Chapter 9

Nanocellulose Scaffolds in Tissue Engineering

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1. Introduction

Regenerative medicine is a broad field that incorporates research on self- healing wherethe body can uses its own systems. The idea of replacement of a damaged body part using biomaterials or materials from natural origin was proposed and applied over 4000 years ago. Recently by the technological advancements the living tissues were engineered, giving rise to the field of tissueengineering (TE). Thus, it is an important interdisciplinary science for regenerative medicine, which involves the development of tissues that reinstate, maintain and enhance the tissue functions. It is the fabrication of bio-artificial tissues *in vitro* and includes the *in vivo* alteration of cell growth and function through the implantation of suitable cells isolated from donor tissues and biocompatible scaffolds. Tissue engineering has already showed remarkable successes in producing avascular tissues and organs (e.g., skin, cartilage, bladder, etc.) and holds great promises for producing tissues and organs containing highlyorganized three-dimensional vascular structures. In the future, tissue engineeringis expected to result in more complex tissues and organs that will be useful in overcoming the need for organ donations, reducing the number of animals used in drug discovery and drug toxicity research, and facilitating the development of patient-specific smart diagnostics and personalized medicine. The nanomedicineapplications in tissue engineering can be used whichcreates a new horizon for the various future developments in the field of tissue engineering.

Tissue engineering involve combination of three main elements, namely cells, scaffolds, and biomechanical or biochemical signals (figure 1). Current research in the field focuses on the development of these three elements to answer basic questions and produce functional living tissue

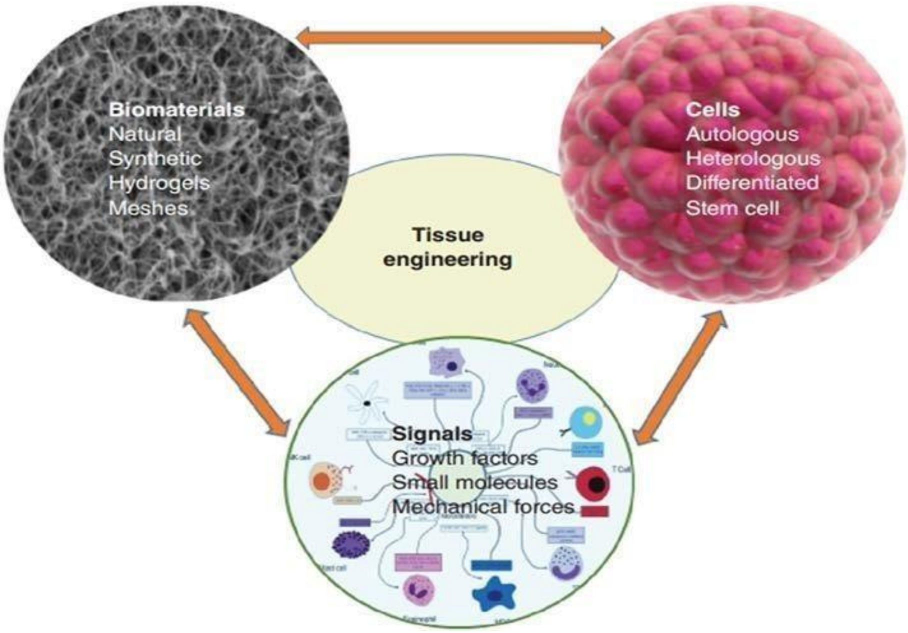

Figure 1. The triads of tissue engineering. Reproduced from ref. [1]

1.Structural and biological conditions for ideal scaffolds in tissue engineering

Scaffolds are polymeric materials that cause desirable cellular interactionsto form newfunctional tissues. The cells are seeded into scaffolds capable of supporting three-dimensionaltissue formation. They are used to support organs and organ systems that may have been damaged after injury or disease in tissue engineering. Scaffolds allow cell attachment and migration and retain cells and biochemical factors. They provide diffusion of vital nutrients and they can influence the behaviour of the cell phase mechanically and biologically.

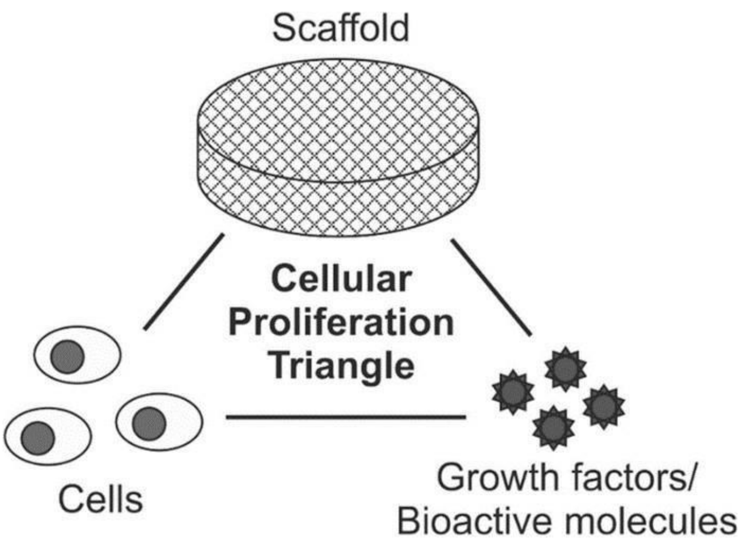

Figure 2. Cellular Proliferation. Reproduced from ref. [2]

Scaffolds prepared from the natural macromolecules has been used in tissue engineering rapidly. Scaffolds used can be directly implanted to promote the *in situ* cell growth, propagation, and regeneration of tissue without previous cell seeding. Scaffolds are of greaterimportance as they promote the tissue maturation and cell growth. Several researches have carried out in the development of usefulscaffolds in tissue engineering [3]. The basicconditions to consider a scaffold useful in tissue engineering are;

1. Biodegradability (easily degradable in normal pathways)
2. Biocompatibility (do not produce any toxic, response when exposed to bodyfluids)
3. Porosity (for the transportation of cell nutrients)
4. Similar mechanical performance
5. Structural morphology to a tissue of requirement in order to imitate the *invivo* nativetissue

2. Nanocellulose

Nanocellulose is cellulose in the form of nanostructures that possess the features not exceeding at least 1 dimension. These nanostructures comprises of the nanofibrils, found in bacterial cellulose; nanofibers, present particularly in the electrospun matrices and nanocrystals. The structures can be assembled further into bigger two-dimensional (2D) and three- dimensional (3D) nano, micro and macro-structures, such as nanoplatelets, membranes, films, microparticles, porous macroscopic matrices, etc. Nanocellulose can be obtained from bacteria,algae and plants. The crystallinity and the length to diameter ratio (L/d) are the parametersthatcontrol the properties of nanocellulose.

