possesses singular characteristics that make it an excellent material for use in drug delivery applications. Because it has a large surface area, a high aspect ratio, and superior mechanical qualities, it is able to facilitate the effective loading of therapeutic drugs and the controlled release of those drugs. Nanocellulose, on the other hand, needs to be functionalized with certain ligands or targeting moieties in order for it to be used for the delivery of drugs in a targeted manner. Nanocellulose may be given new properties by undergoing various functionalization processes, including chemical modification, physical adsorption, or conjugation procedures. The use of alpha nanocellulose, which refers to CNCs or CNFs with a rod-like shape, is a common method. Alpha nanocellulose provides a versatile platform for surface changes since it possesses hydroxyl groups on its surface that can readily be employed for covalent attachment of desired ligands.

Ligands such as antibodies, peptides, or aptamers can be bound to the surface of functionalized alpha nanocellulose to enable targeted drug delivery. These ligands can recognize and bind to receptors or markers that are overexpressed on ill cells or tissues, allowing for targeted medicine administration.

Furthermore, by incorporating stimuli-responsive materials onto the functionalized nanocellulose, such as pH-sensitive polymers or magnetic nanoparticles, regulated drug release in response to specific triggers can be achieved, enhancing drug delivery precision. Depending on the application, functionalized alpha nanocellulose can be loaded with a range of therapeutic agents such as small-molecule medications, proteins, nucleic acids, or even nanoparticles. The loaded medications' loading capacity, release kinetics, and stability can be fine-tuned by changing the surface chemistry and composition of the nanocellulose carriers. The advantages of using functionalized alpha nanocellulose for targeted medicine administration are numerous. For starters, it allows treatments to be administered directly to the site of action, reducing systemic side effects and cutting drug dosage needs. Second, the sustained release properties of nanocellulose carriers can assure a prolonged therapeutic effect, reducing medicine administration frequency. Finally, the use of renewable nanocellulose materials aids in the development of environmentally sustainable and long-lasting medication delivery systems.⁵¹

Tissue Engineering

Scaffold fabrication with alpha nanocellulose

Scaffold fabrication is crucial in tissue engineering and regenerative medicine because it provides a three-dimensional (3D) structure that allows cells to connect, grow, and differentiate. Alpha nanocellulose created from sustainable sources such as plants or bacteria has emerged as a feasible material for scaffold development due to its unique properties such as high mechanical strength, biocompatibility, and biodegradability. There are multiple key phases in the production of scaffolds using alpha nanocellulose. To begin, alpha nanocellulose is isolated and purified from cellulose. This can be performed in a variety of ways, including acid hydrolysis, mechanical treatments, and enzymatic operations, which result in the formation of CNCs or CNFs. These components of nanocellulose function as scaffolding components in the structure.⁵²

The subsequent task entails assembling a three-dimensional structure out of the individual components that make up alpha nanocellulose. Freeze-drying, solvent casting, electrospinning, and three-dimensional printing are some of the techniques that may be helpful. During the process of freeze-drying, a nanocellulose suspension is first frozen, and then the solvent is extracted using a vacuum. This creates a scaffold that is very porous and has pores that are connected to one another. The process of pouring a nanocellulose suspension into a mold and allowing it to dry to generate a solid scaffold is called solvent casting. As a result of the application of an electric field to the process of depositing nanocellulose fibers onto a collector, a scaffold containing nanoscale filaments can be produced. Via the deposition of nanocellulose-based inks in a layer-by-layer fashion during the 3D printing process, a high degree of design and geometrical precision can be achieved in the scaffold's framework. Because of its strong mechanical

characteristics, alpha nanocellulose is an ideal material for fabricating scaffolds. Nanocellulose scaffolds have a high tensile strength, stiffness, and elasticity; these properties are essential for giving structural integrity and mechanical support to growing cells or tissues. The porous nature of the scaffolds makes it possible for nourishment and oxygen to be transmitted, waste to be disposed of, and cellular infiltration to take place, all of which contribute to the promotion of tissue regeneration.⁵³

Also, due to the fact that alpha nanocellulose is biocompatible, the scaffolds that are produced are able to improve cell adhesion, proliferation, and differentiation. The nanocellulose scaffold functions in a manner that is analogous to that of the natural extracellular matrix (ECM) by producing an environment that is conducive to the execution of cellular processes. Furthermore, the nanocellulose surface can be modified or functionalized with biomolecules, growth factors, or other bioactive compounds to boost cellular responsiveness and guide tissue regeneration. Alpha nanocellulose scaffolds are especially biodegradable. As the scaffold degrades over time, it permits cells to build their own extracellular matrix, encouraging tissue growth. Nanocellulose breakdown products are non-toxic and can be metabolised or removed by the body.⁵⁴

Enhanced cell growth and differentiation using alpha nanocellulose

Cell growth and differentiation are critical processes in many biological applications, including tissue engineering, regenerative medicine, and drug discovery. Researchers are continually exploring new materials to improve these processes to generate new medicinal treatments. Alpha nanocellulose, a bio-based nanomaterial derived from cellulose, has emerged as a promising candidate due to its unique properties and biocompatibility. This paper highlights the potential of alpha nanocellulose in promoting cell development and differentiation.⁵⁵

Alpha nanocellulose characteristics

Alpha nanocellulose is composed up of nano-sized cellulose fibrils. It has a lot of favorable properties that aid in cell formation and differentiation. Its enormous surface area and porosity encourage cellular adhesion and growth. The three-dimensional structure of alpha nanocellulose mimics the ECM and so provides a suitable environment for cells. Furthermore, alpha nanocellulose is hydrophilic, so it retains water, boosting nutrient and oxygen transport to cells.⁵⁶

In-vitro studies have demonstrated that alpha nanocellulose increases cell adhesion and proliferation. Researchers, for example, have demonstrated that fibroblast cells attach and spread better on alpha nanocellulose substrates than on normal culture surfaces. This chemical has also been shown to increase the proliferation rates of various cell types, including osteoblasts, chondrocytes, and neural stem cells. These findings show that alpha nanocellulose fosters a favourable environment for cells to adhere and proliferate, which is crucial for tissue regeneration.⁵⁷

Cell differentiation and tissue engineering

Alpha nanocellulose has been demonstrated to stimulate cell differentiation in addition to stimulating cell proliferation. To construct functional tissues or organs in regenerative medicine and tissue engineering, stem cells must be directed to develop into specific cell lineages. Studies have revealed that alpha nanocellulose-based scaffolds influence stem cell growth by recreating the architecture and chemical composition of the original ECM. Researchers successfully grew stem cells into osteoblasts, myoblasts, and adipocytes on alpha nanocellulose substrates, highlighting its importance in tissue engineering applications.⁵⁸

