CHAPTER II
LITERATURE REVIEW

2.1 Lignocellulosic material

Lignocellulosic materials, derived from plant biomass, represent a vast and renewable resource with significant potential for the production of biofuels, biochemicals, and biomaterials. These materials are primarily composed of cellulose, hemicellulose, and lignin, forming a complex and recalcitrant structure. A comprehensive understanding of their composition, structure, and properties is crucial for developing efficient and sustainable utilization strategies.

2.1.1 Main component of lignocellulose

Lignocellulosic materials are composed of three primary components:

2.1.1.1 Cellulose is the most abundant biopolymer on Earth, primarily found in plant cell walls, algae, and some bacteria, consisting of β-D-glucose units linked by β-1,4-glycosidic bonds (Klemm et al., 2005) as show in figure 2.1a It is a crucial structural component that provides mechanical strength and stability to plant tissues. The structure of cellulose consists of linear chains of glucose molecules arranged in a highly ordered manner, forming crystalline and amorphous regions (fig 2.1b) (Moon et al., 2011). The crystalline domains contribute to its mechanical strength, while the amorphous regions provide flexibility and facilitate enzymatic hydrolysis. Cellulose chains are stabilized by extensive intra- and intermolecular hydrogen bonding, which influences its solubility and processability (Habibi et al., 2010). Understanding the hierarchical structure of cellulose is essential for tailoring its properties for various applications, including nanocellulose production, biodegradable materials, and biomedical scaffolds (Dufresne, 2012). Recent studies have focused on modifying cellulose structure through chemical and enzymatic treatments to enhance its functionality and expand its industrial applications.

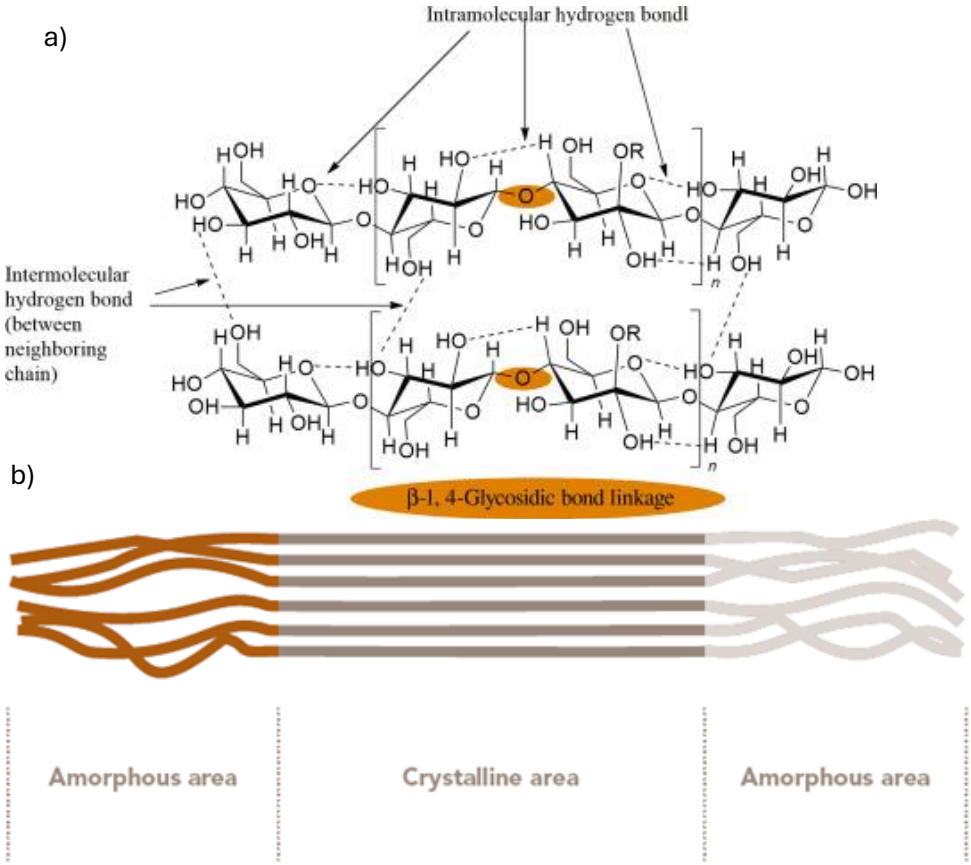


Figure 2.1 Chemical structure of cellulose a) β-D-glucose units linked by β-1,4-glycosidic bonds., b) crystalline and amorphous regions.

Cellulose exists in two primary polymorphic forms: Cellulose I (native cellulose) and Cellulose II (regenerated cellulose). Cellulose I, the naturally occurring form found in plants, bacteria, and algae, consists of parallel β-1,4-linked glucan chains and is highly crystalline, mechanically strong, but difficult to dissolve. It exists in two subtypes: Cellulose Iα, which has a triclinic crystal structure and is predominantly found in bacteria and algae (Sugiyama et al., 1991) as shown in figure 2.2 (a, c) and Cellulose Iβ, which has a monoclinic structure and is primarily present in higher plants and tunicates (Nishiyama et al., 2002) as shown in figure 2.2 (b, d). Due to its superior mechanical properties, Cellulose I plays a crucial role in the production of nanocellulose, which is widely applied in biomedical materials and advanced composites.

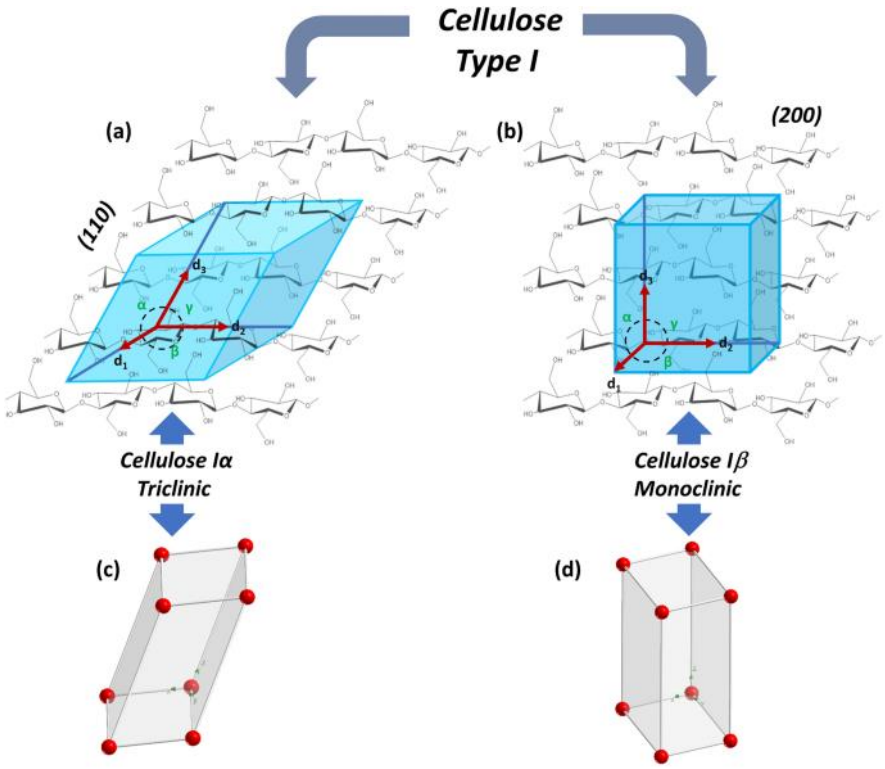


Figure 2.2 Primary polymorphic forms a) Cellulose Iα, b) Cellulose Iβ ,and crystal structure ; c) triclinic pattern , d) monoclinic pattern(Hernández-Varela, J.D. et al., 2021)

Cellulose II, formed through mercerization or regeneration, features an antiparallel chain arrangement with stronger hydrogen bonding, making it the most thermodynamically stable form of cellulose (O'Sullivan, 1997). Unlike Cellulose I, which is naturally occurring, Cellulose II is more soluble, accessible to enzymatic hydrolysis, and easier to process, making it preferable for industrial applications such as biofuel production and textiles (Marrinan and Mann, 1956). The chemical structures of cellulose I and cellulose II were compared, as shown in figure 2.3. Its enhanced solubility and processability contribute to its widespread use in sustainable materials, pharmaceuticals, and advanced cellulose-based composites.