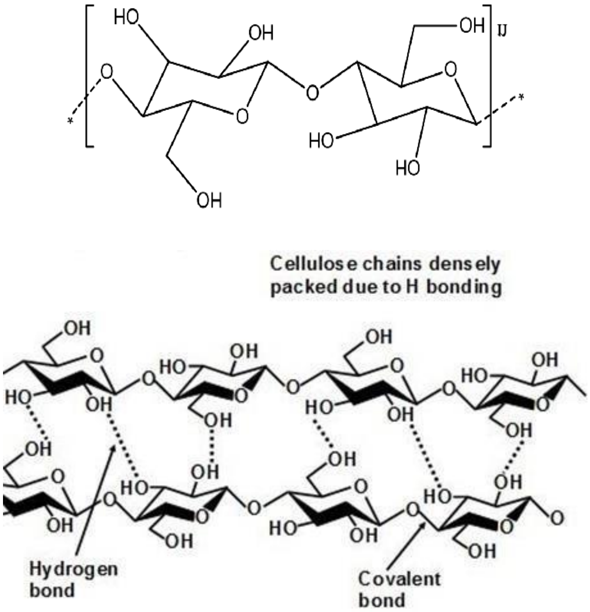

Figure 3. The structure of nanocellulose. Reproduced from ref. [4]

Scaffolds based on Nano-cellulose has direct application in the field of tissue engineering. Nano-cellulose based scaffolds are used due to their biocompatibility, water adsorption and retention and chemo-mechanical properties. The methods to prepare Nano- cellulose based scaffolds are electrospinning, 3D printing, solvent casting and freeze-drying. Nano-cellulose based scaffolds are raw materials for regeneration of different tissues and organsbecause of their distinct features. [5].

Since the β-1, 4-glucose in the molecular chain contains three active hydroxyl groups, NC can easily form hydrogen bond network, which has high mechanical strength and tailorablesurface modification. [6] The scaffolds based on nanocellulose are capable of engineering blood vessels, neural tissue, bone, cartilage, liver, adipose tissue, etc for repairing connective tissue and congenital heart defects. It can be also used for constructing contact lenses and protective barriers.

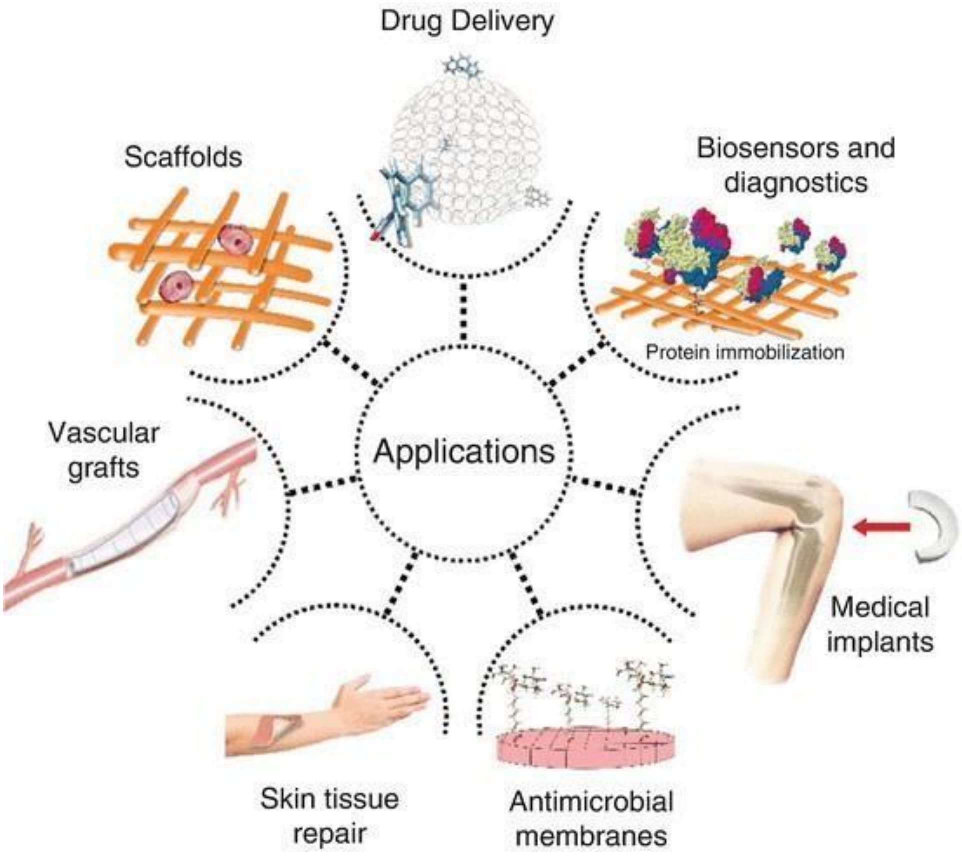

Figure 4. Applications of Nanocellulose Scaffolds. Reproduced from ref. [7]

3.1. Bacterial cellulose

Bacterial nanocellulose is produced by bacteria through the processes polymerization and crystallization. The glucose residues polymerize to β-1, 4 glucan linear chains in the bacterial cytoplasm and are secreted extracellularly. The developed chains are formed which are furthercrystallized to microfibrils. [8]. The microfibrils consolidate to highly pure 3D porous network of entangled nanoribbons of 20–60 nm in width. It is a hydrogel comprised of nanofibrils and imitates the fibrillary component of natural extracellular matrix. Certain mechanical properties of bacterial nanocellulose like elasticity, crystallinity, higher surface area, higher degree of polymerization, strength, Young's Modulus, conformability, water retention assists its use in skin reconstruction [9].

The bacterial nanocellulose is used profoundly in tissue regeneration due to its similarity and resemblance to the soft natural tissues. The advent use of bacterial cellulose as temporary skin substitutes for skin reconstruction was in 1990.The thin films of bacterial cellulose were used as substrates for the cultivation of human transformed skin keratinocyte in2006. From then bacterial nanocellulose are used in skin tissue engineering profoundly [10].