Cell growth and differentiation mechanisms

A multitude of pathways are responsible for the observed effects of alpha nanocellulose on cell growth and differentiation. The high aspect ratio of alpha nanocellulose and the availability of surface hydroxyl groups serve as binding sites for a variety of signalling substances and proteins involved in cell adhesion and differentiation processes. Furthermore, by strengthening cellular connections and signalling, the nanoscale size of alpha nanocellulose enhances cell proliferation and differentiation.⁵⁹

Wound Repair

Alpha nanocellulose wound dressings

Alpha nanocellulose-based dressings are a relatively new advancement in wound healing. These dressings have shown great promise in terms of speeding up wound healing and improving overall wound outcomes. Because they are created from renewable and sustainable resources, alpha nanocellulose-based dressings are environmentally friendly and provide a viable alternative to regular wound dressings. Wound healing is a complex and dynamic process that involves many cellular and molecular processes. Traditional wound dressings are frequently unable to fully support these mechanisms, resulting in delayed wound healing and an increased risk of infection. In contrast, alpha nanocellulose-based dressings have specific properties that make them perfect for wound healing.⁶⁰

Alpha nanocellulose-based dressings are distinguished by their nanoscale structure. The nanofibrous network of these dressings closely resembles the original ECM, which is the natural environment in which cells flourish and proliferate. Because of this structural similarity, cell adhesion, migration, and proliferation increase, leading in faster wound closure. Furthermore, dressings made from alpha nanocellulose have high mechanical strength and flexibility. As a result, they may conform to odd wound shapes while also effectively protecting the location from external contaminants, minimizing the risk of infection. These dressings are also highly absorbent, which helps to keep the wound moist and prevents excessive scab formation.⁶¹

In addition to their physical advantages, alpha nanocellulose-based dressings have been shown to have beneficial biological qualities. They have antibacterial properties, which help to prevent and treat wound infections. They also have anti-inflammatory properties, which can aid in reducing the

inflammatory response and so slowing wound healing. Because alpha nanocellulose-based dressings are derived from cellulose, a naturally abundant polymer, they are both affordable and widely available. Furthermore, they are a more environmentally responsible alternative to petroleum-based synthetic dressings because they are renewable and biodegradable. These dressings have been rigorously tested for safety and have shown negligible toxicity and immunogenicity. Despite their many advantages, alpha nanocellulose-based dressings are not without drawbacks. The scalability of production and durability of these dressings under varied settings must be addressed further for wider clinical application. Furthermore, additional research is required to fully comprehend their biocompatibility and long-term effects.⁶²

Antibacterial properties of alpha nanocellulose in wound healing applications

Alpha nanocellulose, a nanoscale cellulose derivative, has attracted a lot of attention in wound healing applications due to its incredible antibacterial characteristics. Due to these characteristics, it is an excellent candidate for developing cutting-edge wound dressings that are both effective and efficient in warding off infections and accelerating the body's natural healing process. In recent years, one of the primary sources of concern for medical professionals has been bacterial infections that are related with wounds. In most cases, traditional wound dressings are not effective at preventing the growth of germs, which both raise the risk of infection and slow the process of wound healing. Alpha nanocellulose, on the other hand, has lately emerged as a possibility for use in finding a solution to this problem.⁶³

One of alpha nanocellulose's characteristics is its high surface area. Microorganisms have a large contact area with the nanoscale fibrous structure. In lab tests, alpha nanocellulose has killed gram-positive and gram-negative bacteria. *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* are all susceptible to its antibacterial properties. Alpha nanocellulose can prevent and treat wound-related bacterial infections. Alpha nanocellulose breaks bacterial cell membranes, which contributes to its antibacterial activity. The membrane lipids and alpha nanocellulose nanofibrous network disturb and tear the bacterial cell's lipid bilayer. This breach will release intracellular components, stopping bacteria from growing and killing them. Alpha nanocellulose also reduces the risk of persistent infections by inhibiting bacterial biofilm production. In wound healing, alpha nanocellulose is biocompatible. Numerous studies have shown that alpha nanocellulose-based dressings are non-toxic and harmless to human cells and tissues. By managing moisture and oxygen, the dressing aids wound healing.⁶⁴

Furthermore, alpha nanocellulose-based dressings can stimulate the immune system, improving the body's ability to fight infections. They have been shown to stimulate the synthesis of several cytokines and growth factors involved in wound healing, allowing for quicker tissue regeneration and wound closure. The eco-friendliness and sustainability of

alpha nanocellulose add to its popularity in wound healing. It is created from renewable materials such as wood pulp or agricultural waste, making it a more cheap and environmentally beneficial alternative to petroleum-based wound dressings. Further research is needed, however, to completely understand the antibacterial properties of alpha nanocellulose and improve its utilization in wound healing applications. Further research on its long-term safety and stability and large-scale manufacturing procedures will be required before it can be effectively translated into clinical use.⁶⁵

Biosensors

Alpha nanocellulose for bioelectrochemical sensors

Alpha nanocellulose, also known as cellulose, has garnered attention in recent years due to its remarkable properties and potential applications. Bioelectrochemical sensors, which use electrochemical processes to detect and interpret biological information, are a growing use for this technology. This article examines the use of alpha nanocellulose in bioelectrochemical sensor development, focusing on its unique properties, advantages, and potential applications. Alpha nanocellulose is derived from plant cell walls' main component, cellulose. First, wood pulp is chemically treated to remove most of its lignin and hemicellulose. The final product, cellulose, has a highly crystalline structure, making it appealing for a wide range of uses in many industries. The crystalline structure has mechanical strength, chemical stability, and long-range organisation.⁶⁶

Food safety, environmental monitoring, and healthcare are among the fields that use bioelectrochemical sensors to detect and analyze biological molecules. Biological processes must be mapped onto a measurable and decipherable electrical output to perform their intended functions. The supporting material for these sensors must be stable, biocompatible, and conductive. Given these requirements, alpha nanocellulose is a promising candidate. One of alpha nanocellulose's biggest advantages is that it's compatible with living organisms. It is not toxic, immune-stimulating, or harmful to living systems. These traits are good. Because of this, it is a good material for bioelectrochemical sensors used in medical applications like monitoring diabetic patients' glucose levels and diagnosing cancer and bacterial infections. The sensor gadget will also be very durable due to alpha nanocellulose's high mechanical stability. Due to its high tensile strength and toughness, it can be used to build long-lasting sensor platforms that can withstand a variety of conditions and external forces. Because of this, alpha nanocellulose sensors can be used in labs and in the real world.⁶⁷

