Review

Advancement of Natural Cellulosic Scaffolds for Tissue Engineering

Mohammadreza Aghazadeh[1,2,3], Sheyda Delfanian[1], Pouria Aghakhani[2,3] , Shahin Homaeigohar [4], Atefeh Alipour [5*] and Hosein Shahsavarani[2,3*]

[1] Faculty of Chemical Engineering, Babol Noshirvani University of Technology, 47148 Babol, Iran; m.aghazadeh@stu.nit.ac.ir (M.R.A); S.delfanian@stu.nit.ac.ir (S.D)
[2] Department of Cell and Molecular Sciences, Faculty of Life science and Biotechnology, Shahid Beheshti University, Tehran, Iran; pouriaaghakhani97@gmail.com (P.A)
[3] Laboratory of Regenerative Medicine and Biomedical Innovations, Pasteur Institute of Iran, Tehran, Iran
[4] School of Science & Engineering, University of Dundee, United Kingdom shomaeigohar001@dundee.ac.uk (S.H)
[5] Department of Nanobiotechnology, Pasteur Institute of Iran, Tehran, Iran
* Correspondence: hosein.shahsavarani@gmail.com (H.S) a_alipour@pasteur.ac.ir (A.A)

Abstract: In the recent years, tissue engineering researchers have exploited a variety of biomaterials that can potentially biomimic extracellular matrix (ECM) for tissue regeneration. Natural cellulose, mainly obtained from bacterial (BC) and plant-based (PC) sources, can serve as an a high potential scaffold material for different regenerative purposes. Natural cellulose has drawn the attention of researchers due to its advantage over synthetic cellulose in terms of …, exhibiting suitable characteristics in vitro and imitating native tissues. In this article, we will review the recent in vivo and in vitro studies aimed to assess the potentials of natural cellulose for the purpose of soft (skin, nerve, among others) and hard (bone and tooth) tissue engineering.

Keywords: Polymeric biomaterials ; Natural Cellulose ; Tissue Engineering, Differentiation, Microenvironment??

1. Introduction

Despite several successful clinical transplantations of allogeneic tissues and organs to treat diseases caused by failed or defected organs or tissues, shortage of allogeneic donors and medical complications has hindered the number of these types of treatments [1]. As a new field of bioengineering, tissue engineering has created tissue substitutes by combining engineering, cell biology, and material sciences, to imitate the structural and physiological characteristics of native tissues [2]. The organ shortage as a clinical emergence can be conceivably overcome through tissue regeneration via tissue engineering [1].

Cellulose, one of the most exploited natural materials on the planet, has made its way through civilizations from early ages to present [3]. The contribution of this carbohydrate to human life has attracted researchers' attention in various fields of science. From food packaging and energy supply to biomedical and therapeutic applications, it has served as a valuable biopolymer [4].

Biomaterials have been widely investigated for tissue regeneration, particularly as ECM mimicking structures that are biocompatibile, biodegradable, and non-cytotoxic. They are desired to be low cost with proper physicochemical properties and fabricated in a simple manner [5]. Possessing tunable structural and chemical properties, cellulose and its derivatives have found multiple uses in the biomedical field to treat and regenerate damaged tissues such as bone, skin, tendons, cartilage, and blood vessels [6]. The highly organized structure of cellulose consisting of several glucose monomers can endure against biodegradation in the absence of cellulolytic enzymes. Therefore, it can play the role of ECM and mimic the natural microenvironment of the human body, thereby supporting cell growth and tissue regeneration [7]. The structural characteristics of cellulose

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2022 doi:10.20944/preprints202202.0176.v1

vary based on the assemblies of microfibrils and arrangements of the β-(1,4′)-D-glucopyranose monomers. The naturally occurring cellulose I, the native form of cellulose, is characterized by particular hydrogen bonding and van der Waals interactions of two co-existing crystal arrangements; cellulose I_α (triclinic) and cellulose I_β (monoclinic). Two main types of natural cellulose, including bacterial and plant-based cellulose, have been extensively studied for bone and skin tissue regeneration due to their biocompatibility, hydrophilicity, non-toxicity, and other native tissue-mimicking abilities [8, 9]. Bone and skin tissues are not the only regenerative territories that natural cellulose has conquered. It also has been applied for other tissue engineering purposes such as neural, adipose, tendons, lung, and cardiac regeneration [10].