3.2. Plant-based nanocellulose

Plant nanocellulose are the cellulose materials isolated from the plant fibres. The versatile properties of plant nanocellulose paved its use in advanced applications. Cellulose nanofibers and cellulose nanocrystals are the two categories of plant nanocellulose. The cellulose nanocrystal has a rigid rod like structure that is 1–100 nm in diameter and its length is tens to hundreds of nanometer. [11] Whereas fibers possess length in micrometer range. They could be used as reinforcing agents for the development of nanocomposites with polymer matrices. Plant nanocellulose emerged as a promising material for skin tissue engineering. Reinforcing materials from plant-derived nanocellulose in theform of nanocrystals is used as degradable natural and synthetic polymers. [12] Cellulose nanocrystals (CNCs) synthesized by acid hydrolysis of cellulose fibresin HCl or H_2SO_4, have higher elasticity modulus, higher strength, larger surface areas, high crystallinity and bio-compatibilty. CNCs can be widely used to reinforce composites, collagen films and are the promising materials in skin tissue engineering [13].

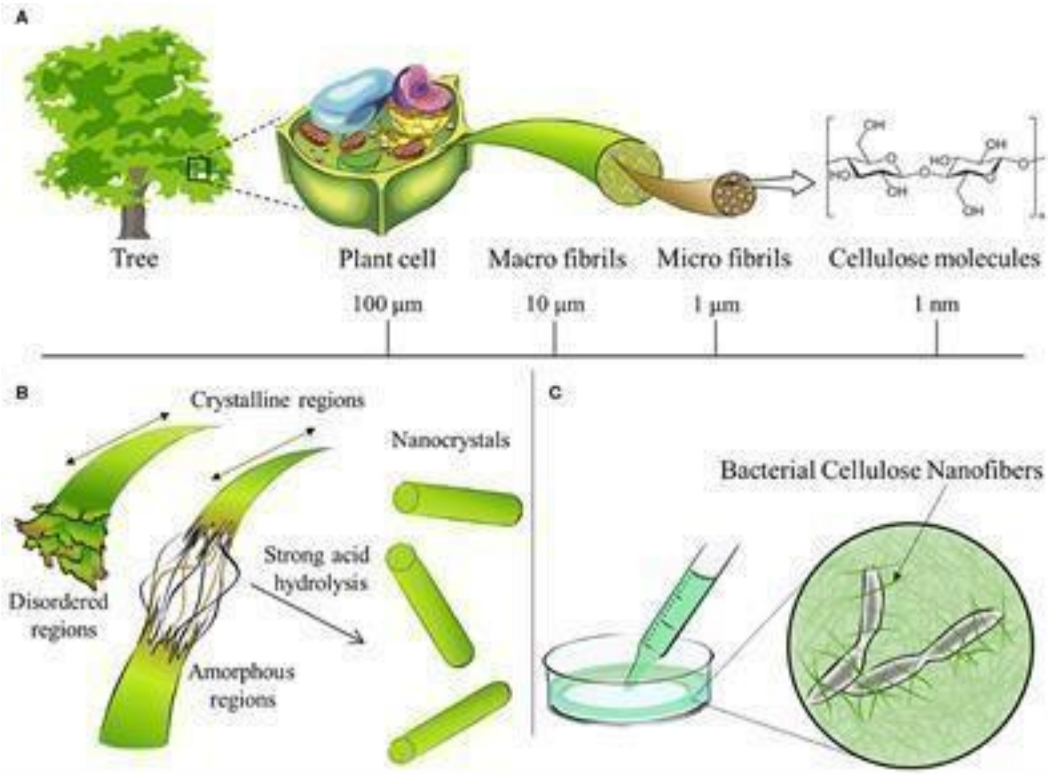

Figure 5. Types of nanocellulose: A) Plant nanocellulose, B) Nanocrystalline cellulose and C) Bacterial Nanocellulose. Reproduced from [14]

Properties of nanocellulose scaffolds

Nano cellulose has emerged as a promising material in the last 14 years. In2011 and 2012, there was a rapid increase in studies on the use of cellulose in technological applications such as bio-technology. In 2013, potential use of nanocellulose in tissue engineering, or the interaction of cells were studied [15]. In this study, composite membranes consisting of bacterial nanocellulose (BNC) and polypyrrole (PPy) were used as a template for seeding PC12rat neuronal cells. During the years from 2014, nanocellulose has been used in the interestingareas of tissue engineering like bone tissue engineering, liver tissue engineering, neural tissue engineering, cartilage tissue engineering, adipose tissue engineering ,vascular tissue engineering etc. [16]. The foremost paper with “nanocellulose“ dedicated to the potential use of the

nanocellulose as a biomaterial for constructing tissue replacements was a study by Kramer in June 2006 [17]. The spark towards the work was inspired by effective properties of nanocellulose such as water absorption capacity, appropriate strength and elasticity, controllable shape, nanofibrous and porous structure, and biocompatibility. The main focus was on developing collagen-likematerials based on the composites of bacterial cellulose and synthetic polymers. The same group of authors published a second review in August 2006 which dealt with technical and biomedical applications of nanocellulose which includes creating artificial blood vessels, cuffs for nerve surgery, animal wound dressings,and cosmetictissues [17]. A very important paper on the use of nanocellulose in tissue engineering focusedon creating bacterial cellulose nanofibrous scaffolds [18]. These scaffolds were promising forvascular tissue engineering as they can promote adhesion of *in vitro* human vascular endothelial cells. The papers on biomedical application of nanocellulose with a review article by Klemm et al. discussed the types, sources, modes of preparation and properties of nanocellulose [18]. It dealt with bacterial cellulose and suggested that it can be a suitable material for organ implants, wound dressing, replacements of blood vessels and bone tissues. The future applications in the biomedical field by bacterial nanocellulose were reviewed in 2010 [19].

In vitro and *in vivo* studies suggest that nanocellulose scaffolds are non- toxic or minimally toxic. The implanted nanocellulose and injected nanocellulose are considered haemo-compatible and biocompatible. Biomaterial scaffolds should be biodegradable *in vivo* after the formation and regeneration of new tissue but nanocellulose scaffolds do not fully biodegrade [20]. Porosity is the crucial and important feature in the tissue engineering context.This arose the possibilityof tailoring porous 3D architectures while maintaining structural integrity and makes the use of nanocellulose effectively. The inter connectivity of the pores helps in the diffusion of cell nutrients and Though nanocellulose scaffolds have very high porosity, the mechanical strength of thenanocellulose is lower for its applications in stiffer tissues like cartilage and bone and load bearing locations and sites. The mechanical strength can be enhanced by the covalent cross- linking of nanocellulose maintaining highporosity. The cross linkers used can be tannic acid, 1, 2, 3, 4-butanetetracarboxylic acid (BTCA), sodium (meta) periodate. Structural morphologyof scaffolds play a crucial role for numerous factors like protein adsorption and cell adhesion which promote natural cellular functions. The scaffold anisotropy should be such that it should effect the cell growth significantly. Example, myoblast cells prefer to grow in the direction of nanocellulose alignment.