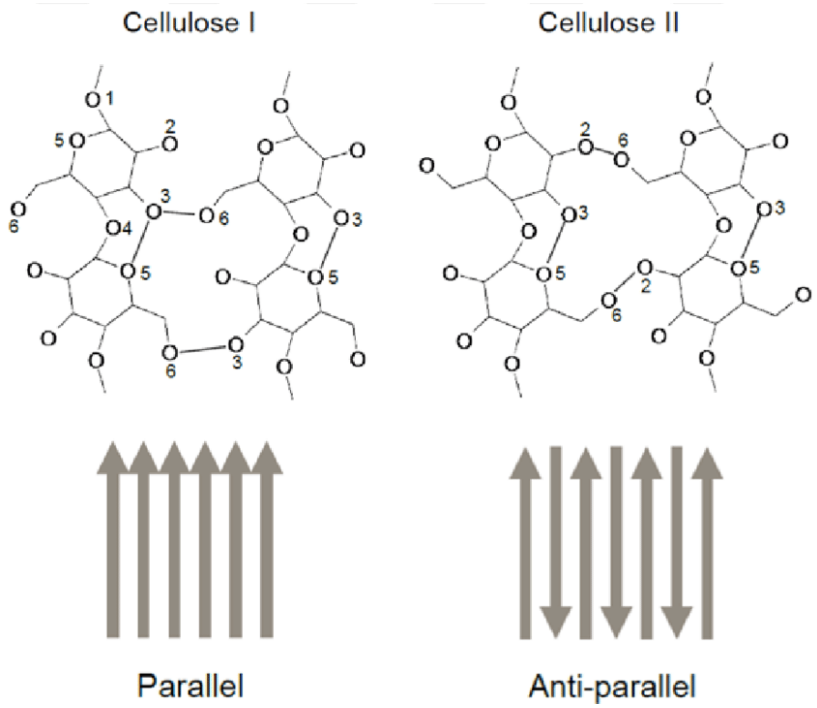
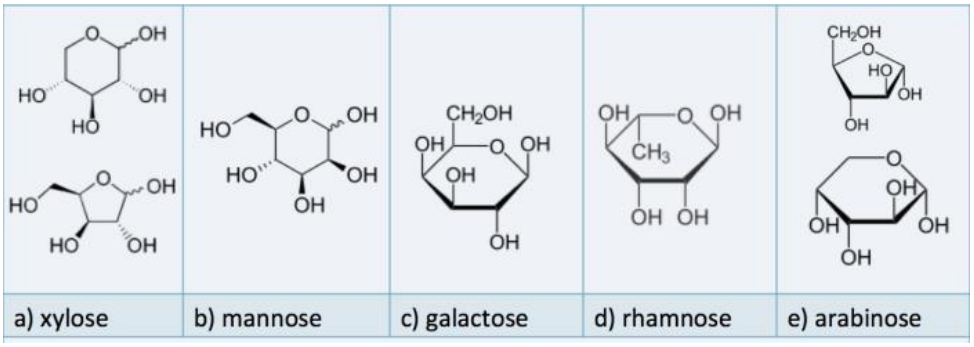
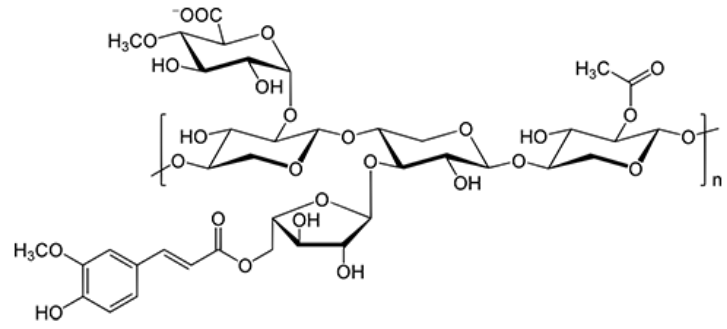


Figure 2.3 Comparison of chemical structures cellulose I and cellulose II (Kushan E., 2020)

2.1.1.2 Hemicellulose is a heterogeneous polysaccharide that, unlike cellulose, consists of branched chains of various sugar monomers (fig 2.4 a-e) such as xylose, mannose, arabinose, and galactose (Scheller and Ulvskov, 2010). It forms a matrix with cellulose and lignin in plant cell walls, providing flexibility and linking structural components. The amorphous nature of hemicellulose makes it more susceptible to hydrolysis compared to cellulose, allowing for easier breakdown in biorefinery processes (Peng et al., 2012). The composition and structure of hemicellulose vary among plant species, with xylans being the predominant type in hardwoods and glucomannans in softwoods. Due to its hydrophilic nature, hemicellulose has potential applications in biodegradable films, food additives, and biofuels, where structural modifications improve its processability and functional properties (Ebringerová, 2006).





f)

Figure 2.4 various sugar monomers a) xylose, b) mannose, c) galactose, d) rhamnose, e) arabinose and f) structure of hemicellulose (xylan) (Pennsylvania State University, 2024).

2.1.1.3 Lignin is an amorphous, highly branched aromatic polymer as shown in Fig 2.5 that provides rigidity and hydrophobicity to the plant cell wall (Boerjan, Ralph, and Baucher, 2003). Composed mainly of phenylpropanoid units, such as guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) monomers, lignin acts as a binding agent between cellulose and hemicellulose, offering structural support and resistance against microbial degradation (Ralph et al., 2004). Its complex and heterogeneous structure makes lignin difficult to degrade, posing challenges in biomass processing and biofuel production. However, lignin has significant potential for valorization, including applications in bioplastics, adhesives, and carbon-based materials (Laurichesse and Avérous, 2014). Ongoing research focuses on efficient lignin extraction and modification strategies to enhance its functionality and integration into various industries.

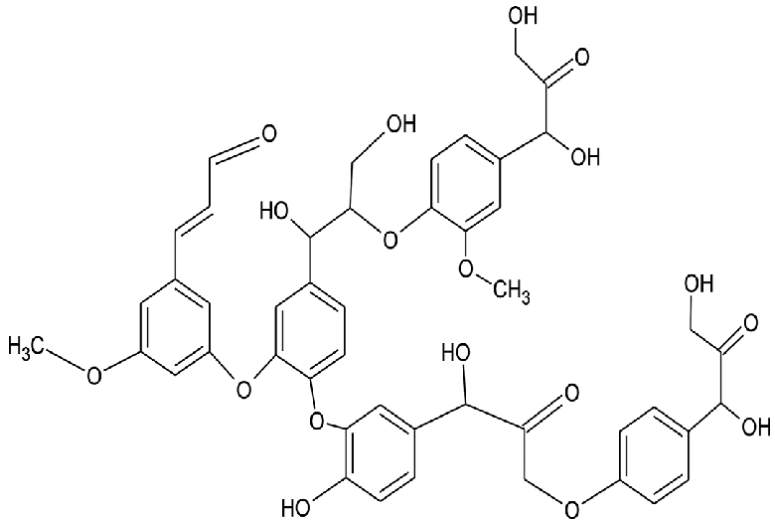


Figure 2.5 Chemical structure of lignin (Mahmood et al. 2018)

2.1.2 Structural Interactions and Recalcitrance

Lignocellulosic materials exhibit significant structural recalcitrance due to the intricate interactions between cellulose, hemicellulose, and lignin as shown in Fig 2.6 which hinder enzymatic hydrolysis and biomass conversion (Zhao et al., 2012). The crystalline nature of cellulose, the complex branching of hemicellulose, and the hydrophobic, cross-linked lignin network create a rigid, protective barrier that resists microbial and enzymatic degradation (Himmel et al., 2007). Lignin, in particular, acts as a physical shield around cellulose microfibrils, limiting enzyme accessibility and contributing to biomass recalcitrance (Li et al., 2014). Additionally, strong covalent and non-covalent interactions between lignin and polysaccharides, such as ester and ether linkages, further enhance this resistance (Rahikainen et al., 2013). Various pretreatment strategies, including chemical, enzymatic, and mechanical processes, have been explored to disrupt these interactions and enhance the efficiency of lignocellulosic biomass conversion into biofuels and biochemicals (Sun and Cheng, 2002). Understanding these structural interactions is crucial for optimizing biomass deconstruction methods and improving the cost-effectiveness of lignocellulosic biorefineries.

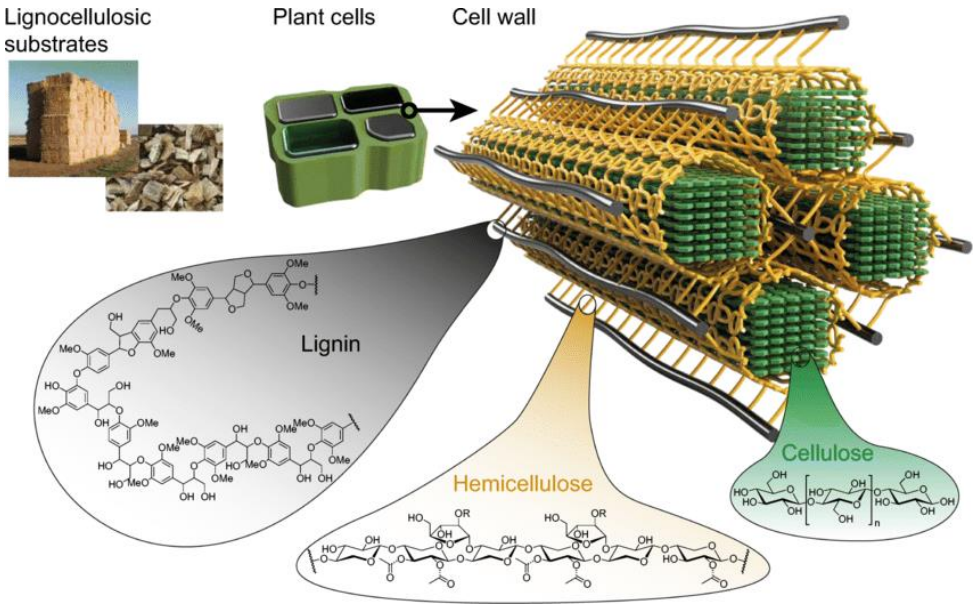


Figure 2.6 Components and structure of lignocellulosic materials (Brethauer et al., 2020)

2.1.3 Pretreatment and Bioconversion Strategies

Effective pretreatment and bioconversion strategies are crucial for overcoming the recalcitrance of lignocellulosic biomass, enhancing enzymatic hydrolysis, and improving the efficiency of biofuel and biochemical production (Mosier et al., 2005). Pretreatment methods include physical (e.g., milling and grinding), chemical (e.g., acid, alkaline, and organosolv treatments), physicochemical (e.g., steam explosion and liquid hot water), and biological approaches (e.g., microbial and enzymatic treatments) (Alvira et al., 2010). These strategies aim to break down the complex lignocellulosic structure by disrupting lignin-carbohydrate linkages, increasing cellulose accessibility, and reducing hemicellulose complexity (Mood et al., 2013). Bioconversion processes, such as enzymatic saccharification and microbial fermentation, play a key role in transforming pretreated biomass into fermentable sugars and valuable bio-products (Chandra et al., 2007). Recent advancements in consolidated bioprocessing (CBP), genetic engineering of microorganisms, and enzyme cocktail optimization have further improved conversion efficiency and economic feasibility (Lynd et al., 2008). Continued research on integrating pretreatment and bioconversion strategies is essential to advancing sustainable bioenergy and bioproduct industries.