This review discusses the recent studies and developments of natural cellulose for bone and skin tissue regeneration. Additionally, the last section addresses the application of bacterial and plant-based cellulose in the other tissue engineering areas.

2. Cellulose Sources

Generally, cellulose can be derived from nature or obtained as a synthetic material. The crystal structure of cellulose differs based on its synthetic or natural origin. In this regard, natural cellulose consists of cellulose I crystal structure while the synthetic ones have cellulose II and III configurations [6].

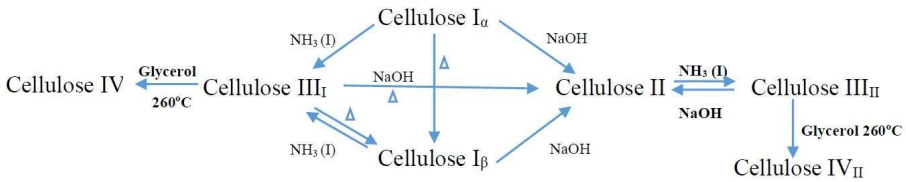


Figure 1. Scheme of the transformation cycle of cellulose crystals [8].

2.1. Natural cellulose

All cellulose-synthesizing organisms, including bacteria, algae, tunicates, and higher plants, contain cellulose synthase proteins, which catalyze glucan chain polymerization. Despite the fact that the catalytic domains of cellulose synthases are preserved across all cellulose-synthesizing organisms, the vast differences in the organisms' lifestyles and the structure of the cellulose they produce suggest that the regulatory proteins and underlying mechanisms for cellulose synthesis may have evolved independently [11]. Bacterial cellulose and plant-derived cellulose are the two main classes of cellulose that are used in natural polymer tissue engineering scaffolds.

2.1.1. Bacterial cellulose

Bacterial cellulose (BC) is a biomaterial produced naturally by bacteria. It has a unique cellulose nanofiber-weaved 3D network structure that gives it exceptional mechanical qualities, water holding capacity, and suspension stability. It also has a high purity, a high degree of crystallinity, excellent biocompatibility, and biodegradability. Accordingly, BC has attracted a lot of attention from both academia and industry [12]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2022 doi:10.20944/preprints202202.0176.v1

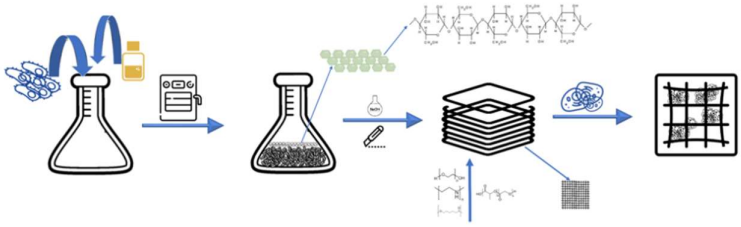


Figure 2. Schematic illustration of the fabrication procedure of Bacterial cellulose scaffolds: 1. Bacterial inoculolation[Ref???], 2. Purification and slicing[Ref???], 3. Modification, [Ref???]4. Cell Culture[Ref???].

2.1.2. Plant- derived cellulose

Plant-derived cellulose is typically mixed with hemicellulose, lignin, pectin, and other compounds, whereas bacterial cellulose is almost pure, possesses a significantly higher water content, and shows a notably improved tensile strength due to its longer chain length.[ref???]