Cellulose nanofibrils were modified either by two ways profoundly. A negative electrical charge can be introduced by TEMPO-mediated oxidation or a positive charge introduced by 2, 3-epoxy propyl trimethyl ammonium chloride. It had been proven that the cellperformance on anionic-CNF (a-CNF) is better than cationic-NCF (c-CNF) [20].

1. Some limitations and methods to overcome

5.1. There are some limitations to the use of plant nanocellulose in skin tissue engineering,at times there is limited degradation of nanocellulose that can cause skin irritation and skin defects. The retention of non-degradable components cause scar formation. However, degradability can be induced by incorporation ofcellulase enzymes [21]. The introduction of N-acetyl glucosamine residues into the cellulose during the synthesis can yield degradable cellulose. These residues can be degraded in the human body by the wide spread enzyme, lysozyme [22]. The other method to yield degradable nanocellulose scaffolds is oxidation. The implantation of oxidized acetyl cellulose sponges in rats had degradation of about47% whereas ethyl cellulose had degradation of about 18% after 60 weeks. Regenerated cellulose, 2, 3-dialdehydecellulose (DAC) are degradable nanocellulose scaffold. **Skin tissue engineering**

Skin is the largest organ of the human body that stores water, fat, vitamin D. It has significant role in protection, sensation, immunization, and regulation. It is made up of two main layers epidermis and dermis. The hypodermis is present beneath the dermis. Skin tissue engineering is the reconstruction of the superficial skin layer epidermis, formed by keratinocytes and the skin inner layer dermis formed by fibroblasts.

The surface structured bacterial nanocellulose films provided an excellentplatform forthe growth, spreading and migration in keratinocytes by formation ofclusters. It formed a surface-structured 3D network of bacterial cellulose nanofibers. The biologically active molecules combined with bacterial nanocellulose accelerates the adhesion and growth of skincells. E.g., chitosan enriches the adhesion of the human keratinocytes on bacterial cellulose films [24].

Poly-pyrrole and polyaniline the electroactive composites of bacterial cellulose and conducting polymers are promising for skin tissue engineering.

Bacterial nanocellulose exhibitwound healing and speeds up infection prevention. Tuning the physical properties of bacterial nanocellulose with the combination of cellulose nanowhiskers with hydrogels helps in the drug release in the respective tissues. The silver nanoparticles along with the nanocellulose scaffoldsis used toavoid and reduce bacterial and microbial infections in the tissues [25].

The plant nanocellulose can be chemically modified by its conversion to cellulose acetate or to hydroxyethyl cellulose. It enhanced the electro spinnabilityof cellulose that supported the growth of mouse subcutaneous fibroblasts. Skin tissue engineering and wound dressing is highly benefitted by the blend of the cellulose acetate with gelatine. 3D Cellulose acetate scaffolds produced by spin-printing stimulated the metabolic activity of human

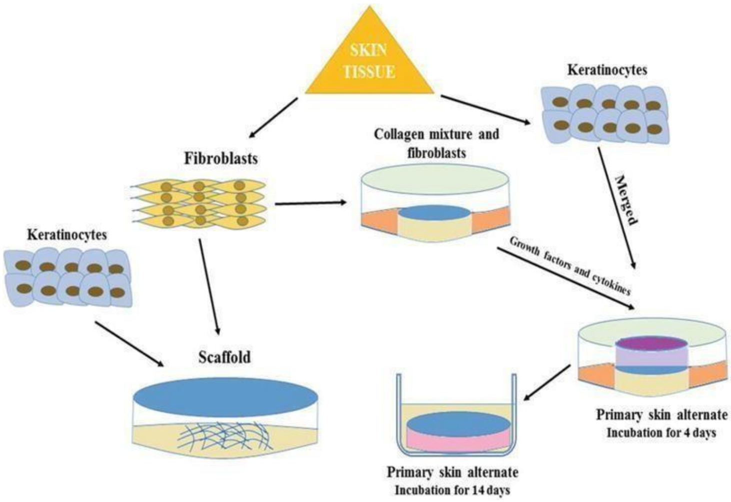

Figure 7. Depiction of the skin tissue engineering process by nanocellulose, [25].

5.1.Skeletal muscle tissue engineering

The skeletal muscles are those organs of the muscular system that are mostly attached by tendons to the bones of the skeleton system in the human body. The skeletal muscle consistsof uni-axially aligned muscle fibres assembled into fascicles. They propagate force on the bones by contraction in the same direction.The skeletal muscle cells are soft and fragile. The contractive forces are withstandby the connective tissue which furnish and assist the delicate muscle cells. Skeletal muscle play an important role in the physiological activities of blood vessels and nerves and their functionalities. Their coverings are routes of blood vessels and nerves.

An ideal tissue engineered skeletal muscle fibre should possess the contraction ability.Their functionalities and potentials should be similar to the native musclefiber. Electrospun cellulose nanocomposites and nanofibers (ECCNN) synthesized by electrospinning could be used for the skeletal tissue engineered fibre. When simulated human conditions are applied, the mechanical characteristics of the system can be compared to native tendons and ligaments.

These nanocomposites of engineered skeletal tissue possessed stable performance for cyclic loading and unloading [27]. Also, the irregular defects in the skeletal muscle can be rectified somewhat by the nanocellulose composites combined with gels. This can be used for the modification of the mechanical properties to clear the defects in the tissue engineered skeletalmuscle. The mechanically stable hydrogels can be prepared by cross-linking of hydrazide- functionalized POEGMA (poly (oligoethylene) glycol methacrylate) and aldehyde-functionalized NCC (A-NCC) and enhanced the dimensional stability.