2.1.4 Industrial Applications

Biomass for industrial applications is sourced from agricultural residues (such as straw, husks, and bagasse), forestry waste (including wood chips, sawdust, and bark), dedicated energy crops (like switchgrass and miscanthus), and organic waste (municipal solid waste, animal manure, and food waste). These feedstocks serve as raw materials for bio-based products, including biofuels, bioplastics, and biofertilizers. However, their availability and sustainability depend on land use, climate conditions, and advancements in biomass processing technologies. As industries emphasize renewable resources, optimizing biomass utilization is crucial for enhancing energy production, reducing carbon emissions, and promoting eco-friendly material development (U.S. Department of Energy, 2024; National Renewable Energy Laboratory, 2024).

Lignocellulosic materials have widespread industrial applications, ranging from biofuels and biochemicals to composite materials and biomedical products. The conversion of lignocellulosic biomass into bioethanol, biodiesel, and biogas offers a renewable alternative to fossil fuels, contributing to sustainable energy solutions (Ragauskas et al., 2006). In addition, lignocellulose-derived nanocellulose and lignin-based materials are used in packaging, coatings, and environmentally friendly plastics (Dufresne, 2012). The pharmaceutical and biomedical fields have also explored the use of lignocellulosic materials in drug delivery systems, wound dressings, and tissue engineering scaffolds due to their biocompatibility and mechanical strength (Chinga, 2018). Furthermore, lignocellulosic byproducts serve as animal feed, soil conditioners, and raw materials for high-value chemicals such as furfural, levulinic acid, and biopolymers (Zoghlami and Paës, 2019). With ongoing research and advancements in processing technologies, lignocellulosic materials continue to emerge as versatile, sustainable resources across multiple industries as shown in figure 2.7.

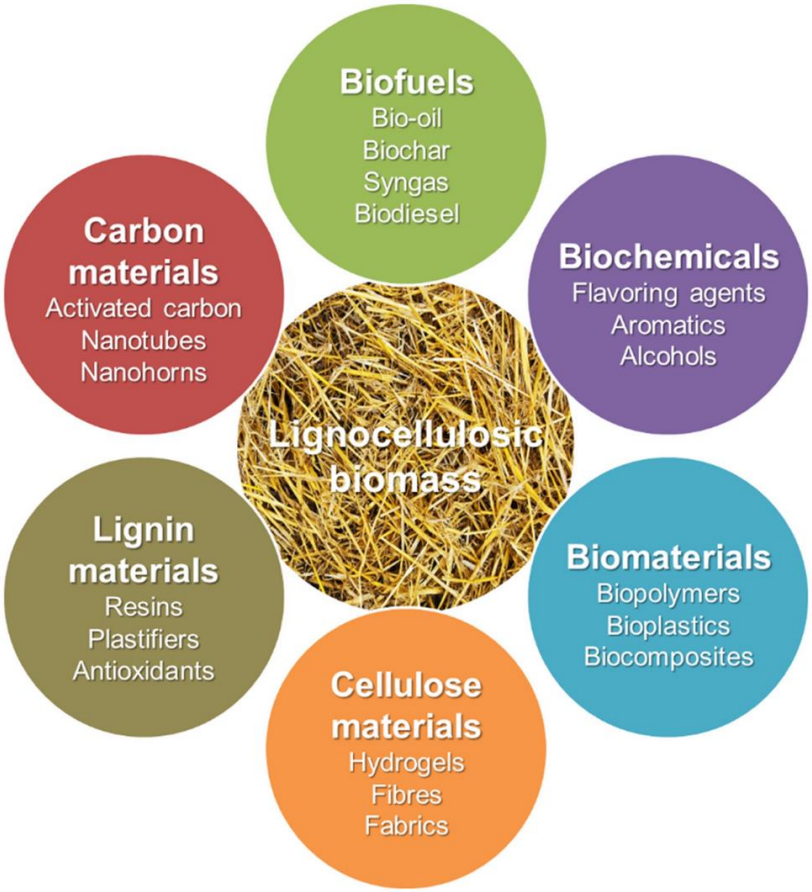


Figure 2.7 different industrially relevant bioproducts (Okolie *et al*., 2021)

2.2 Nanocellulose based Biopolymers

2.2.1 Nanocellulose

In the realm of biopolymers, cellulose stands out as the most abundant, forming a major structural component of plant cell walls. It's a linear homopolysaccharide composed of β-glucose units linked by 1,4-glycosidic bonds. When cellulose undergoes specific processing techniques, it can be broken down into nanoscale structures known as nanocellulose. Nanocellulose generally has at least one dimension in the range of 1-100 nm.

Cellulose exhibits a hierarchical structure, ranging from the macroscopic level down to the nanoscale. As depicted in Figure 2.8(A), cellulose fibers are composed of bundles of smaller fibrils. These fibrils further comprise crystalline and amorphous regions, as illustrated in Figure 2.8(B). Crystalline regions feature a highly ordered arrangement of cellulose chains, contributing to the material's stiffness and strength.

Conversely, amorphous regions exhibit a less organized structure, influencing the flexibility of cellulose (Phanthong *et al*., 2018).

Nanocellulose refers to cellulose broken down into nanoscale structures, typically with at least one dimension in the range of 1-100 nm. This nanoscale reduction significantly impacts the physical and mechanical properties of cellulose, offering improved characteristics compared to its bulk form. Nanocellulose was categorized into three main types:

- Cellulose Nanofibrils (CNF): These possess high aspect ratios (length-to-diameter) and excellent flexibility. CNFs are typically extracted using mechanical processes.
- Cellulose Nanocrystals (CNC): CNCs are highly crystalline and possess rod-like structures. They are often obtained through chemical treatment methods.
- Bacterial Nanocellulose (BNC): BNC is produced by specific bacteria and exhibits high purity and excellent biocompatibility.

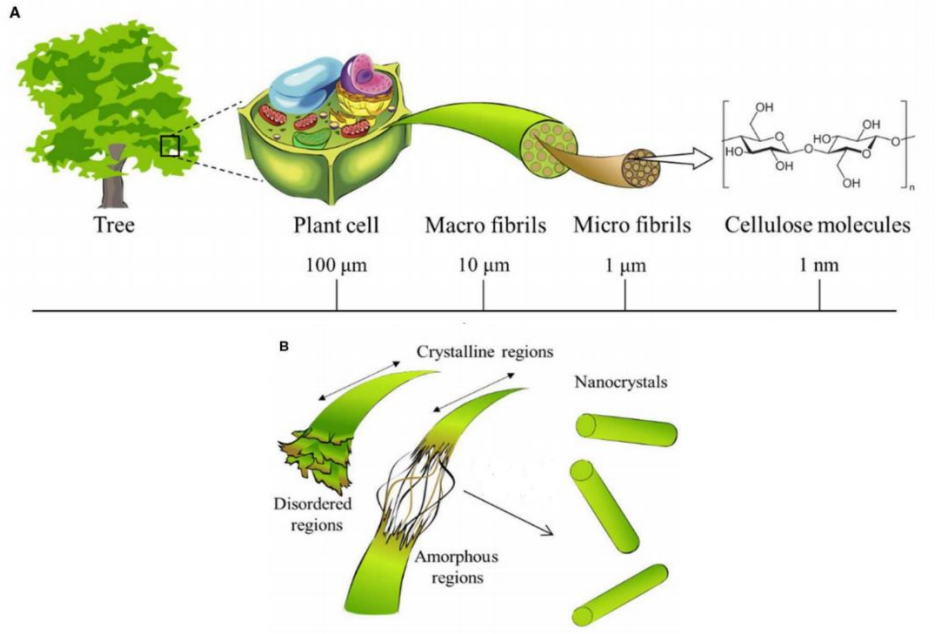


Figure 2.8 Cellulose contained in plants or trees has a hierarchical structure from the meter to the nanometer scale, as shown in (A). The crystalline and amorphous regions of cellulose is shown in (B) (Trache *et al*., 2020)

While all types of nanocellulose share a similar chemical composition (β-1,4-glycosidic linked glucose units), they differ in morphology (shape), particle size, and crystallinity due to variations in source material and extraction methods (Phanthong *et al*., 2018). These differences influence their specific properties and potential applications. The structure of nanocellulose was illustrated using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), as shown in Figure 2.9 These techniques allow for the visualization of the morphology and size distribution of the nanocellulose particles. The key characteristics of different nanocellulose types was summarized in table 2.1, including their production methods and size ranges.