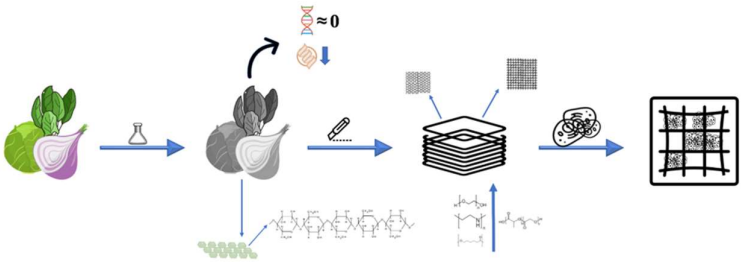
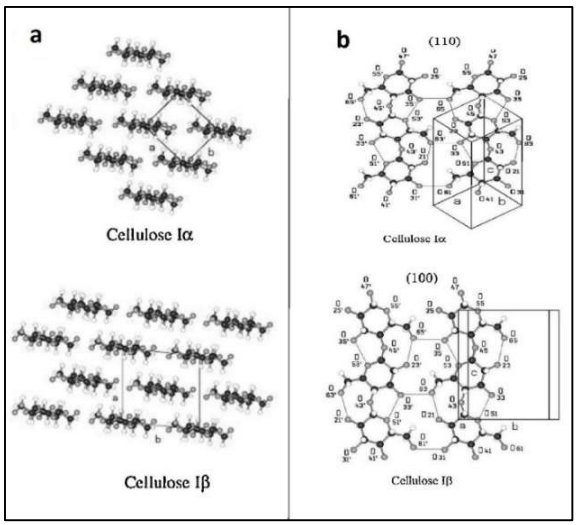


Figure 3. Fabrication process of the plant- derived cellulosic scaffolds: 1. Plant decellularization, 2. Slicing, 3. Modification, 4. Cell culture[Ref???]

The native cellulose structure is composed of two crystalline forms of Iα and Iβ. Since the natural cellulose is obtained from different origins, these crystalline forms are found in various proportions. Algal and bacterial cellulose possess a rich content of Iα. While, animal and higher plant cellulose are rich of Iβ, which is thermodynamically more stable [13].



 doi:10.20944/preprints202202.0176.v1

Figure 2. (a) Projections of the cellulose I crystal packings. (b) Two single sheets of cellulose Iα and cellulose Iβ on the (100) and (110) crystallographic planes, respectively [13].

3. Natural Cellulose for Bone Tissue Engineering

Over 2.2 million bone grafts are used per year to treat bone injuries and defects, thus notably affecting patients welfare and life quality. Despite several disadvantages, administering bone grafts is yet the standard strategy for reconstructing bone tissues in clinical practices [14]. Both allo- and auto-grafting strategies might encounter multiple clinical concerns such as limited amounts of graft material, antigenicity, short-term viability, infection, and unpredicted graft resorption. However, recent considerations have overcome some of these complications at the expense of important grafts properties such as osteoconductivity, osteoinductivity, and mechanical strength [15]. In the recent years, remarkable progress in bone tissue engineering has proposed efficient treatments to encourage bone regeneration. In this regard, it is now possible to tailor the physicochemical characteristics of scaffolds using nanotechnology to mimic native bone tissue behavior [14].