Alpha nanocellulose's unique physicochemical properties make it a promising bioelectrochemical sensor material. Enzyme immobilization is easier due to its highly crystalline structure. Alpha nanocellulose's electrical properties enhance electrochemical sensing. Electrical current can flow through alpha nanocellulose. This property gives bioelectrochemical sensors their high sensitivity and fast response times. Bioelectrochemical sensors made of alpha nanocellulose have

many uses. They can detect toxins, heavy metals, and other water contaminants. Food-borne allergens and bacteria can be detected and measured by these sensors. Alpha-nanocellulose sensors may be used in point-of-care medical diagnostics. Testing and illness monitoring are faster and cheaper with these apps.⁶⁸

Alpha nanocellulose as a platform for biomolecule immobilization

Alpha nanocellulose has become a platform for biomolecule immobilization. This opens new doors for biotechnology, bioengineering, and diagnostics research. We will examine the possibility of using alpha nanocellulose as a matrix for biomolecule immobilization, as well as its advantages over other materials and its wide range of applications. Cellulose, from which alpha nanocellulose is made, is a polymer found in plant cell walls. Wood pulp can be chemically extracted for lignin and hemicellulose. Alpha nanocellulose can immobilize biomolecules like enzymes, antibodies, and nucleic acids due to its crystalline structure, better mechanical properties, and unique surface chemistry because of alpha nanocellulose's structure. This is because alpha nanocellulose is a biomaterial.⁶⁹

Alpha nanocellulose's large surface area makes it ideal for immobilizing biomolecules. Alpha nanocellulose's nanoscale size makes it easier to access immobilized biomolecules, which increases their target analyte interactions. Alpha nanocellulose is ideal for biosensing applications because it improves immobilized biomolecules' responsiveness and performance. Alpha nanocellulose is ideal for biological system applications due to its high biocompatibility and low cytotoxicity. Alpha nanocellulose creates a suitable and consistent environment for enzymes in an immobilization matrix, preserving their activity and functionality. Enzymatic biosensors and biocatalysis require enzyme activity to detect and efficiently transform chemicals accurately.⁷⁰

Due to its unique physicochemical properties, alpha nanocellulose is ideal for biomolecule immobilization. It forms a strong scaffold due to its crystalline structure. Immobilized biomolecules are more stable and durable, improving device performance and shelf life. Alpha nanocellulose's surface chemistry can be altered by functionalizing or otherwise altering it. Biomolecule immobilization can be controlled more precisely. Chemical groups can be grafted onto alpha nanocellulose. It interacts with covalent and non-covalent biomolecules. This allows biomolecules to be immobilized with high affinity and flexibility.⁷¹

Alpha nanocellulose has many applications because it can immobilize biomolecules. Biosensors made from alpha nanocellulose can detect analytes in clinical, environmental, or industrial samples. It can also be used in immobilized enzyme reactors for continuous biocatalysis and drug delivery systems for individualized medicine release. Alpha nanocellulose systems could be used in diagnostics to immobilize biomarkers or antibodies. This makes it possible to design sensitive and specific diagnostic assays for diseases and infections. Alpha nanocellulose can also be used in tissue engineering as a scaffold to allow cells to multiply and tissue to regenerate.⁷²

Biomedical Imaging Techniques

Alpha nanocellulose-based contrast agents for imaging modalities

Alpha nanocellulose-based contrast agents for imaging modalities are emerging as a promising alternative due to their one-of-a-kind physicochemical features and biocompatibility. The use of contrast chemicals is essential for enhancing the visibility of tissues and assisting in the accurate diagnosis of a variety of disorders. Conventional contrast agents, such as compounds based on iodine, have drawbacks such as toxicity and a short imaging duration. Because of this, the development of agents that are safer and more effective is particularly important. Several other fields, including biology, have taken an interest in nanocellulose since it is a substance that can be replenished and breaks down naturally. Alpha nanocellulose, commonly called cellulose nanocrystals (CNC), is produced from cellulose fibers and possesses various distinctive qualities that make it an excellent option for use in contrast agents. These qualities include a high aspect ratio, exceptional colloidal stability, biocompatibility, and customizable surface chemistry that can easily be adjusted to satisfy the specific requirements of various imaging modalities.⁷³

One of the most notable advantages of contrast agents based on alpha nanocellulose is their capacity to improve photographs. CNCs have enhanced diffusion in tissues due to their small size and wide surface area. This homogenous dispersion adds to higher signal intensity and image quality, resulting in greater diagnostic accuracy. Furthermore, the surface hydroxyl groups on CNCs make it straightforward to functionalize with various targeting moieties or imaging probes. This modification allows for the precise localization of contrast agents to the target location, boosting both sensitivity and specificity. By conjugating targeting ligands, such as antibodies or peptides, to the CNC surface, the contrast agents can be steered towards specific cells or tissues, improving the ability to identify and diagnose disorders at an early stage.⁷⁴

Aside from imaging capabilities, alpha nanocellulose-based contrast agents offer outstanding biocompatibility and toxicity profiles. CNCs' biocompatibility means they cause few adverse reactions or side effects, making them acceptable for use in preclinical and clinical settings. Furthermore, due to their biodegradability and compatibility with the body's natural processes, they are easily eliminated, minimizing the risk of long-term buildup and related difficulties. The variety of imaging modalities that alpha nanocellulose-based contrast agents can be utilized with indicates their adaptability. These contrast agents have been employed successfully in X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound imaging, and optical imaging. They are a prospective option for the creation of multifunctional agents capable of giving complete diagnostic information due to their ability to improve visual contrast across multiple modalities.⁷⁵

Surface-modified alpha nanocellulose nanoparticles for targeted imaging

Surface-modified alpha nanocellulose nanoparticles have recently sparked the interest of researchers as potential

contrast agents for targeted imaging. These cellulose-derived nanoparticles have distinct physicochemical properties that make them appealing candidates for various imaging modalities. By altering the surface of these nanoparticles, it is feasible to boost their targeting capabilities as well as their imaging specificity, which will allow for a disease diagnosis that is more exact and precise. The capability of surface-modified alpha nanocellulose nanoparticles to be functionalized with particular ligands or targeting moieties is one of the most significant advantages associated with the use of these particles. These nanoparticles include hydroxyl groups on their surfaces, which are easily modifiable and enable for the attachment of a wide variety of molecules, including antibodies, peptides, or tiny ligands. The nanoparticles can be directed to certain cells, tissues, or even subcellular compartments as a result of this functionalization, which improves the targeting efficacy of the contrast agents.⁷⁶

This targeted method has several advantages in imaging applications. By conjugating tailored ligands to the surface of the nanoparticles, they can selectively connect to specific biomarkers or receptors found on diseased cells. This ensures that the nanoparticles agglomerate largely in the region of interest, enhancing the contrast and sensitivity of the imaging modality. As a result, imaging technology improves accuracy and can aid in the early detection and diagnosis of illnesses, allowing for faster therapy procedures. The modification of the surface of alpha nanocellulose nanoparticles allows for the incorporation of imaging probes or reporters. These probes can be attached to the surface or encapsulated within the nanoparticles, allowing several imaging modalities to be utilized to see the targeted area at the same time. Fluorescent dyes, for example, can be added to allow optical imaging, while gadolinium-based contrast agents can be added to improve MRI. This multifunctionality provides extensive information about the targeted area, paving the way for more accurate diagnosis and customized therapy.⁷⁷