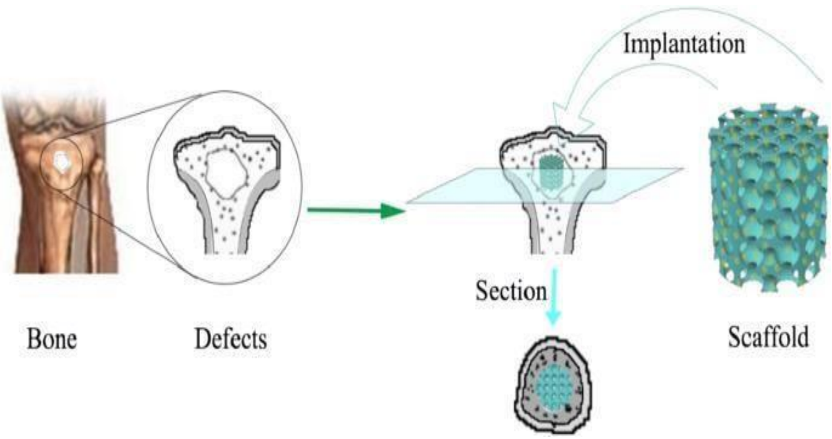

Figure 8. The representation of bone tissue engineering by nanocellulose scaffolds.

Reproduced from ref. [28].

Hydrogels can be used as bio-mimic scaffolds due to its remarkable properties of biocompatibility, controllable hydrogel anisotropy and tunable morphologies. The hydrogels used to make artificial muscle tissue by the monomer N-isopropylacrylamide and modified maleic anhydride NFC (MANFC) as the cross linker. The hydrogels formed were extensivelystretchable to more than 20 times theoriginal length. MANFC fibres are oriented largely to comprehend with the deformations which as a whole increases the tensile strength [29], https://www.sciencedirect.com/science/article/abs/pii/S0032386116304645].

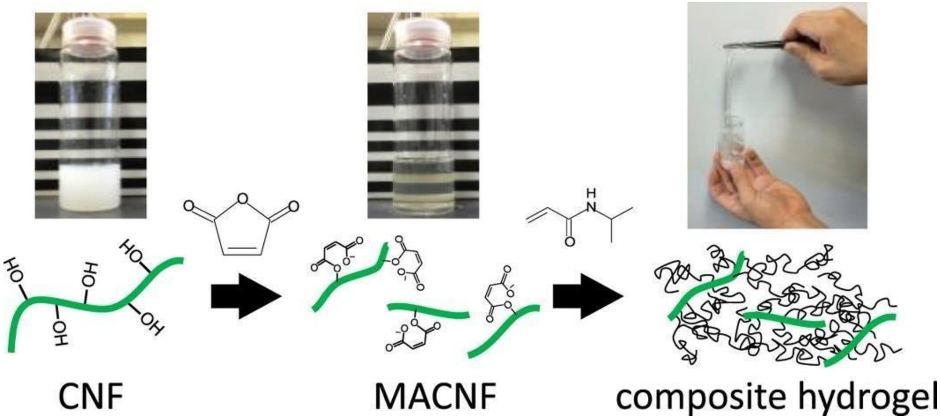

Figure 9. Surface modification of CNF with MA and the following hydrogel formation viainsitu polymerization of NIPAM. Reproduced fromref. [30].

5.2.Cardiac tissue engineering

Cardiac muscle tissue keeps the heart pumping through involuntary movements. The specialised cells that are pacemaker cells control the contractions of the heart. Cardiovascular tissue plays an important role in circulating blood for the transport of oxygen, carbon dioxide, nutrients, blood cells, and hormones and conserves the homeostasis of human body. Vascular grafts with nanostructured surfaces enhance the cell adhesion and proliferation. Cardio vascular diseases like heart valve disease, coronary artery disease, myocardial infarction, heart failure, pericardial disease, cardio myopathy. Simpledrug therapy is crucial and difficult to control theend- stage cardiac disease.

The composites prepared manifests promising applications in heart valves, cardiovascular substitutes and tissue engineered scaffolds. The 3D scaffolds for the tissue engineered cardiac should have the stability, flexibility and cyto- compatibility. The mechanicalstrength can be improved by crosslinking in NC based cardiac scaffolds. The potential toxicity of certain cross linkers can be avoided by single-component bioinks based on acetylated NFC (AceNFC) developed for direct ink writing which do not need cross-linking. The AceNFC bioinks required low concentration of nanofibrils and had favourable fidelity in dry and wet conditions. It possessed remarkable stability, biocompatibility with cardiac myoblast cells.

In another work a low-solid scaffold based on NFC, PLA were fabricated by 3D printing and manifested dryable and rehydratable properties deformation. NFC can accord to the printability and structural fidelity. Bio-inks should possess conductivity to restore normal conduction in damaged tissues for diseases such as arrhythmia. Conductive CNT patches wereprepared by 3D printed NFC/s ingle-walled CNT inks on BC. The patches formed are flexible, stretchable, and conductive in wet and dry conditions [31].

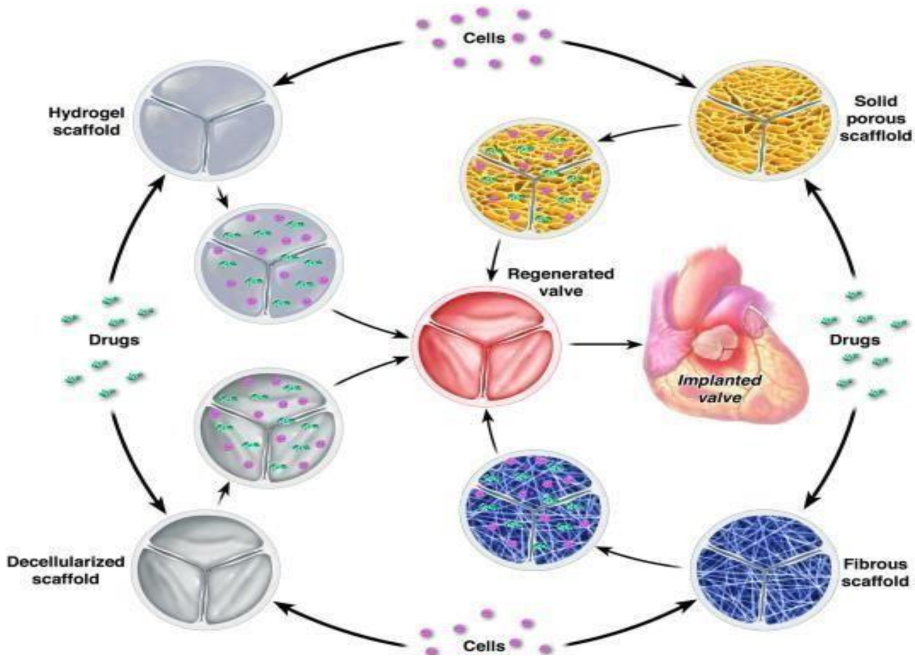

Figure 10. The cycle of processes in the cardiac tissue engineering [32]

5.3. Ophthalmic tissue engineering

The eye is a sensory organ that collects light, converts it into nerve impulses then the optic nerve transmits these signals to the brain and forms an image thereby providing sight. The human eyes comprises the two eyeballs surrounded by the orbit, the bony sockets of the skull. The eyes are protected by the fatty and fibrous tissue in the orbits. The eyelids, the conjunctiva, the lacrimal glands and the fibrous tunic (the outer coating layer of the eye).