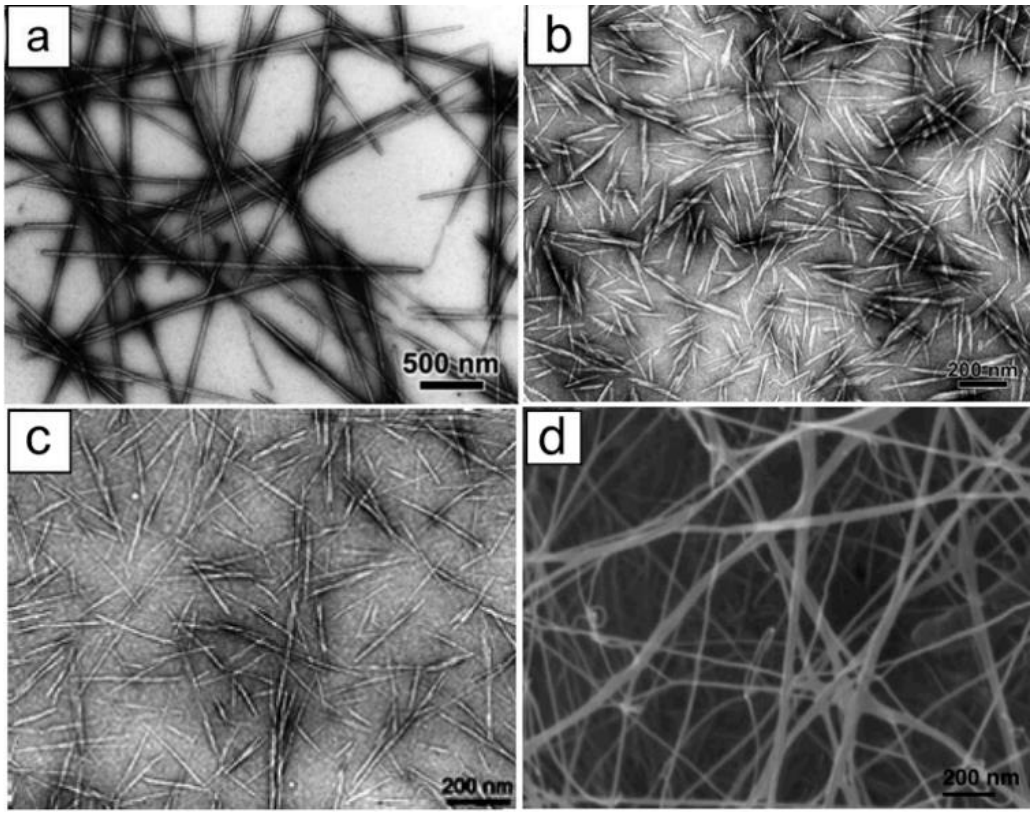


Figure 2.9 TEM images of a dried dispersion of cellulose nanocrystals (CNC) derived from (a) tunicate, (b) ramie, and (c) sisal, and (d) SEM image of nanofibrils of bacterial cellulose (BC) (Parry, 2016).

Table 2.1 Type of nanocelluloses, method of production and sizes (Parry, 2016).

Types of nanocelluloses	Typical sources	Method of production	Average Dimensions
Cellulose nanocrystals (CNC)	Wood, cotton, tunicate, ramie, bacterial cellulose, bamboo	Acid Hydrolysis	Diameter 3-50 nm Length: 30nm to -300 nm (wood and cotton based) 100 nm to several microns (tunicate and bacterial cellulose based)
Nanofibrillated Cellulose (NFC)	Wood, cotton, potato hemp, flax	High pressure homogenization, Microfluidization, Grinding, Cryocrushing	Diameter 5-60 nm Length: several microns regardless of the cellulose source
Bacterial Cellulose (BC)	*Acetobacter/ Glucobacter xylinum*	Biosynthesis of glucose and alcohol	Diameter 10-100 nm Length: Mostly several tens of micrometers up to a mm.

Sugarcane bagasse, a fibrous by-product generated during sugarcane processing, represents a significant portion of agricultural waste. This residue is a complex lignocellulosic material with a composition of approximately 35-45% cellulose, 26.2-35.8% hemicellulose, 11.4-25.2% lignin, and 2.9-14.4% other components (Chandel et al., 2012). Traditionally, sugarcane residues have found applications in ethanol production, paper and board manufacturing, animal feed, and electricity generation. However, due to its high cellulose content, sugarcane bagasse emerges as a promising and sustainable feedstock for nanocellulose production.

2.2.1.1 Chemical methods for nanocellulose extraction

Chemical methods are widely employed for nanocellulose extraction, with acid hydrolysis being a prominent technique. Sulfuric acid is frequently used, as exemplified by the hydrolysis of sugarcane bagasse with 50% sulfuric acid at a 1:25 cellulose-to-acid ratio, at 40°C for 10 minutes (Trache et al., 2020; Wulandari et al., 2016). Other acids, such as hydrochloric acid, can also be utilized, as demonstrated by the extraction of nanocellulose from ramie fibers using various concentrations (6, 8, and 10 M) at 45°C for 70 minutes with a 1:20 g/ml cellulose-to-acid ratio (Akbar et al., 2020). Additionally, organic acids like citric acid are gaining traction in biorefinery approaches. (Bondancia et al.,2020) reported a maximum CNC yield of 23% (diameter: 9 nm, length: 215 nm) after a 6-hour reaction at 120°C. While dialysis remains the primary method for acid removal and nanocellulose purification, its lengthy processing

time is a disadvantage. TEMPO-mediated oxidation presents an alternative chemical approach. (Hastuti et al., 2019) successfully derived cellulose nanofibers from oil palm empty fruit bunches (OPEFB) using a TEMPO/NaBr/NaClO system at pH 10 and room temperature for 2 hours. However, a major drawback associated with chemical methods is the generation of wastewater from the washing process, requiring proper treatment and disposal practices for environmental sustainability.

2.2.1.2 Mechanical methods for nanocellulose extraction

Mechanical processes utilize high shear forces to break down cellulose fibers into nanofibrils. Common techniques include ball milling, ultrasonication, and high-pressure homogenization.

- Ball Milling: This method employs a rotating jar containing milling balls of various sizes. The cellulose material gets crushed and refined due to collisions between the balls and the jar walls (Trache et al., 2020; Phanthong et al., 2016). Ball milling can be used in combination with mild acid hydrolysis or as a pretreatment step for enzymatic nanocellulose production (Squinca et al., 2020).
- Ultrasonication: This technique utilizes sound waves to generate cavitation (bubble formation and collapse) within the cellulose suspension. The resulting high-energy forces contribute to the defibrillation of cellulose fibers (Phanthong et al., 2018). Ultrasonication often serves as an assisting technique, frequently combined with chemical methods (acid hydrolysis or oxidation) for nanocellulose production (Perdoch et al., 2020).
- High-Pressure Homogenization: This method involves forcing a cellulose slurry through a narrow chamber at high pressure. The intense shear forces generated within the fluid effectively cleave cellulose microfibrils into nanofibrils (Phanthong et al., 2018). As an example, nanocellulose was successfully isolated from soy pulp using high-pressure homogenization at pressures ranging from 100-140 MPa for three passes (Wu et al., 2020).

2.2.1.3 Enzymatic Hydrolysis for Nanocellulose Production

Enzymatic hydrolysis offers a promising bio-based approach for nanocellulose extraction. This method utilizes enzymes to selectively break down

non-cellulosic components in lignocellulosic biomass, such as sugarcane bagasse and straw. Compared to chemical methods, enzymatic hydrolysis provides a more environmentally friendly alternative with minimal environmental impact and reduced energy consumption. Several enzymes are employed in enzymatic hydrolysis for nanocellulose production. Key enzymes include:

- Endoglucanase: This enzyme cleaves cellulose chains internally, increasing accessibility for other enzymes.
- Beta-glucosidase: This enzyme breaks down cellobiose, a disaccharide byproduct of cellulose hydrolysis, into glucose monomers.
- Xylanase: This enzyme degrades xylan, a hemicellulose component present in lignocellulosic biomass, facilitating the isolation of cellulose.

The effectiveness of enzymatic hydrolysis depends on various factors, including enzyme loading, reaction time, and temperature. Studies have reported successful nanocellulose production using commercial enzyme cocktails at temperatures around 50°C and reaction times ranging from 42 to 96 hours (Martelli et al., 2016; Squinca et al., 2020). Pretreatment of lignocellulosic biomass can significantly enhance the efficiency of enzymatic hydrolysis. Ball milling, for example, has been shown to improve the accessibility of cellulose to enzymes, leading to higher nanocellulose yields (Squinca et al., 2020). Enzymatic hydrolysis can achieve moderate yields of nanocellulose. Studies have demonstrated yields of approximately 11.3% and 12% for sugarcane bagasse and straw, respectively, after a 96-hour enzymatic hydrolysis process (de Aguiar et al., 2020) as shown in figure 2.10.

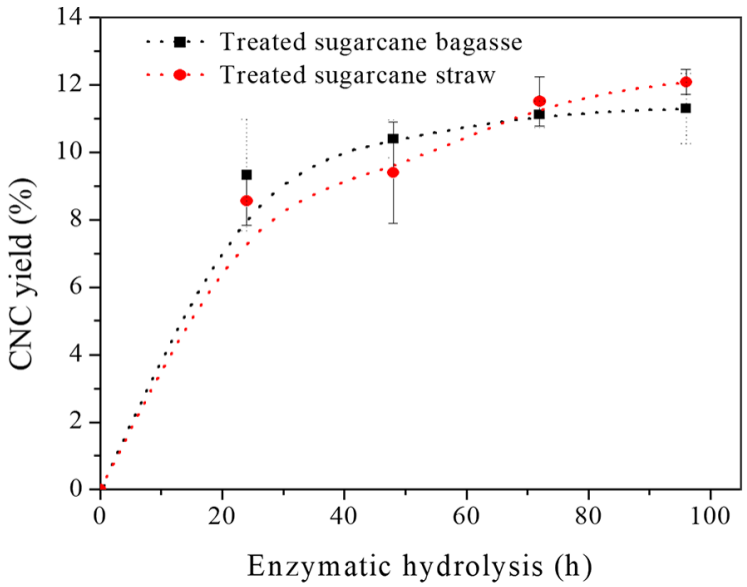


Figure 2.10 Yields of enzymatic nanocellulose from treated sugarcane bagasse and straw, using 10% (w/v) solids loading and enzyme 10 mg protein/g of solid at 50 °C. (de Aguiar *et al*., 2020)

2.2.1.4 Bacterial Nanocellulose Production

Bacterial nanocellulose (BNC) offers a unique route for nanocellulose production through a bioprocess mediated by specific bacteria. Unlike other extraction methods that rely on harsh chemicals or intensive mechanical forces, BNC production leverages fermentation, a generally more environmentally friendly approach. Notably, *Gluconacetobacter xylinus* (formerly known as *Acetobacter xylinum*) stands out as a highly efficient and non-pathogenic bacterium for producing cellulose nanofibrils.