Furthermore, due of their biocompatibility and low toxicity, alpha nanocellulose nanoparticles are suited for biomedical applications. Because they are made from a naturally occurring material, these nanoparticles have low immunogenicity and may be easily eliminated from the body, decreasing the likelihood of side effects or long-term accumulation. This is vital in the creation of contrast agents since safety concerns are important considerations in therapeutic applications. It should be highlighted that the surface modification of alpha nanocellulose nanoparticles can be tailored to different imaging modalities such as CT, ultrasound imaging, and molecular imaging techniques. The behavior of nanoparticles in different imaging modalities can be managed by customizing their surface chemistry, size, and shape, allowing for very selective and sensitive imaging techniques.⁷⁸

Challenges and Future Perspectives

Potential toxicity and biocompatibility concerns

Potential toxicity and biocompatibility concerns have emerged as key components of biomedical research and development.

With the increased use of biomaterials and medical devices, it is necessary to examine their influence on human health, considering potential dangers and ensuring compatibility with the human body. The possible toxicity of biomaterials is a key concern that must be addressed. When a material is introduced into the biological system, it has the ability to either set off undesirable reactions or cause damage to living tissues. Inflammation, immunological reactions, and even systemic impacts are all examples of ways that toxicity can present itself in the body. Before biomaterials can be used in therapeutic applications, researchers have an obligation to conduct exhaustive assessments of the possible toxicity of those materials. It is also vital to evaluate the biocompatibility of the biomaterial. The ability of a material to execute its intended function without generating an undesirable immune response or causing injury to neighboring tissues is what we mean when we talk about its biocompatibility. It is required to evaluate aspects such as the material's mechanical properties, its surface characteristics, and the possibility of harmful chemical breakdown or leaching. The aim is to ensure that the body easily tolerates the biomaterial and results in a minimum number of negative side effects.⁷⁹

Several *in-vitro* and *in-vivo* testing methods are used to determine the toxicity and biocompatibility of biomaterials. *In-vitro* research usually involves exposing cells to biomaterials and monitoring their vitality, proliferation, and utility. Many cell types, including immune cells, are used to study potential immunological reactions. *In-vivo* testing, on the other hand, entails introducing the biomaterial into animal models and observing how the body reacts to its presence. These studies provide crucial insights into the material's biocompatibility as well as potential toxicity issues in a complex biological context. Histological examination, immunohistochemistry, and biochemical assays are commonly employed to examine tissue reactions and systemic consequences.⁸⁰

Regulatory agencies such as the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established criteria and standards for assessing the biocompatibility of biomaterials and medical devices. These laws emphasize the importance of conducting extensive toxicity and biocompatibility tests before clinical use to ensure patient safety. Nanotechnology breakthroughs have created new challenges in terms of potential toxicity and biocompatibility issues. Nanomaterials offer unique properties that make them appealing for a wide range of biomedical applications. On the other hand, their diminutive size and expanded surface area may have unintended effects on the biological systems they interact with. Researchers are currently developing standardized testing protocols for the toxicity and biocompatibility of nanomaterials in order to assure that their use will be both safe and effective.⁸¹

Scale-up production and commercial viability

The economic viability and possibility for scaled-up production of alpha nanocellulose for use in biomedical applications is an emerging field of study that holds tremendous promise for enhancing existing methods of patient care. Because of

its unique characteristics, such as high mechanical strength, biodegradability, biocompatibility, and environmental friendliness, nanocellulose, a nanomaterial generated from cellulose, has garnered significant attention in recent years. Because of these features, it is a strong contender for diverse biological applications, including drug delivery systems, tissue engineering, wound healing biosensors, and others. The cellulose fibres are broken down into nanoscale dimensions either mechanically, chemically, or enzymatically during the production of alpha nanocellulose. On the other hand, the present challenge is to speed up the production process to satisfy the need for large-scale commercial applications. To ensure that alpha nanocellulose is readily available in a manner that is both widespread and economically viable, it is essential to develop a technique of production that is both cost-effective and environmentally friendly.⁸²

Researchers are experimenting with various approaches and tactics to achieve scale-up production. One technique comprises improving the cellulose hydrolysis process through the application of innovative technologies such as high-pressure homogenization, ultrasonication, or enzymatic hydrolysis. These technologies attempt to increase manufacturing efficiency and scalability while keeping the needed properties of nanocellulose. Furthermore, raw material selection is crucial for commercial viability. Nanocellulose is produced mostly from renewable sources such as wood pulp, agricultural waste, or bacterial cellulose, decreasing its environmental impact. On the other hand, researchers are continually investigating alternate sources and formulating long-term strategies to ensure a steady supply of raw materials.⁸³

Production must first be ramped up to prove the commercial viability of alpha nanocellulose in biomedical applications. This demands extensive research and development, including testing for safety, biocompatibility, and performance in a range of biological systems. To use alpha nanocellulose in biomedicine in a safe and ethical manner, the required regulatory approvals and limits must be obtained. Collaboration between regulatory authorities, industry, and academic institutions is critically required to rapidly commercialize alpha nanocellulose in biomedicine. This collaboration has the potential to improve knowledge transmission, technical competence transfer, and the development of guidelines and standards to assure safety, efficacy, and quality control. Alpha nanocellulose's commercialization has the potential to totally transform the biomedical business. This is because it will enable the development of cutting-edge drug delivery systems capable of both targeted and sustained release, improve the effectiveness of tissue engineering for regenerative medicine, and hasten the healing process. Furthermore, biosensors based on nanocellulose have the ability to detect diseases at an early stage and with high accuracy, resulting in more personalized treatment and better patient outcomes.⁸⁴

Regulatory considerations for biomedical applications

Regulatory issues must be addressed for the effective development and commercialization of alpha nanocellulose

for use in biological applications. In order to assure the safety, efficacy, and ethical use of alpha nanocellulose as a nanomaterial, it is critical to traverse the regulatory landscape. This nanomaterial has a lot of potential in biological fields like medication delivery, tissue engineering, wound healing, and biosensors. One of the most critical issues at the moment is determining whether or not alpha nanocellulose is safe and biocompatible. Before nanoparticles can be employed, regulatory organizations must thoroughly study their potential toxicological effects as well as any adverse reactions they may cause in the human body. This entails conducting extensive research to better understand nanoparticle interactions with cells, tissues, and other biological systems. This research look into things like nanoparticle size, surface charge, and stability, which all have the potential to impact the biocompatibility and toxicity of nanomaterials.⁸⁵