Tissue engineered opthalmic tissue solve the problems by furnishing corneal regeneration implants, vision-corrective lenses and intraocular lenses. The requirement of ophthalmic tissue engineering is materials that possess excellent optical properties, efficient oxygen permeability, mechanical strength, better biocompatibility. Nanocellulose can be usedas an ideal material for the ophthalmic tissue engineering due to the properties like prominent water absorption, water

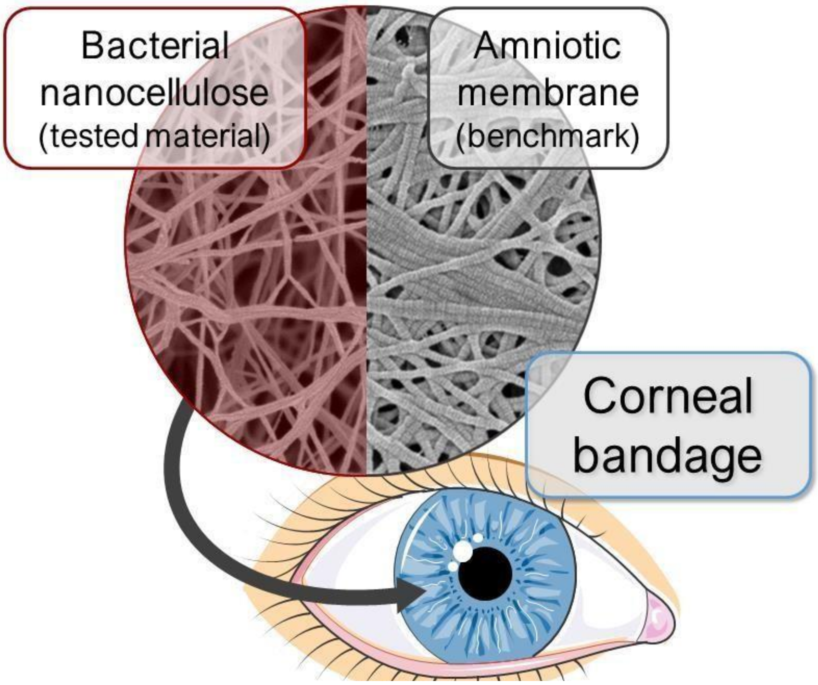

Figure 11. Bacterial nanocellulose corneal bandage. Reproduced from ref. [34].

retention, mechanical strength good compatibility and optical transparency due to the presence of large number of hydrogen bonds [33].

The hydrogel produced as such can promote and enhance the growth of human corneal epithelial cells. This acquires the potential for ophthalmic prostheses, disposable contact lenses, and corneal implants. For instance, the incorporation of PVA with NC can be used to produce a largely transparent macroporous hydrogel with more than 90 % water content. The visual acuity of ophthalmic devices can be improved by the NCC-PVA as it exhibited low light scattering when completely wetted. The excellent optical properties are shown by NCC or NFC in PVA hydrogel which are self-supporting hydrogels with large water content. The macroporous structures filled with water of hydrogel has a significant role in the provision of high oxygen permeability, low protein adsorption, and high wearing comfort. The viscoelastic and mechanical characteristics of hydrogels were influenced by the enhancement of NCC and solvent compositions.

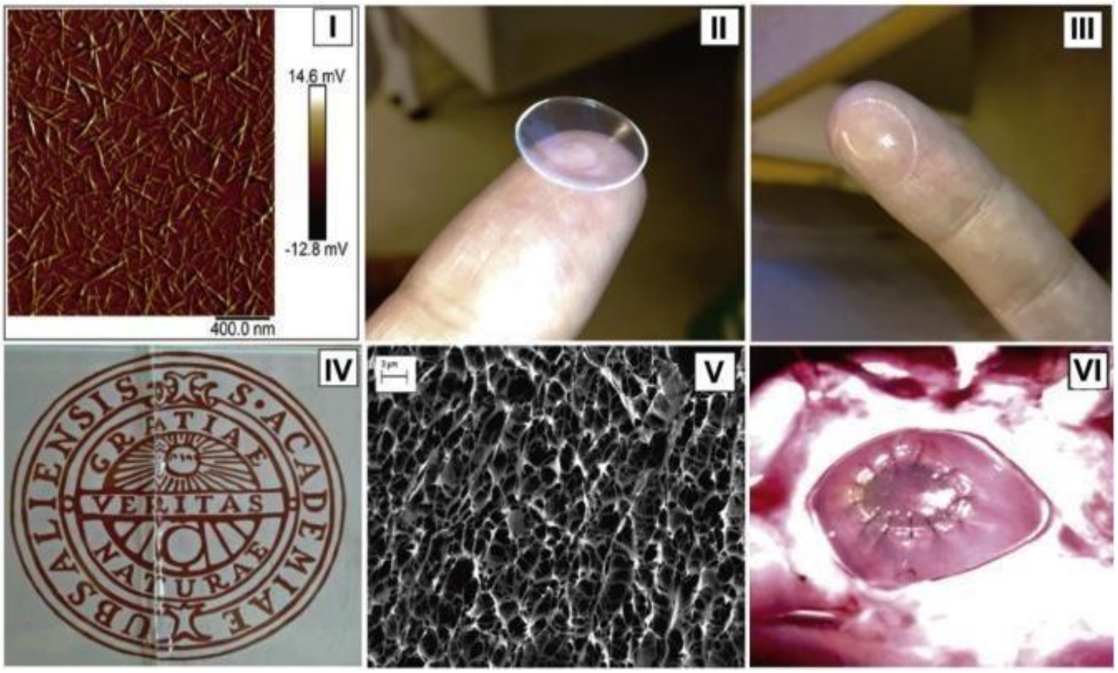

Figure 12. Tissue engineered eye lens. Reproduced from ref. [35]

5.1.Neural tissue engineering

The nervous system is the principal part of the human body that coordinates the behaviour and transmits signals between the body parts. The nervous systemis made up of a special type of cell called neurons at the cellular level. Neurons send signals in the form of electrochemical waves and travels through the thin fibres called axons. It releases the chemicalneurotransmitters at the junctions of other neurons called synapses. The nervous controls the internal environmental equilibrium and responds to the changes in the external environment. Itis dividedinto PNS which is the Peripheral nervous system and CNS which is the Central nervous system.