The cultivation of *G. xylinus* for BNC production typically employs Hestrin & Schramm (HS) medium. This medium provides an optimal environment for bacterial growth and cellulose production. HS medium is formulated with glucose as the primary carbon source, fueling the metabolic processes of the bacteria. Additionally, a combination of peptone and yeast extract serves as a rich source of nitrogen, essential for bacterial growth and cellulose biosynthesis. The fermentation process usually adopts a stationary phase approach, allowing the bacteria to maximize cellulose production within a defined timeframe. Fermentation times typically range from 4 to 15 days, depending on the desired yield and targeted properties of the BNC.

The specific details of BNC production and corresponding fermentation times using HS medium can be found in Table 2.2.

Table 2.2 Bacterial nanocellulose (BNC) production in Hestrin & Schramm (HS) medium (Corujo *et al*., 2016)

strain	fermentation time (day)	production (g/L)
Komagataeibacter hansenii MCM B-967	7	0.3
Gluconacetobacter xylinus ATCC 10788	7	0.4
Komagataeibacter sp. PAP1	7	1.2
Komagataeibacter sucrofermentans DSM 15973	15	1.2
Gluconacetobacter hansenii UAC09	7	1.5
Gluconacetobacter medellinenses	13	1.9
Gluconacetobacter sacchari	4	2.5-2.7

2.2.2 Polylactic acid

Lactic acid is a versatile molecule found naturally in organisms. Its unique chemical structure, featuring hydroxyl and carbonyl groups, comprises three forms as shown in Figure 2.11 This structure makes lactic acid a valuable precursor for other compounds. Notably, its pure L and D forms are particularly in demand. Most importantly, L-lactic acid serves as the foundation for poly(lactic acid), a useful polymer with various industrial applications.

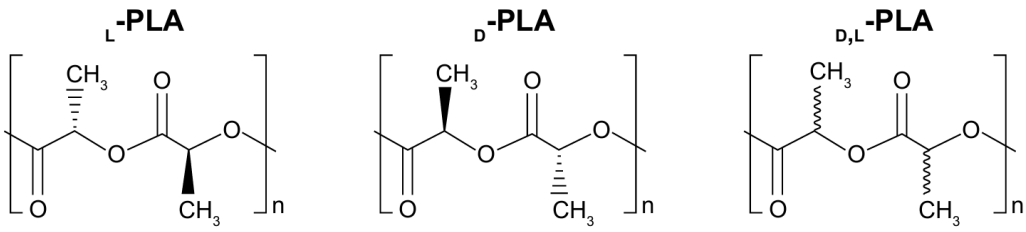


Figure 2.11 Structure of poly(lactic acid) isomers (L-PLA, D-PLA, D,L-PLA) (Song et al., 2018)

Lactic acid bacteria can be categorized into two primary groups based on their fermentation products: homofermenters and heterofermenters. Homofermenters specialize in lactic acid production, efficiently converting glucose into lactate via the glycolysis pathway and utilizing enzymes like lactate dehydrogenase for this primary output (Castañeda et al., 2023). Conversely, heterofermenters exhibit greater metabolic versatility, utilizing glucose to generate a wider range of products including acetic acid, carbon dioxide, and ethanol alongside some lactic acid (Castañeda et al.). For example:

Homofermenters: Preferred for industrial production of high-purity lactic acid used in plastics, food additives, and pharmaceuticals. They are also essential for yogurt production.

Heterofermenters: Used in various food fermentations like sourdough bread, kimchi, sauerkraut, and some sausages. Their ability to produce flavor compounds and tolerate some oxygen makes them suitable for these applications.

Polylactic acid (PLA) possesses a unique combination of properties ideal for industrial applications. The physical properties of PLA were summarized in Table 2.3. Moreover, PLA offers excellent transparency, low-temperature stability, and resistance to grease and oil. Importantly, these properties can be strategically manipulated by adjusting D-content and molecular weight (Ranakoti et al., 2022). For example, increasing molecular weight enhances strength but reduces crystallinity. The ratio of D- and L-content directly influences crystallinity and biodegradation.

Table 2.3 Physical properties of PLA (Ranakoti et al., 2022)

Property	Values
Specific Gravity	1–1.5
Surface Energy (dynes)	36–40
Melting Temperature (°C)	140–210
Molecular Weight (Daltons)	Approx. 1.6 × 105
Melt Flow Index (g/10 min)	4–22
Crystallinity (%)	5–35
Glass Transition Temperature (°C)	50–75

The degradation is crucial for the long-term effects of PLA materials. In the field of biomedicine, a slow degradation rate of PLA is a drawback. Therefore, blending PLA with other materials has been used to develop properties. However, achieving the ideal balance between degradation speed, strength, and other properties for safe tissue growth and implant function remains a challenge.

2.2.3 Polybutylene succinate

Polybutylene succinate (PBS), a commercially available biodegradable and sustainable bioplastic since 1993, has attracted continuous research efforts to develop eco-friendly fermentation methods using microorganisms as an alternative feedstock (Rafiqah et al., 2021). PBS was synthesized through polycondensation of succinic acid (or dimethyl succinate) with 1,4-butanediol (BDO). The flowchart of PBS production was presented in Figure 2.12 Polybutylene succinate (PBS) synthesis involves a two-step process:

- Esterification/Transesterification: This step reacts succinic acid (or dimethyl succinate) with BDO (with a slight BDO excess) to form oligomers. The reaction occurs at 160-190°C under nitrogen and removes water (or methanol) as a byproduct.
- Polycondensation: The oligomers undergo further reaction at higher temperatures (220-240°C) and high vacuum to remove BDO and form high-molecular-weight PBS. Various catalysts like tin(II) chloride, distannoxane, and titanium-based catalysts are used in this step.

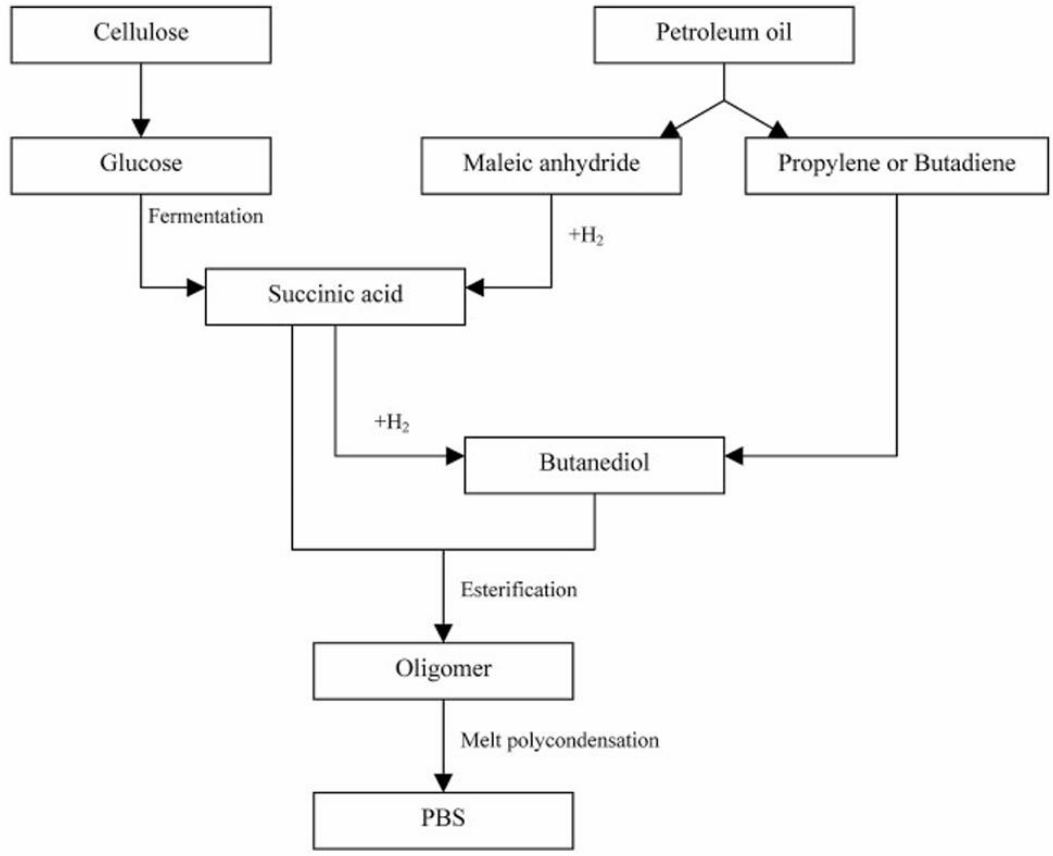


Figure 2.12 PBS production flowchart (Xu and Guo, 2010)

PBS is a versatile bioplastic with a semi-crystalline structure, making it desirable for various applications. It exhibits good elongation and properties as shown in Table 2.4. However, PBS degrades in water and lacks some properties like softness. To mitigate these limitations, PBS is often blended with other materials.