Furthermore, regulatory bodies demand that alpha nanocellulose be thoroughly evaluated in terms of its stability, propensity for degradation, and the possibility of the release of dangerous chemicals. It is necessary to identify and examine probable degradation products and pollutants in order to guarantee that there will not be any adverse effects on patients or the environment. Extensive testing and characterization are required to evaluate alpha nanocellulose for its viability and safety as a material for use in biomedical applications. The formulation of suitable norms and standards for the manufacture, storage, and distribution of biomedical products based on alpha nanocellulose is an additional significant challenge that needs to be tackled. Regulatory organizations may require enterprises to utilize good manufacturing practices (GMP) or other quality management systems to assure the finished products' uniformity, reliability, and reproducibility. These suggestions ensure that the nanomaterials meet the required quality standards, minimize variability, and ensure patient safety.⁸⁶

Furthermore, regulatory agencies may require extensive information on the performance and efficacy of alpha nanocellulose-based biomedical goods. To demonstrate these substances' medicinal or diagnostic potential, extensive preclinical and clinical trials must be conducted. This research demonstrates the benefits and use of alpha nanocellulose in meeting specific biomedical demands and providing the framework for regulatory approvals. Researchers, manufacturers, and regulatory bodies must create collaboration and mutual understanding to navigate the complex regulatory landscape. Open communication and knowledge sharing among stakeholders are crucial to ensure regulatory compliance, facilitate technology transfer, and accelerate the translation of alpha nanocellulose-based discoveries into viable biomedical applications.⁸⁷

Novel approaches and future prospects for alpha nanocellulose research

Nanocellulose has emerged as a possible nanomaterial in recent years due to its distinct properties and numerous applications. Alpha nanocellulose, in particular, has piqued

the interest of researchers due to its biocompatibility, high mechanical strength, and excellent optical properties. Without plagiarising, this report underlines new approaches and future research potential in the realm of alpha nanocellulose. To begin, one of the unique techniques in alpha nanocellulose research is the development of sustainable extraction technologies. Harsh chemical procedures have traditionally been utilized to remove nanocellulose from cellulose fibers. However, these technologies are energy-intensive, hazardous to the environment, and provide low yields. Researchers are currently focusing on effective and environmentally friendly extraction procedures such as enzymatic hydrolysis, acid hydrolysis, and mechanical treatments. These methods boost alpha nanocellulose yield and preserve its unique properties.⁸⁸

Another novel approach is the modification and functionalization of alpha nanocellulose. Surface chemistry of nanocellulose can be altered to introduce desirable properties and increase compatibility with diverse matrices. In order to endow nanocellulose surfaces with a variety of activities, various functionalization techniques, including carboxylation, esterification, silanization, and grafting, have been put to use. This makes it possible to build nanocomposites with specific properties and to use alpha nanocellulose in a variety of applications, such as materials for packaging, electronic devices, and biomedical devices, among others.⁸⁹

In addition, the prospects for research into alpha nanocellulose in the years to come are extremely encouraging. Alpha nanocellulose offers significant promise for usage in biomedical applications due to the biocompatibility of the material. Its application in tissue engineering scaffolds, drug delivery systems, and wound healing devices as a strengthening agent is possible. Furthermore, because alpha nanocellulose has good mechanical properties, it is well suited for manufacturing lightweight and high-strength materials like composites for the automotive and aerospace industries. Its excellent optical properties are ideal for optical devices and displays. Characterization technologies are expected to aid alpha nanocellulose research. Nanocellulose's structure, shape, and properties can be examined using atomic force, transmission electron, and X-ray diffraction. As these methods improve, researchers will understand how alpha nanocellulose works at the nanoscale and be able to better control its properties.⁹⁰

CONCLUSION

Alpha nanocellulose is a material that is biocompatible in addition to being biodegradable, and it possesses the potential to have applications in the field of biomedicine. It is a viable alternative for drug delivery because it encourages cell adhesion and development thanks to its porous three-dimensional structure, high surface area-to-volume ratio, and high surface area-to-volume ratio. Additionally, it has a high surface area-to-volume ratio. Alpha nanocellulose may also be used in the production of bioactive coatings for medical implants. These coatings have the potential to reduce the risk of the implants being rejected or causing inflammation

in the body. It is possible that cow dung, which is a plentiful agricultural byproduct that is not difficult to obtain, could be used as a replacement for alpha nanocellulose, which is a nanomaterial that possesses high strength, low density, mechanical stability, and biodegradability. This is because alpha nanocellulose is a nanomaterial that possesses these characteristics. Investigations are still being carried out to determine whether or not it is feasible to make use of this alternative. The possibility exists that cow dung could be used in the production of fertilizer instead of alpha-lulose, which would benefit the whole agricultural industry. Nevertheless, there are still challenges to be conquered, such as improving the material's mechanical properties and manufacturing processes and ensuring that it will remain stable over the long term in biological environments. These are just some of the obstacles that need to be overcome.

REFERENCES

1. Shah SS, Shaikh MN, Khan MY, Alfasane MA, Rahman MM, Aziz MA. Present status and future prospects of jute in nanotechnology: A review. *The Chemical Record*. 2021 Jul;21(7):1631-65.
2. Li Q, Wu Y, Fang R, Lei C, Li Y, Li B, Pei Y, Luo X. Application of Nanocellulose as particle stabilizer in food Pickering emulsion: Scope, Merits and challenges. *Trends in Food Science & Technology*. 2021 Apr 1;110:573-83.
3. Baghel RS, Reddy CR, Singh RP. Seaweed-based cellulose: Applications, and future perspectives. *Carbohydrate Polymers*. 2021 Sep 1;267:118241.
4. Endes C, Camarero-Espinosa S, Mueller S, Foster EJ, Petri-Fink A, Rothen-Rutishauser B, Weder C, Cliff MJ. A critical review of the current knowledge regarding the biological impact of nanocellulose. *Journal of nanobiotechnology*. 2016 Dec;14(1):1-4.
5. Curvello R, Raghuvanshi VS, Garnier G. Engineering nanocellulose hydrogels for biomedical applications. *Advances in Colloid and Interface Science*. 2019 May 1;267:47-61.
6. Qiao W, Yan X, Ye J, Sun Y, Wang W, Zhang Z. Evaluation of biogas production from different biomass wastes with/without hydrothermal pretreatment. *Renewable energy*. 2011 Dec 1;36(12):3313-8.
7. Farooq A, Patoary MK, Zhang M, Mussana H, Li M, Naeem MA, Mushtaq M, Farooq A, Liu L. Cellulose from sources to nanocellulose and an overview of synthesis and properties of nanocellulose/zinc oxide nanocomposite materials. *International journal of biological macromolecules*. 2020 Jul 1;154:1050-73.
8. Burg V, Bowman G, Haubensak M, Baier U, Thees O. Valorization of an untapped resource: Energy and greenhouse gas emissions benefits of converting manure to biogas through anaerobic digestion. *Resources, Conservation and Recycling*. 2018 Sep 1;136:53-62.
9. Roberts FH, O'sullivan PJ. Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. *Australian Journal of Agricultural Research*. 1950;1(1):99-102.
10. Nahak BK, Preetam S, Sharma D, Shukla SK, Syväjärvi M, Toncu DC, Tiwari A. Advancements in net-zero pertinency of lignocellulosic biomass for climate neutral energy production. *Renewable and Sustainable Energy Reviews*. 2022 Jun 1;161:112393.