The rise of neural tissue engineering is due to the restricted capability of neural tissue to self- repair and regenerate after damage. The axonal development, the maintenance of the axon channel, the promotion of the neural stimulation and the activity can be carried out by theneural tissue engineered scaffolds. The cell behaviour can be induced correctly by the scaffoldswith eminent performances and these are of larger importance to the tissue engineered neural tissue.

Neural tissue engineered tissue can be made effectively from the electrospun NC- based scaffolds as they have controllable porosity, mechanical strength, orientation, and flexibility. 3D printing of neural tissue engineering scaffolds can be done by conductive CNF or carbon nanotubes. It has an electrical conductivityof 3.8×10^{-1} S/cm to which the neural cells preferably attach firmly and propagate and differentiate [36].

The elastic modulus and tensile strength of the electrospun nanocellulose can be increased by the addition of 20% weight of NCC (based on nanocellulose). The direction of itsfibre orientation can be increased by almost 171.6 and 101.7% and enhances the thermal stability. The electrical conductivity of printed NFC/CNT (carbon nanotube) were 0.38 S/cm with the diameter less than 1 mm. They should incorporate to enhance and improve the development of neuronal cells and their nourishment. These developments are to be done in neuronal cells that tend to adhere, propagate and differentiate effectively. The *in situ* electricalstimulation and improvement in the fidelity of the 3D scaffolds improve the performance of the scaffolds used in the neural tissue engineering.

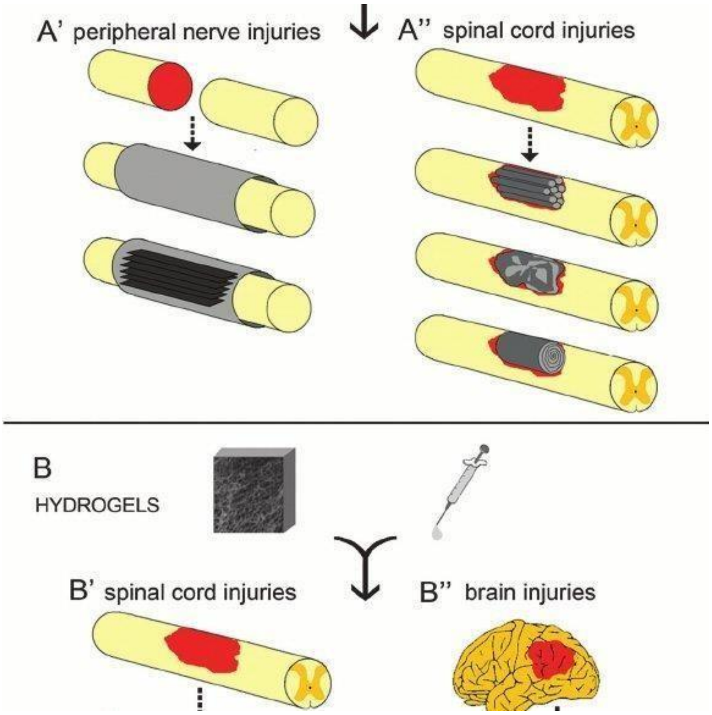

Figure 13. Treatment of nervous injuries by hydrogels. Reproduced from ref [37].

1. Future Prospects and Challenges of Nanocellulose Scaffolds in Tissue Engineering

The establishment of nanocellulose based scaffolds for tissue engineeringhas undergone a significant and substantial progress. The simpler scaffold formation methods andtheir mechanical features created numerous possibilities for the future prospects and applications of nanocellulose. The major challenges that occurred in the formation of scaffoldsis the combination of high porosity and apt mechanical performance. There is a gap between the research of nanocellulose scaffolds and their use in the commercial field. Therefore *in vivo* and *in vitro* studies should be done to bridge this gap. There is clinical demand, which enhance the development of tissue engineering technology for the treatment of the sorelydamaged tissues or organs. NC based scaffolds meet the requirements of an

effective tissue engineered though it has superior water absorption, water retention, biocompatibility, and mechanical properties.

The synthesis of nanocellulose scaffolds by additive manufacturing and biofabricationis effective for the emergent tissue engineering techniques. The different shape and size of thematerials can be customized by 3D printing and it is highly useful in the biomedical purposes. 3D printed materials can be used in the bone regeneration like collagen-based scaffolds loadedwith bone marrow stem cells set out as *ex-situ* miRNA delivery systems. But a major challengearises in this is there is shrinkage or swelling in the resultant materials after 3D printing.

In skin tissue engineering, the direct use of nanocellulose is not much advantageous due to its non- degradability in human organism. The scaffolds thatpersists in the skin lead to scar formation and complications. Therefore, nanocellulose can be used as a temporary carrierfor the delivery of cells into the wounds and it can be eliminated when the cells have adhered to the wounded region. But, the *in vitro* construction of artificial skin used for the experimental purposes such as for metabolism, vascularization of skin tissue, effects of drugs on tissues of various organs in the body and various studies in biology. Nanocellulose can be used as a material for the fabrication of epidermal electronics. It can be used as an advanced dressing material for systematic utilization of various drugs, transparent dressing material to allow, permit the direct inspection of wounds.

References

[1] Computational and structural biotechnology Journal 17

[2] Scaffolds for tissue engineering: A state of the art review concerningtypes,properties,materials, processing and characterization

[3] https://doi.org/10.1016/B978-0-08-100979-6.00001-X

[4] Nanocellulose in thin films,coatings and plies for packaging applications

[5] Basu et al., 2017, Fereshteh, Fathi, Bagri, & Boccaccini, 2016

[6] Dai et al., 2017; Shao et al., 2019; Yang, Lu, Li, Zhang, & Cha, 2016

[7] Electrospun Cellulose and Nanocellulose Composites as a Biomaterials

[8] Khazeni, S.; Hatamian-Zarmi, A.; Yazdian, F.; Mokhtari-Hosseini, Z.B.; Ebrahimi-Hosseinzadeh, B.; Noorani, B.; Amoabedini, G.; Soudi, M.R. Production of nanocellulose in miniature-bioreactor: Optimization and characterization. Prep. Biochem. Biotechnol.