Table 2.4 Physical properties of PBS (Su et al., 2019).

properties	PBS
Glass transition temperature (°C)	-32
Melting point (°C)	114
Heat distortion temperature (°C)	97
Modulus of elasticity (MPa)	550-700
Tensile strength (MPa)	34
Elongation at break (%)	560

2.2.4 Properties of nanocellulose based biopolymer

Nanocellulose has attracted great interest in the preparation of nanocomposites with polymer matrices due to its interesting properties. Nanocellulose-based biopolymers exhibit exceptional mechanical strength, high surface area, and tunable rheological properties, making them suitable for a wide range of applications (Dufresne, 2012). These biopolymers demonstrate remarkable biodegradability, biocompatibility, and non-toxicity, which are essential for biomedical applications such as wound healing, drug delivery, and tissue engineering (Kargarzadeh et al., 2017). Additionally, nanocellulose enhances the thermal stability and barrier properties of polymer matrices, improving their performance in packaging and coatings (Eyholzer et al., 2010). The high aspect ratio and hydroxyl-rich surface of nanocellulose allow for chemical modifications that tailor its functionality for specific industrial needs (Lin & Dufresne, 2014). Ongoing research focuses on optimizing nanocellulose integration into composite materials to expand its commercial viability and sustainable applications (Trache et al., 2020).

Mechanical testing studies on nanocellulose-reinforced biopolymers have demonstrated significant property enhancements across various research efforts. In PLA composites, tensile strength increases by 21% with 5 wt% cellulose nanofibrils (CNF) (Jonoobi et al.,2010)., while tensile strength achieved a 54% improvement using 1 wt% cellulose nanocrystals (CNC) with surfactant (Fortunati, et al., 2012). More dramatic results were observed in modulus and strength (up to 58% and 210%, respectively) with well-dispersed CNC (Wang and Drzal, 2012). However, cellulose nanofibrils CNF led to higher strength and modulus than CNC at the same fiber concentration (Xu et al., 2016). For PBS matrices, even more substantial improvements were recorded, and the tensile modulus increased with the addition of CNF or CNC (Lin et al.2011). Overall, these studies highlight the effectiveness of nanocellulose as a reinforcing agent for biopolymers. Relatively low concentrations of nanocellulose (typically 1-5 wt%) can substantially enhance mechanical properties, particularly modulus. This reinforces the potential of nanocellulose-reinforced biopolymers for various applications, including packaging, biomedical devices, and tissue engineering.

2.2 Tissue engineering

2.2.1 Key Components of Tissue Engineering

2.2.1.1 Stem cells

Stem cells are categorized by potency, which determines their ability to differentiate into various cell types, as summarized in Table 2.5. Totipotent stem cells (e.g., zygote) can develop into any body or extraembryonic cell, while pluripotent stem cells (e.g., ESCs, iPSCs) can generate all body cells but not placenta. Multipotent stem cells (e.g., MSCs, HSCs) differentiate into a limited range of related cells, making them valuable for tissue engineering, especially for bone, cartilage, and blood. Oligopotent stem cells (e.g., myeloid and lymphoid progenitors) give rise to only a few specific cell types, while unipotent stem cells (e.g., muscle satellite cells) specialize in a single cell type but retain self-renewal properties. Pluripotent stem cells offer the highest potential but pose tumor risks, whereas multipotent stem cells are safer and widely used in regenerative medicine.

Table 2.5 types of stem cell

Potency	Differentiation Potential	Examples	Applications	Limitations
Totipotent	All body + extraembryonic cells	Zygote, Morula	Early development studies	Ethical issues, rare
Pluripotent	All body cells (not placenta)	ESCs, iPSCs	Organoids, regenerative medicine	Tumor risk, ethical concerns
Multipotent	Multiple related cell types	MSCs, HSCs, NSCs	Bone, cartilage, blood cell engineering	Limited differentiation
Oligopotent	Few related cell types	Myeloid, Lymphoid progenitors	Blood/immune cell therapy	Very restricted potential
Unipotent	One specific cell type	Satellite cells, Epidermal cells	Skin grafts, muscle repair	Least versatile

Stem cells are undifferentiated cells with the ability to self-renew and differentiate into specialized cell types, making them a cornerstone of regenerative medicine and tissue engineering (Singh et al., 2021). They are categorized into embryonic stem cells (ESCs), adult stem cells, and induced pluripotent stem cells (iPSCs). ESCs, derived from the inner cell mass of blastocysts, possess pluripotency, allowing them to differentiate into all three germ layers (Thomson et al., 1998). Adult stem cells, such as mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), are multipotent and primarily contribute to tissue repair and homeostasis (Pittenger et al., 1999). iPSCs, generated by reprogramming somatic cells through the introduction of key transcription factors, provide an ethical alternative to ESCs with promising applications in disease modeling and personalized medicine (Takahashi & Yamanaka, 2006). Stem cells have demonstrated potential in treating neurodegenerative disorders, cardiovascular diseases, and musculoskeletal injuries through their regenerative properties and immunomodulatory effects (Trounson & McDonald, 2015). However, challenges such as tumorigenic risks, immune rejection, and standardization of differentiation protocols remain significant barriers to clinical translation (Bianco et al., 2013). Advances in biomaterials, gene editing, and 3D bioprinting continue to drive innovations in stem cell-based therapies, paving the way for more effective regenerative medicine strategies.

2.2.1.1.1 Mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) are a type of adult stem cell possessing the unique ability to differentiate into various cell types of mesenchymal origin, such as bone, cartilage, and fat cells. This multipotency makes them a promising tool in regenerative medicine. Additionally, MSCs exhibit immunomodulatory properties, meaning they can interact with the immune system in a way that minimizes rejection by the recipient's body. A diverse array of tissues, including bone marrow, umbilical cord, and adipose tissue, were identified as suitable sources for MSC isolation (Figure 2.13). Moreover, The isolation process typically involves a density gradient separation for bone marrow, adipose, and blood-derived MSCs, while enzymatic digestion with collagenase was used for other tissues (Table 2.6).

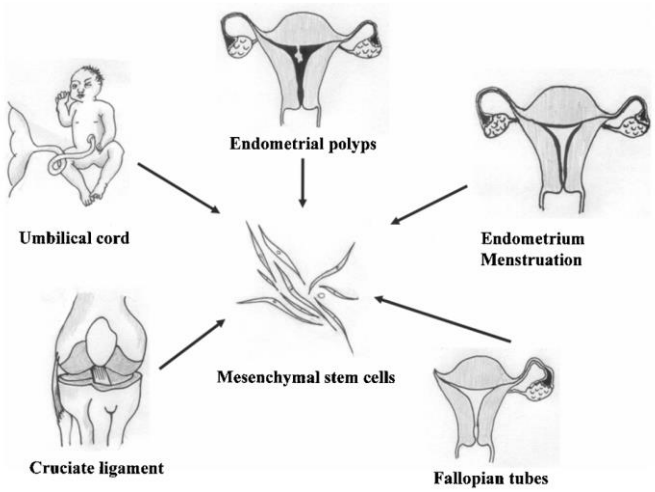


Figure 2.13 Various sources of MSCs (Ding, Shyu, and Lin, 2011)

Table 2.6 The isolation process of MSCs (Ullah, Subbarao and Rho, 2015)

Source	Method of isolation
Bone marrow	Ficoll density gradient method Novel marrow filter device
Adipose tissue	Digestion method Membrane filtration method
Amniotic fluid and membrane	Density gradient method Digestion method
Dental tissues	Digestion method
Endometrium	Digestion method
Limb bud	Digestion method
Peripheral blood	Ficoll density gradient
Placenta and fetal membrane	Digestion method
Salivary gland	Digestion method(Ringer solution)
Skin and foreskin	Digestion method
Sub amniotic umbilical cord lining membrane	Digestion method
Synovial fluid	Ficoll density gradient method
Wharton's jelly	Enzymatic digestion method

Wharton's Jelly, a component of the umbilical cord matrix, was identified as a source for MSCs. Wharton's Jelly Cell (WJC) have been proven to differentiate into neuronal and glial cells in vitro. Moreover, these cells can migrate to the site of injury and differentiate into neuronal and glial cells in stroke rats. When compared to bone marrow-derived MSCs, WJ-derived MSCs are characterized by faster growth and greater expansion potential (Ding, Shyu, and Lin, 2011). The suitability of WJ-derived MSCs as an alternative source for various therapeutic applications was proposed, highlighting the necessity for further investigation through long-term clinical trials. However, the multipotency of MSCs makes them a promising candidate for future clinical applications in various diseases, warranting further research on their differentiation, transplantation, and immune response.

2.2.1.2 Biomaterial scaffold

Biomaterial scaffolds play a crucial role in tissue engineering by providing a three-dimensional (3D) framework that supports cell attachment, proliferation, and differentiation, mimicking the extracellular matrix (ECM) to facilitate tissue regeneration (O'Brien, 2011). These scaffolds can be fabricated from natural biomaterials such as collagen, gelatin, chitosan, and alginate, or synthetic polymers like polylactic acid (PLA), polycaprolactone (PCL), and poly(lactic-co-glycolic acid) (PLGA) (Hutmacher, 2000). The choice of scaffold material influences properties such as biodegradability, mechanical strength, and biocompatibility, which are essential for successful tissue integration (Murphy et al., 2010).

Numerous materials have been explored as scaffolds for tissue engineering applications. To achieve optimal functionality, a critical aspect is the close match between the scaffold and the newly generated tissue. Various scaffolds, derived from a wide range of biomaterials, have been developed to promote the regeneration of different tissues and organs within the body. When designing or evaluating the suitability of a scaffold for tissue engineering, several key considerations are paramount: biocompatibility, biodegradability, and mechanical properties (O'Brien, 2011).

- Biocompatibility: Biocompatibility, a material's ability to elicit a favorable host response in a specific application (i.e., non-toxic and non-

immunogenic) (Anderson, 2012), is crucial for scaffold design. A biocompatible scaffold should primarily support cell attachment, normal function, migration (both on the surface and throughout the scaffold), and proliferation before the cells start laying down new extracellular matrix (O'Brien, 2011). Factors influencing biocompatibility include the material's chemistry, scaffold structure, and morphology. Additionally, the biomaterial synthesis process, the scaffold manufacturing method, and the sterilization conditions can significantly impact biocompatibility.