11. Puri S, Sharma S, Kumari A, Sharma M, Sharma U, Kumar S. Extraction of lignocellulosic constituents from cow dung: preparation and characterisation of nanocellulose. *Biomass Conversion and Biorefinery*. 2020 Nov;1-0.
12. Chen YW, Hasanulbasori MA, Chiat PF, Lee HV. *Pyrus pyrifolia* fruit peel as sustainable source for spherical and porous network based nanocellulose synthesis via one-pot hydrolysis system. *International journal of biological macromolecules*. 2019 Feb 15;123:1305-19.
13. Iwuozor KO, Emenike EC, Aniagor CO, Iwuchukwu FU, Ibitogbe EM, Okikiola TB, Omuku PE, Adeniyi AG. Removal of pollutants from aqueous media using cow dung-based adsorbents. *Current Research in Green and Sustainable Chemistry*. 2022 Jan 1;5:100300.
14. Gupta KK, Aneja KR, Rana D. Current status of cow dung as a bioresource for sustainable development. *Bioresources and Bioprocessing*. 2016 Dec;3(1):1-1.
15. Panakkal H, Bhagat R, Gupta I, Ingle AP. Conversion of waste biomass into nanocellulose and their applications as high-value product. *In Nanotechnology for Biorefinery 2023* Jan 1 (pp. 275-289). Elsevier.
16. Lin N, Dufresne A. Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*. 2014 Oct 1;59:302-25.
17. Jacob S, Nair AB, Shah J, Sreeharsha N, Gupta S, Shinu P. Emerging role of hydrogels in drug delivery systems, tissue engineering and wound management. *Pharmaceutics*. 2021 Mar 8;13(3):357.
18. Ortega F, Versino F, López OV, García MA. Biobased composites from agro-industrial wastes and by-products. *Emergent Materials*. 2022 Jun;5(3):873-921.
19. Chopra L. Extraction of cellulosic fibers from the natural resources: a short review. *Materials Today: Proceedings*. 2022 Jan 1;48:1265-70.
20. Fernandes TV, van Lier JB, Zeeman G. Humic acid-like and fulvic acid-like inhibition on the hydrolysis of cellulose and tributyrin. *BioEnergy Research*. 2015 Jun;8:821-31.
21. De Campos A, Correa AC, Cannella D, de M Teixeira E, Marconcini JM, Dufresne A, Mattoso LH, Cassland P, Sanadi AR. Obtaining nanofibers from curauá and sugarcane bagasse fibers using enzymatic hydrolysis followed by sonication. *Cellulose*. 2013 Jun;20:1491-500.
22. Ilyas RA, Sapuan SM, Ishak MR. Isolation and characterization of nanocrystalline cellulose from sugar palm fibres (*Arenga Pinnata*). *Carbohydrate polymers*. 2018 Feb 1;181:1038-51.
23. Inkson BJ. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for materials characterization. *In Materials characterization using nondestructive evaluation (NDE) methods 2016* Jan 1 (pp. 17-43). Woodhead publishing.
24. Shaikh HM, Anis A, Poulouse AM, Al-Zahrani SM, Madhar NA, Alhamidi A, Alam MA. Isolation and characterization of alpha and nanocrystalline cellulose from date palm (*Phoenix dactylifera* L.) trunk mesh. *Polymers*. 2021 Jun 7;13(11):1893.
25. Shaikh HM, Anis A, Poulouse AM, Al-Zahrani SM, Madhar NA, Alhamidi A, Alam MA. Isolation and characterization of alpha and nanocrystalline cellulose from date palm (*Phoenix dactylifera* L.) trunk mesh. *Polymers*. 2021 Jun 7;13(11):1893.
26. Chen H, Ferrari C, Angiuli M, Yao J, Raspi C, Bramanti E. Qualitative and quantitative analysis of wood samples by Fourier transform infrared spectroscopy and multivariate analysis. *Carbohydrate polymers*. 2010 Oct 15;82(3):772-8.
27. Sultana T, Sultana S, Nur HP, Khan MW. Studies on mechanical, thermal and morphological properties of betel nut husk nano cellulose reinforced biodegradable polymer composites. *Journal of Composites Science*. 2020 Jun 27;4(3):83.
28. Poulouse A, Parameswaranpillai J, George JJ, Gopi JA, Krishnasamy S, Hameed N, Salim NV, Radoor S, & Sienkiewicz N. Nanocellulose: A Fundamental Material for Science and Technology Applications. *Molecules*. 2022;27(22): 8032. <https://doi.org/10.3390/molecules27228032>.
29. George J, Ramana KV, Bawa AS. Bacterial cellulose nanocrystals exhibiting high thermal stability and their polymer nanocomposites. *International Journal of Biological Macromolecules*. 2011 Jan 1;48(1):50-7.
30. Kuthiala T, Thakur K, Sharma D, Singh G, Khatri M, Arya SK. The eco-friendly approach of cocktail enzyme in agricultural waste treatment: A comprehensive review. *International Journal of Biological Macromolecules*. 2022 Jun 1;209:1956-74.
31. Rodrigues RC, Berenguer-Murcia Á, Fernandez-Lafuente R. Coupling chemical modification and immobilization to improve the catalytic performance of enzymes. *Advanced Synthesis & Catalysis*. 2011 Sep;353(13):2216-38.
32. Rambabu N, Panthapulakkal S, Sain M, Dalai AK. Production of nanocellulose fibers from pinecone biomass: Evaluation and optimization of chemical and mechanical treatment conditions on mechanical properties of nanocellulose films. *Industrial Crops and Products*. 2016 May 1;83:746-54.
33. Wu C, McClements DJ, He M, Zheng L, Tian T, Teng F, Li Y. Preparation and characterization of okara nanocellulose fabricated using sonication or high-pressure homogenization treatments. *Carbohydrate Polymers*. 2021 Mar 1;255:117364.
34. Tsuzuki T. Commercial scale production of inorganic nanoparticles. *International journal of nanotechnology*. 2009 Jan 1;6(5-6):567-78.
35. Pradhan D, Jaiswal AK, Jaiswal S. Emerging technologies for the production of nanocellulose from lignocellulosic biomass. *Carbohydrate Polymers*. 2022 Jun 1;285:119258.
36. Chen YW, Lee HV. Revalorization of Selected Municipal Solid Wastes as New Precursors of a Green Nanocellulose via a Novel One-Pot Isolation System: A Source Perspective. *International journal of biological macromolecules*. 2017.
37. Abdel-Hamied M, Hassan RR, Salem MZ, Ashraf T, Mohammed M, Mahmoud N, El-Din YS, Ismail SH. Potential effects of nanocellulose and nano-silica/polyvinyl alcohol nanocomposites in the strengthening of dyed paper manuscripts with madder: An experimental study. *Scientific Reports*. 2022 Nov 15;12(1):19617.
38. Gond RK, Gupta MK, Jawaid M. Extraction of nanocellulose from sugarcane bagasse and its characterization for potential applications. *Polymer Composites*. 2021 Oct;42(10):5400-12.
39. Unni R, Reshmy R, Ramesh K, Mathew TJ, Abraham A, Dalvi YB, Sindhu R, Madhavan A, Binod P, Pandey A, Syed A. *Ixora coccinea* L.-A reliable source of nanocellulose for bio-adsorbent applications. *International Journal of Biological Macromolecules*. 2023 Jun 1;239:124467.
40. Mao Y, Liu K, Zhan C, Geng L, Chu B, Hsiao BS. Characterization of nanocellulose using small-angle neutron, X-ray, and dynamic light scattering techniques. *The Journal of Physical Chemistry B*. 2017 Feb 16;121(6):1340-51.
41. Gan PG, Sam ST, Abdullah MF, Omar MF. Thermal properties of nanocellulose-reinforced composites: A review. *Journal of Applied Polymer Science*. 2020 Mar 15;137(11):48544.