[9] Mahdavi, M.; Mahmoudi, N.; Rezaie Anaran, F.; Simchi, A. Electrospinning ofnanodiamond-modified polysaccharide nanofiberswith physico-mechanical properties close to natural skins

[10] Kramer, F.; Klemm, D.; Schumann, D.; Heßler, N.; Wesarg, F.; Fried, W;Macromol.
Symp. 2006, 244, 136–148

[11] Li, W.; Guo, R.; Lan, Y.; Zhang, Y.; Xue, W.; Zhang, Y. Preparation andPreparation of nanocrystals reinforced collagen composite films. J. Biomed.

[12] Mo, Y.; Guo, R.; Liu, J.; Lan, Y.; Zhang, Y.; Xue, W.; Zhang, Y. Preparation andproperties of PLGA nanofiber membranes reinforced with cellulose nanocrystals. Colloids Surf. B Biointerfaces 2015, 132, 177–184.

[13] Shefa, A.A.; Amirian, J.; Kang, H.J.; Bae, S.H.; Jung, H.-I.; Choi, H.; Lee,S.Y.; Lee,B.-T. In vitro and in vivo evaluation of effectiveness of a novel TEMPO-oxidized cellulose nanofiber-silk fibroin scaffold in wound healing. Carbohydr. Polym. 2017, 177, 284–296

[14] Bhattacharya, M.; Malinen, M.M.; Lauren, P.; Lou, Y.-R.; Kuisma, S.W.; Kanninen,L.; Lille, M.; Corlu, A.; GuGuen-Guillouzo, C.; Ikkala, O.; et al. Nanofibrillar cellulose hydrogel promotes three-dimensional liver cellculture. J. Controll. Release 2012, 164, 291–298.

[15] Recent trends in the preparation, characterization and applications of nanocellulose

[16] Mondal, S. Preparation, properties and applications of nanocellulosic materials.
Carbohydrate Polym. 2017, 163, 301–316\
[17] Klemm, D.; Schumann, D.; Kramer, F.; Heßler, N.; Hornung, M.; Schmauder, H.-P.; Marsch, S. Nanocelluloses as innovative polymers in research and application. In
Polysaccharides II; Klemm, D., Ed.; Springer: Berlin/Heidelberg, Germany, 2006; Volume205, pp. 49–96, ISBN 978-3-540-37102-1
[18] Bodin, A.; Ahrenstedt, L.; Fink, H.; Brumer, H.; Risberg, B.; Gatenholm, P. Modification of nanocellulose with a xyloglucan–RGD conjugate enhances adhesion and proliferation of endothelial cells: Implications for tissue engineering. Biomacromolecules2007, 8, 3697–3704.
[19] Gatenholm, P.; Klemm, D. Bacterial nanocellulose as a renewable material forbiomedical applications. MRS Bull. 2010, 35, 208–213.
[20] Weishaupt, R.; Heuberger, L.; Siqueira, G.; Gutt, B.; Zimmermann, T.; Maniura- Weber, K.; Salentinig, S.; Faccio, G. Enhanced antimicrobial activity and structural transitions of a nanofibrillated cellulose–nisin biocomposite suspension. ACS Appl. Mater.
Interfaces 2018, 10, 20170–20181
[21] Hu, Y.; Catchmark, J.M. In vitro biodegradability and mechanical properties of bioabsorbable bacterial cellulose incorporating cellulases. ActaBiomater. 2011, 7, 2835–2845.
[22] Hu, Y.; Catchmark, J.M. Integration of cellulases into bacterial cellulose:Toward bioabsorbable cellulose composites. J. Biomed. Mater. Res. Part B Appl. Biomater. 2011,97B, 114–123.
[23] Yadav, V.; Paniliatis, B.J.; Shi, H.; Lee, K.; Cebe, P.; Kaplan, D.L. Novelin vivo- degradable cellulose-chitin copolymer from metabolicall

engineered Gluconacetobacterxylinus. App. Environ. Microbiol. 2010, 76,6257–6265.

[24] Khan, S.; Ul-Islam, M.; Ikram, M.; Islam, S.U.; Ullah, M.W.; Israr, M.; Jang, J.H.; Yoon, S.; Park, J.K. Preparation and structural characterization of surface modifiedmicroporous bacterial cellulose scaffolds: A potential material for skinregeneration applications in vitro and in vivo. Int. J. Biol. Macromol. 2018, 117,1200–1210.

[25] Shi, Z.; Li, Y.; Chen, X.; Han, H.; Yang, G. Double network bacterial cellulosehydrogel to build a biology- Device interface.

[26] Tissue Engineering for skin replacement methods; doi 10.5772/interchopen.69905

[27] He et al., 2014 Scaffolds for tissue engineering biomicromolecules

[28] Recent advances in additive manufacturingtechnology for bone tissueEngineering

[29] https://www.sciencedirect.com/science/article/abs/pii/S0032386116304 6 45

[30] https://www.sciencedirect.com/science/article/abs/pii/S0032386116304 6 45

[31] Hoagland, Santos, Poelzing, & Gourdie, 2019 Pedrotty et al., 2019 Therole of thegap junction perinexus in cardiac conduction

[32] Scaffolds for tissue engineering of cardiacvalves doi.org/10.1016/j.actbio.2014.03.014

[33] Tummala, Joffre, Rojas, Persson, & Mihranyan, 2017 ; The role of solvent onStructure, coelasticity, and mechanical compressibility in nanocelllulose reinforced hydrogels.

[34] Tummala, Bachi, & Mihranyan, 2019 train-induced stiffening of nanocellulose- reinforced poly(vinyl alcohol) hydrogels mimicking collagenous soft tissues. Soft Matter,13(21), 3936–3945.

[35] Advances in tissue engineering of nanocelllulose based scaffolds

[36] Sustainable resource based hyperbranched epoxy nanocomposite an infectionresistant, biodegradable, implantable muscle scaffold

[37] Conductive microfibers derived from wet spun lignin/ micro nanocellulosehydrogels

[38]Surface modification of electrospun scaffolds for endothelialisation of tissueengineered vascular grafts using human cord blood derived endothelial cells.

[39] Advanced materials through assembly of nanocelluloses

[40] Three dimensional printed polycaprolactone microcrystalline cellulose