- Biodegradability: Furthermore, the scaffold should exhibit biodegradability to allow cells to produce their own extracellular matrix and allow their byproducts to be safely eliminated (O'Brien, 2011).
- Mechanical Properties: Ideally, the scaffold's mechanical properties should match those of the targeted implantation site. The scaffold must possess sufficient mechanical strength to withstand surgical manipulation during implantation. However, achieving high strength often comes at the expense of reduced porosity, another crucial property for optimal cell function (O'Brien, 2011).

A critical aspect of designing scaffolds for tissue engineering applications was achieved a close match between the mechanical properties of the scaffold and the targeted implantation site. Sufficient mechanical strength was necessitated for the scaffolds to endure surgical manipulation during implantation. However, an inherent trade-off was identified, whereby high strength could only be achieved at the expense of reduced porosity, another crucial parameter for optimal cellular function within the scaffold (O'Brien, 2011).

Advanced fabrication techniques, including electrospinning, 3D bioprinting, and freeze-drying, have been employed to create scaffolds with controlled porosity and architecture, enhancing cellular infiltration and vascularization (Jakab et al., 2010). Research focuses on functionalizing biomaterial scaffolds with bioactive molecules, growth factors, and nanomaterials to improve their regenerative potential (Dvir et al., 2011). Despite significant progress, challenges remain in optimizing scaffold degradation rates, mechanical properties, and immune responses for clinical applications (Liu et al., 2016). Future advancements in biomaterials and scaffold fabrication technologies hold great promise for developing personalized and bioactive scaffolds for tissue engineering and regenerative medicine.

2.2.1.3 Bioactive Molecules

Bioactive molecules play a critical role in biomedical applications by modulating cellular behavior, promoting tissue regeneration, and enhancing the functionality of biomaterials (Langer and Tirrell, 2004). These molecules include growth factors, peptides, cytokines, and small molecules that influence cell proliferation, differentiation, and extracellular matrix synthesis (Lutolf and Hubbell, 2005). Growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), and bone morphogenetic proteins (BMPs) have been extensively used in tissue engineering to stimulate angiogenesis, osteogenesis, and wound healing (Chen et al., 2007). Bioactive molecules can be incorporated into scaffolds via surface modifications, chemical conjugation, or controlled release systems to ensure localized and sustained therapeutic effects (Lee et al., 2011). Advances in nanotechnology and biomaterials have enabled the development of smart delivery systems, including nanoparticles and hydrogels, that improve the bioavailability and stability of these molecules (Yoo et al., 2011). Despite their therapeutic potential, challenges such as degradation kinetics, immune responses, and precise dose control must be addressed for clinical translation. Future research focuses on optimizing bioactive molecule delivery strategies to enhance regenerative medicine and targeted therapies.

2.2.2 Applications of nanocellulose based biopolymer in Tissue Engineering

Tissue engineering focuses on creating functional tissues to repair or replace damaged organs in the body. Each tissue type serves a specific purpose. For example, skin provides a protective barrier, bones and cartilage offer structural support, and the pancreas plays a crucial role in biochemical production. In tissue engineering, a patient's own cells are often cultured in a lab environment and then seeded onto specially designed scaffolds. These cultured scaffolds are then implanted into the patient's body, where the scaffolds serve as a template for the cells to grow and differentiate into the desired tissue.

A diverse array of natural and synthetic polymers were investigated for the fabrication of scaffolds for tissue regeneration. Each polymer offered distinct advantages in terms of mechanical strength and structural form.

Common scaffold architectures included sponge-like structures, fibrous matrices, and gel-type constructs suitable for cell culture (Sridhar et al., 2011). When selecting biopolymers for scaffold production, paramount considerations were placed on biocompatibility, biodegradability, and the ability to achieve the desired mechanical properties.

Nanocellulose-based scaffolds have emerged as promising materials for tissue engineering due to their unique properties. These properties include biocompatibility, water absorption and retention, optical transparency, and suitable chemo-mechanical characteristics. Nanocellulose scaffolds offer potential applications in repairing, improving, or replacing various damaged tissues and organs, including skin, blood vessels, nerves, skeletal muscle, heart, liver, and those in the field of ophthalmology (Luo et al., 2019).

Cellulose nanocrystals was incorporated with polyvinyl alcohol (PVA) to create tissue engineering scaffolds. The impact of these scaffolds on human skin cells in a laboratory setting was subsequently evaluated (Lam et al., 2017). Additionally, cellulose nanofibrils (CNFs) was blended with gelatin (gel) and cross-linked for application in bone tissue engineering. The structure of these cross-linked scaffolds was analyzed using scanning electron microscopy (SEM), and a 3D model was generated by microcomputed tomography (m-CT) (Figure 2.14).

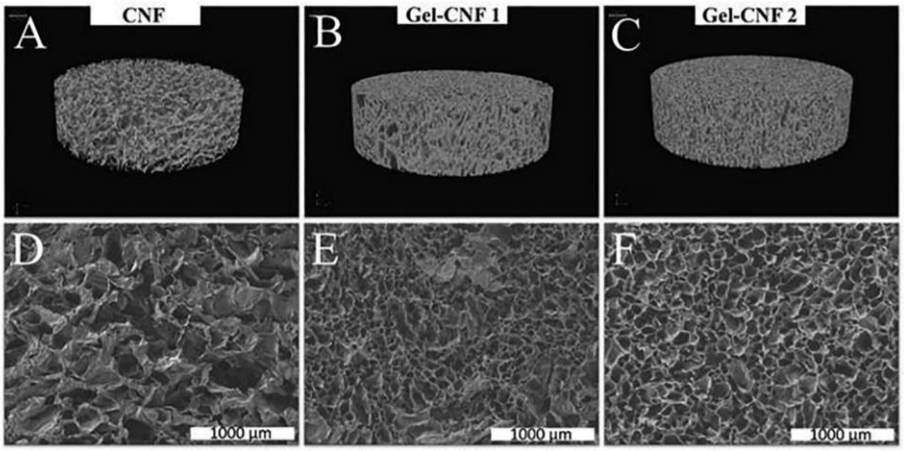


Figure 2.14 Structural surface characterization by m-CT (A-C) and SEM (D-F) (Carlström *et al.,* 2020).

Polylactic acid (PLA) emerged as a prominent biopolymer classified within the aliphatic polyester family. Notably, PLA could be derived from sustainable resources such as sugarcane, corn, and potatoes. Electrospinning was employed to create PLA-based scaffolds and their blends, finding various applications in the medical field, as detailed in Table 2.7. Additionally, studies reported that the properties of the solvent used in electrospinning PLA fibers, such as boiling point, viscosity, conductivity, and surface tension, significantly affect the process efficiency, morphology, and diameter distribution of the resulting PLA nanofibers (Figure 2.15).

Table 2.7 Details of various electrospun based PLA for biomedical applications. (Kanmaz *et al*., 2018)

Polymers and Blends	Molecular	Solvents weight (g/mol)	Average fiber diameter (nm)	Fillers	Application
PLA	200,000	DMAc and CHL	971 ± 274	Cur	Wound healing
PLLA and PVDF	90,000	DMAc and AC	2,290 – 4,620	Cur and Enro	Wound healing
PLGA	120,000-190,000	Trifluoroethanol and CHL	777 ± 249	Ciprofloxacin and sodium alginate	Wound healing
PLLA	300,000	DCM	3000-5000	DMOG and DS	Wound healing
PLGA	75,000	1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)	~1,000	-	Cardiac
PLGA	110,000	HFIP	720 ± 350	Elastin and collagen	Cardiac
PLA	75,000-120,000	DMF, DCM and methanol	~556	Dipyridamole	Cardiac
PLLA	100,000	HFIP	100–500	Laminin	Nerve
PLLA	100,000	HFIP and hexamethyl-disilazane (HMDS)	860 ± 110	Coll and HA	Bone
(PPDO:PLLA:-PEG)	42,000	DMF and DMC	~1,400	-	Skin
PLA and PEVA	205,000	CHL and methanol	1,000–3,000	Tetracycline	Drug release
PLLA	-	CHL, 1,2-dichlorethane and ethyl acetate	290 - 539	Cyclosporine A (CsA)	Drug release
PLA	470,000	DMF and DCM	549.7 ± 153.1	Graphene oxide (GO) and rhodamine B	Drug release
PLLA and chitosan	low molecular weight	AC and DCM	2 760 ± 720	-	Dental