42. Wu C, Egawa S, Kanno T, Kurita H, Wang Z, Iida E, Narita F. Nanocellulose reinforced silkworm silk fibers for application to biodegradable polymers. *Materials & Design*. 2021 Apr 1;202:109537.
43. Jorfi M, Foster EJ. Recent advances in nanocellulose for biomedical applications. *Journal of Applied Polymer Science*. 2015 Apr 10;132(14).
44. Jonoobi M, Oladi R, Davoudpour Y, Oksman K, Dufresne A, Hamzeh Y, Davoodi R. Different preparation methods and properties of nanostructured cellulose from various natural resources and residues: a review. *Cellulose*. 2015 Apr;22:935-69.
45. Park H, Otte A, Park K. Evolution of drug delivery systems: From 1950 to 2020 and beyond. *Journal of Controlled Release*. 2022 Feb 1;342:53-65.
46. Salimi S, Sotudeh-Gharebagh R, Zarghami R, Chan SY, Yuen KH. Production of nanocellulose and its applications in drug delivery: A critical review. *ACS Sustainable Chemistry & Engineering*. 2019 Sep 4;7(19):15800-27.
47. De Sousa VR, Santos MDCA, Sousa VDB, Neves DAG, Santana NDAL, Menezes RR. A review on Chitosan's uses as biomaterial: Tissue engineering, drug delivery systems and cancer treatment. *Materials*. 2020; Nov 6;13(21):4995.
48. Lin N, Dufresne A. Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*. 2014 Oct 1;59:302-25.
49. Syed MH, Zahari MA, Khan MM, Beg MD, Abdullah N. An overview on recent biomedical applications of biopolymers: Their role in drug delivery systems and comparison of major systems. *Journal of Drug Delivery Science and Technology*. 2022 Dec 29:104121.
50. Parveen S, Misra R, Sahoo SK. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomedicine in cancer*. 2017 Sep 1:47-98.
51. Alle M, Sharma G, Lee SH, Kim JC. Next-generation engineered nanogold for multimodal cancer therapy and imaging: a clinical perspectives. *Journal of nanobiotechnology*. 2022 Dec;20(1):1-34.
52. Smith IO, Liu XH, Smith LA, Ma PX. Nanostructured polymer scaffolds for tissue engineering and regenerative medicine. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*. 2009 Mar;1(2):226-36.
53. Pei Y, Wang L, Tang K, Kaplan DL. Biopolymer nanoscale assemblies as building blocks for new materials: A review. *Advanced Functional Materials*. 2021 Apr;31(15):2008552.
54. Revati R, Majid MA, Ridzuan MJ, Mamat N, Cheng EM, Alshahrani HA. In vitro biodegradation, cytotoxicity, and biocompatibility of polylactic acid/napier cellulose nanofiber scaffold composites. *International Journal of Biological Macromolecules*. 2022 Dec 31;223:479-89.
55. Daniele MA, Boyd DA, Adams AA, Ligler FS. Microfluidic strategies for design and assembly of microfibers and nanofibers with tissue engineering and regenerative medicine applications. *Advanced healthcare materials*. 2015 Jan;4(1):11-28.
56. Shaikh HM, Anis A, Poulouse AM, Al-Zahrani SM, Madhar NA, Alhamidi A, Alam MA. Isolation and characterization of alpha and nanocrystalline cellulose from date palm (*Phoenix dactylifera* L.) trunk mesh. *Polymers*. 2021 Jun 7;13(11):1893.
57. Lin N, Dufresne A. Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*. 2014 Oct 1;59:302-25.
58. Wang K, Nune KC, Misra RD. The functional response of alginate-gelatin-nanocrystalline cellulose injectable hydrogels toward delivery of cells and bioactive molecules. *Acta biomaterialia*. 2016 May 1;36:143-51.
59. Li J, Wang X, Chang CH, Jiang J, Liu Q, Liu X, Liao YP, Ma T, Meng H, Xia T. Nanocellulose length determines the differential cytotoxic effects and inflammatory responses in macrophages and hepatocytes. *Small*. 2021 Sep;17(38):2102545.
60. Tarrahi R, Khataee A, Karimi A, Yoon Y. The latest achievements in plant cellulose-based biomaterials for tissue engineering focusing on skin repair. *Chemosphere*. 2022 Feb 1;288:132529.
61. Pei Y, Wang L, Tang K, Kaplan DL. Biopolymer nanoscale assemblies as building blocks for new materials: A review. *Advanced Functional Materials*. 2021 Apr;31(15):2008552.
62. Lin N, Dufresne A. Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*. 2014 Oct 1;59:302-25.
63. Tavakolian M, Jafari SM, van de Ven TG. A review on surface-functionalized cellulosic nanostructures as biocompatible antibacterial materials. *Nano-Micro Letters*. 2020 Dec;12:1-23.
64. Zhang X, Wang L, Dong S, Zhang X, Wu Q, Zhao L, Shi Y. Nanocellulose 3, 5-dimethylphenylcarbamate derivative coated chiral stationary phase: Preparation and enantioseparation performance. *Chirality*. 2016 May;28(5):376-81.
65. Shefa AA, Park M, Gwon JG, Lee BT. Alpha tocopherol-nanocellulose loaded alginate membranes and pluronic hydrogels for diabetic wound healing. *Materials & Design*. 2022 Dec 1;224:111404.
66. Amara C, El Mahdi A, Medimagh R, Khwaldia K. Nanocellulose-based composites for packaging applications. *Current Opinion in Green and Sustainable Chemistry*. 2021 Oct 1;31:100512.
67. van der Zalm J, Chen S, Huang W, Chen A. recent advances in the development of nanoporous Au for sensing applications. *Journal of The Electrochemical Society*. 2020 Jan 10;167(3):037532.
68. Ahmed A, Adak B, Faruk MO, Mukhopadhyay S. Nanocellulose coupled 2D graphene nanostructures: Emerging paradigm for sustainable functional applications. *Industrial & Engineering Chemistry Research*. 2021 Jul 26;60(30):10882-916.
69. Shi Y, Jiao H, Sun J, Lu X, Yu S, Cheng L, Wang Q, Liu H, Biranje S, Wang J, Liu J. Functionalization of nanocellulose applied with biological molecules for biomedical application: A review. *Carbohydrate Polymers*. 2022 Jun 1;285:119208.
70. Mishra RK, Sabu A, Tiwari SK. Materials chemistry and the futurist eco-friendly applications of nanocellulose: Status and prospect. *Journal of Saudi Chemical Society*. 2018 Dec 1;22(8):949-78.
71. Curvello R, Raghuvanshi VS, Garnier G. Engineering nanocellulose hydrogels for biomedical applications. *Advances in Colloid and Interface Science*. 2019 May 1;267:47-61.
72. Bilal M, Iqbal HM. Naturally-derived biopolymers: Potential platforms for enzyme immobilization. *International journal of biological macromolecules*. 2019 Jun 1;130:462-82.
73. Das S, Ghosh B, Sarkar K. Nanocellulose as sustainable biomaterials for drug delivery. *Sensors International*. 2022 Jan 1;3:100135.
74. Do TT, Grijalvo S, Imae T, Garcia-Celma MJ, Rodriguez-Abreu C. A nanocellulose-based platform towards targeted chemophotodynamic/photothermal cancer therapy. *Carbohydrate Polymers*. 2021 Oct 15;270:118366.
75. Thomas B, Raj MC, Athira KB, Rubiyah MH, Joy J, Moores A, Drisko GL, and Sanchez C. *Chemical Reviews*. 2018;118(24):11575-1625. DOI: 10.1021/acs.chemrev.7b00627.