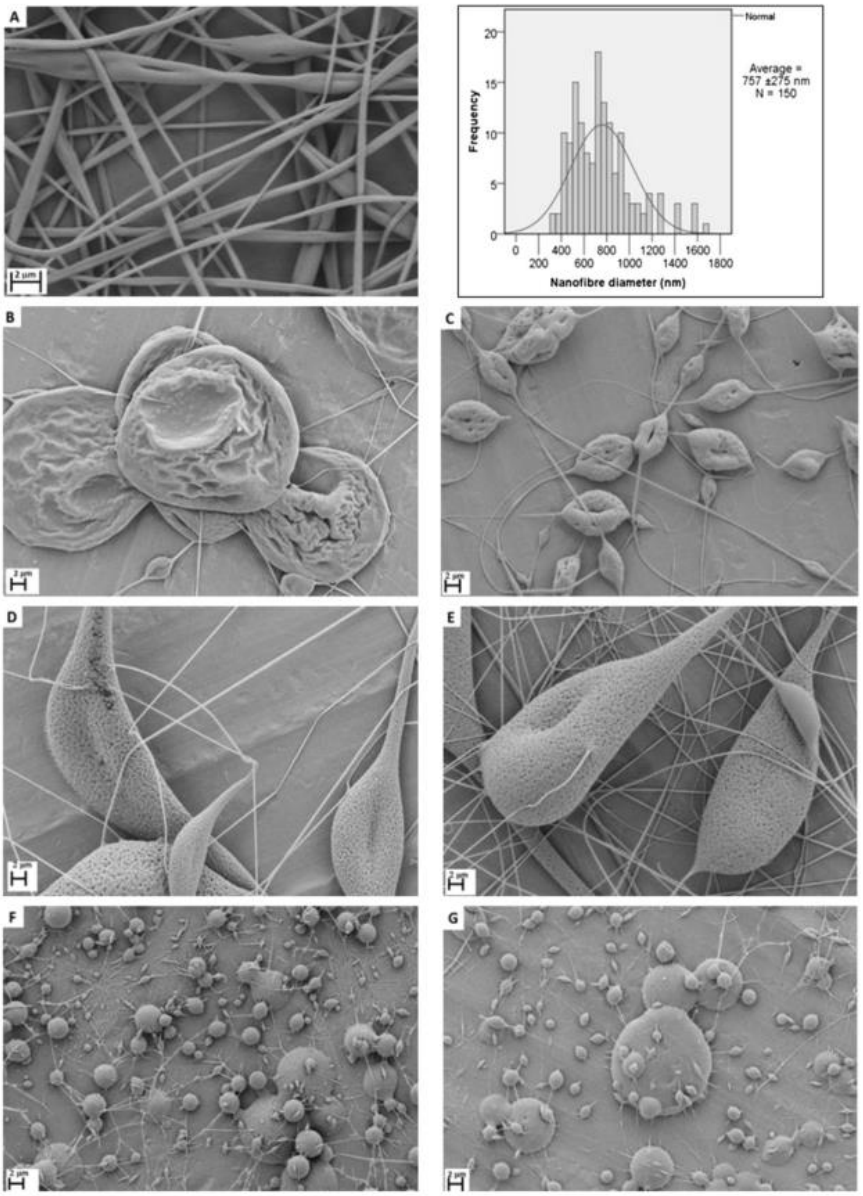


Figure 2.15 Effect of single solvent systems on nanofibre morphology: scanning electron micrographs of PLA nanofibres from solutions of 10% (w/v) of PLA in: (a) acetone with nanofibre diameter distribution, (b) 1,4-dioxane, (c) tetrahydrofuran, (d) dichloromethane, (e) chloroform, (f) dimethylformamide and (g) dimethylacetamide (Casasola *et al*., 2014).

Investigations were undertaken to enhance the properties of scaffold materials through the blending of polylactic acid (PLA) with polybutylene succinate (PBS). A progressive improvement in the flexibility of the composite material was observed with increasing PBS content within the blend, as detailed in Table 2.8

Furthermore, research efforts focused on the incorporation of cellulose (CNF) into PLA/PBS composites. The addition of CNF was reported to further enhance the mechanical performance of electrospun scaffolds. Notably, an increase in both tensile strength and elastic modulus was observed with a rising nanocellulose content. This improvement can be attributed to the reinforcing effect exerted by the CNF, a consequence of strong molecular interactions established between the polymer matrix and the CNF (Abudula et al., 2019).

Table 2.8 Characterization summary of PLA/PBS scaffolds in the different ratio (Abudula *et al.*, 2019)

PLA/ PBS ratio	Fiber diameter (nm)	Elastic modulus (Mpa)	Tensile Strength (Mpa)	Strain at break (%)	Indentation modulus (Mpa)	Water contact angle (°)	Protein Adsorption (μg/mg)
100/0	1019 ± 75	129 ± 5.1	1.68 ± 0.17	62.4 ± 2.3	2525 ± 40	113.7 ± 3.1	60.3 ± 3.3
75/25	948 ± 80	112 ± 3.8	2.42 ± 0.14	71.5 ± 3.5	1825 ± 26	107.7 ± 1.7	98.0 ± 5.6
60/40	631 ± 64	108 ± 2.5	2.52 ± 0.2	76.5 ± 2.6	1619 ± 75	98.6 ± 1.9	120.2 ± 3.0
50/50	409 ± 51	98.6 ± 4.6	2.77 ± 0.23	110.3 ± 1.6	1537 ± 32	83.5 ± 2.3	138.4 ± 1.9
40/60	460 ± 60	58 ± 2.6	2.64 ± 0.15	112.5 ± 1.3	1319 ± 55	91.8 ± 1.8	142.2 ± 2.3
25/75	_	29.7 ± 2.3	2.43 ± 0.15	105.6 ± 2.4	1158 ± 24	109.2 ± 1.2	147.2 ± 3.6
0/100	_	11 ± 2.3	1.54 ± 0.1	115.3 ± 1.8	210 ± 11	113.8 ± 2.1	163.9 ± 1.8

Nanocellulose has been extensively studied for its biomedical applications, with various in vivo studies evaluating their safety profiles. A study on cellulose nanofibrils (CNF) administered to rats over five weeks showed no significant toxic effects, as assessments of hematology, serum markers, and organ histology revealed no adverse changes, suggesting that ingested nanocellulose is likely non-hazardous in small quantities (DeLoid et al., 2019). PLA implants typically induce mild inflammatory responses that resolve over time, though localized acidosis from degradation products remains a concern (Bernardo et al., 2022; Hietala et al., 2001). Similar results occurred for PBS and PBS/PLA, while in the case of PLA, the inflammation was still occurring to some extent (Gigli et al., 2016). However, Numerous in vivo studies on biopolymers have revealed generally favorable biocompatibility profiles with manageable side effects.

2.2.3 Strategies in Tissue Engineering

Tissue engineering integrates principles from biology, engineering, and material science to develop functional tissues for regenerative medicine. Various strategies have been explored to enhance tissue regeneration, including scaffold-based approaches, cell-based therapies, and bioactive molecule delivery (Langer and Vacanti, 1993). Scaffold-based tissue engineering involves the use of biomaterial scaffolds that provide structural support and a microenvironment conducive to cell attachment, proliferation, and differentiation (O'Brien, 2011). These scaffolds can be fabricated from natural polymers such as collagen, alginate, and chitosan or synthetic polymers like poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) (Murphy et al., 2010).

Cell-based strategies, particularly the use of stem cells, have gained significant attention due to their ability to self-renew and differentiate into multiple cell types (Zakrzewski et al., 2019). Mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) have been widely used for tissue repair and regeneration due to their regenerative potential and immunomodulatory properties (Chen et al., 2019). Advances in genetic engineering and cell reprogramming have further improved the efficiency of stem cell-based therapies in tissue regeneration (Takahashi and Yamanaka, 2016).

Another critical strategy in tissue engineering is the delivery of bioactive molecules, such as growth factors, peptides, and small molecules, to stimulate cell behavior and promote tissue regeneration (Mooney and Vandenburgh, 2008). Controlled release systems, including nanoparticles, hydrogels, and microspheres, have been developed to enhance the stability and bioavailability of these molecules (Lee et al., 2011). Advancements also include applying 3D bioprinting and biofabrication techniques, which enable the precise spatial arrangement of cells and biomaterials to create complex tissue constructs (Murphy and Atala, 2014).

Despite these advancements, challenges such as vascularization, immune response, and long-term functionality of engineered tissues remain critical areas for further research (Pashuck and Stevens, 2012). Future efforts aim to integrate advanced biomaterials, smart drug delivery systems, and bioprinting technologies to improve tissue engineering outcomes and facilitate clinical translation.

2.2.4 Challenges and Future Directions

Despite significant advancements, tissue engineering faces several challenges that hinder its clinical translation and widespread application. One of the primary obstacles is vascularization, as engineered tissues require an adequate blood supply to sustain cell viability and function (Zhao et al., 2016). The lack of functional capillary networks in larger tissue constructs limits nutrient and oxygen diffusion, leading to necrosis in the core regions of engineered grafts (Novosel et al., 2011). Current strategies, including the use of angiogenic growth factors, endothelial cell seeding, and 3D bioprinting, aim to address this issue, but achieving fully integrated vascular networks remains a challenge (Zhang et al., 2021).

Another major limitation is immune response and biocompatibility. The host immune system often reacts to implanted scaffolds, cells, or bioactive molecules, leading to inflammation, fibrosis, or rejection (Anderson et al., 2008). The development of immune-evasive biomaterials, immunomodulatory strategies, and patient-specific cell therapies holds promise for overcoming these barriers (De Vries et al., 2019). Additionally, stem cell-based tissue engineering faces challenges related to cell sourcing, differentiation efficiency, and long-term functionality. Ensuring consistent differentiation and integration of stem cells into host tissues remains a crucial hurdle (Trounson and McDonald, 2015).

Scaffold degradation and mechanical stability also present critical concerns. The degradation rate of biomaterial scaffolds must be synchronized with new tissue formation to prevent premature collapse or excessive persistence that hinders remodeling (O'Brien, 2011). Balancing mechanical properties with biodegradability remains a key focus in scaffold design. Furthermore, large-scale manufacturing and standardization pose challenges in translating lab-scale successes to clinical applications. Regulatory approvals, cost-effective production, and reproducibility of tissue-engineered products require significant advancements in biofabrication techniques (Murphy and Atala, 2014).

Looking ahead, the future of tissue engineering lies in emerging technologies such as 3D bioprinting, gene editing, and smart biomaterials. 3D bioprinting enables precise fabrication of complex tissue architectures, incorporating multiple cell types and growth factors (Groll et al., 2016). Gene editing tools like

CRISPR-Cas9 offer potential for genetic modifications to enhance cell functionality and immune tolerance (Hendriks et al., 2023). Additionally, bioactive and responsive biomaterials that can adapt to dynamic physiological environments will revolutionize tissue engineering applications (Sant et al., 2021). Addressing these challenges through interdisciplinary collaboration and technological advancements will drive the next generation of regenerative medicine and organ bioengineering.