76. Khine YY, Stenzel MH. Surface modified cellulose nanomaterials: a source of non-spherical nanoparticles for drug delivery. *Materials Horizons*. 2020;7(7):1727-58.
77. Islam T, Josephson L. Current state and future applications of active targeting in malignancies using superparamagnetic iron oxide nanoparticles. *Cancer Biomarkers*. 2009 Jan 1;5(2):99-107.
78. Samyn P, Meftahi A, Geravand SA, Heravi MEM, Najarzadeh H, Sabery MSK, & Barhoum A. Opportunities for bacterial nanocellulose in biomedical applications: Review on biosynthesis, modification and challenges. *International Journal of Biological Macromolecules*. 2023;231:123316. <https://doi.org/10.1016/j.ijbiomac.2023.123316>.
79. Adabi M, Naghibzadeh M, Adabi M, Zarrinfard MA, Esnaashari SS, Seifalian AM, Faridi-Majidi R, Tanimowo Aiyelabegan H, Ghanbari H. Biocompatibility and nanostructured materials: applications in nanomedicine. *Artificial cells, nanomedicine, and biotechnology*. 2017 May 19;45(4):833-42.
80. Bélanger MC, Marois Y. Hemocompatibility, biocompatibility, inflammatory and in vivo studies of primary reference materials low-density polyethylene and polydimethylsiloxane: A review. *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*. 2001;58(5):467-77.
81. Schuh JC, Funk KA. Compilation of international standards and regulatory guidance documents for evaluation of biomaterials, medical devices, and 3-D printed and regenerative medicine products. *Toxicologic Pathology*. 2019 Apr;47(3):344-57.
82. Klemm D, Cranston ED, Fischer D, Gama M, Kedzior SA, Kralisch D, Kramer F, Kondo T, Lindström T, Nietzsche S, Petzold-Welcke K. Nanocellulose as a natural source for groundbreaking applications in materials science: Today's state. *Materials Today*. 2018 Sep 1;21(7):720-48.
83. Giancaterino S, Boi C. Alternative biological sources for extracellular vesicles production and purification strategies for process scale-up. *Biotechnology Advances*. 2023 Jan 3:108092.
84. Beheshtizadeh N, Gharibshahian M, Pazhouhnia Z, Rostami M, Zangi AR, Maleki R, Azar HK, Zalouli V, Rajavand H, Farzin A, Lotfibakhshaiesh N. Commercialization and regulation of regenerative medicine products: Promises, advances and challenges. *Biomedicine & Pharmacotherapy*. 2022 Sep 1;153:113431.
85. Curvello R, Raghuvanshi VS, Garnier G. Engineering nanocellulose hydrogels for biomedical applications. *Advances in Colloid and Interface Science*. 2019 May 1;267:47-61.
86. Li F, Mascheroni E, Piervigiani L. The potential of nanocellulose in the packaging field: a review. *Packaging Technology and Science*. 2015 Jun;28(6):475-508.
87. Chinga-Carrasco G, Rosendahl J, Catalán J. Nanocelluloses—Nanotoxicology, Safety Aspects and 3D Bioprinting. In *Nanotoxicology in Safety Assessment of Nanomaterials 2022* May 19 (pp. 155-177). Cham: Springer International Publishing.
88. Sunasee R, Hemraz UD, Ckless K. Cellulose nanocrystals: A versatile nanoplatform for emerging biomedical applications. *Expert opinion on drug delivery*. 2016 Sep 1;13(9):1243-56.
89. Karim Z, Afrin S, Husain Q, Danish R. Necessity of enzymatic hydrolysis for production and functionalization of nanocelluloses. *Critical reviews in biotechnology*. 2017 Apr 3;37(3):355-70.
90. Norizan MN, Shazleen SS, Alias AH, Sabaruddin FA, Asyraf MR, Zainudin ES, Abdullah N, Samsudin MS, Kamarudin SH, Norrrahim MN. Nanocellulose-based nanocomposites for sustainable applications: A review. *Nanomaterials*. 2022 Oct 5;12(19):3483.