Bacterial cellulose, an abundant, inexpensive, slowly degradable, and biocompatible biomaterial, possesses unique characteristics that have drawn the attention of bone tissue engineering researchers [16]. In a recent study, Dubey et al. [year]combined osteoinductive low-dose BMP-2 primed murine mesenchymal stem cells with osteoconductive 3-D structured bacterial cellulose scaffolds to propose a cost-effective bone tissue engineering strategy. They prepared ECM-mimicking 3-D macro/microporous-nanofibrous bacterial cellulose (mNBC) scaffolds. The initial studies on the interaction of the scaffold material with the unprimed C3H10T1/2 cells confirmed the scaffolds' ability to provide proper cell adhesion, growth, and infiltration. For osteogenic studies, the cells were preconditioned with 50 ng/mL BMP-2 for 15 min and then were cultured on the mNBC scaffolds for up to three weeks. The results showed that the mNBC scaffolds could partially promote the mineralization of the cells. Also, the scaffolds seeded with the low-dose BMP-2 primed cells have displayed remarkably improved bone matrix secretion and maturation compared to unprimed ones, which demonstrates osseointegration of this method [17]. Cakmak et al. [year]developed a novel 3D printed porous scaffold using a blend of polycaprolactone (PCL), gelatin (GEL), and bacterial cellulose (BC) reinforced with different concentrations of hydroxyapatite (HA) nanoparticles. To obtain an optimal pore size and the uniform blending ratio, they fabricated four different composites whose infill rates varied from fifty to eighty percent. The ideal pore size for bone tissue-mimicking ECM was achieved at the 80% infill rate and resulted in more than 90% uniformity ratio. BC-containing composites showed a lower tensile strength and higher cell viability compared to the ones without BC. Furthermore, including 0.25% HA in the blends enhanced cell adhesion and viability more than other composites. The comparative studies on these printed scaffolds demonstrated their potential application as bone implants [18]. In another study, Aki et al. . [year] investigated the effects of incorporating bacterial cellulose into Hexagonal Boron Nitride (hBN) reinforced polyvinyl alcohol (PVA) to fabricate 3D printed bone tissue engineering scaffolds. The results showed that although the tensile strength decreased in 0.5 wt.% BC, the cell viability and adhesion significantly increased. Moreover, the DSC analysis confirmed that the crystalline structure of PVA was not disrupted by the additives [19]. A 3D porous microsphere was fabricated by Zhang et al. using chemically synthesized collagen (COL) and bacterial cellulose with additional Bone morphogenetic protein 2 (BMP-2). This highly porous scaffold was shown to have a particle size ranging from 8 to 12 microns, a pore volume of 0.59 cm^3/g, and an average pore diameter of was 198.5 nm. The in vitro studies revealed that these scaffolds promoted the proliferation, adhesion, and osteogenic differentiation of mice MC3T3-EL cells, thus offering a promising capacity as biocompatible 3D-COL/BC/BMP-2 microsphere based scaffolds for bone tissue engineering [20]. Codreanu et al. . [year] assessed the in vitro biocompatibility and in vivo osteoblast differentiating ability of bacterial cellulose-modified

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2022 doi:10.20944/preprints202202.0176.v1

polyhydroxyalkanoates (PHB/BC) scaffolds. The in vitro study confirmed the biocompatibility and the supporting role of the PHB/BC scaffolds for the proliferation of the 3T3-L1 preadipocytes. Furthermore, BC contributed to differentiation of osteoprogenitor cells to osteogenic cells in the initial stages. An in vivo study on a critical-size mouse calvarial defect showed an intensified OSX expression and enhanced ALP activity in the first four weeks after implantation of the scaffolds. Thus, these results indicated that the BC-PHB scaffold could promote osteoblast differentiation in vivo and eventually induce new bone formation as verified by X-ray and histology/histomorphometry analysis [21].

As a natural cellulose-based material, decellularized cabbage (DCb) has been investigated for bone tissue engineering by Salehi et al. . [year]They decellularized CB via an organ perfusion method using SDS, Triton-X100 and sodium hypochlorite, then washed it by deionized water, normal hexane, and PBS. The as-prepared DCB scaffold had an irregular geometry, whereby entrapping the seeded cells. Both BET and tensile test indicated its potential for mimicking spongy bone tissues. Moreover, the in vitro studies showed a significant increase in ALP activity and mineralization of BM-MSCs cultured on the scaffold compared to the control group. The gene expression analysis implied the higher expression of osteocalcin, collagen-1 (Col-I), Runx2, and ALP in the cells cultured on DCb leaves compared to those cultured on the petri dish. Conclusively, taking into account the osteogenic capacity and the bone healing potential of the scaffold, DCb was proven to be a remarkable ECM-mimicking structure for bone tissue engineering applications [22].

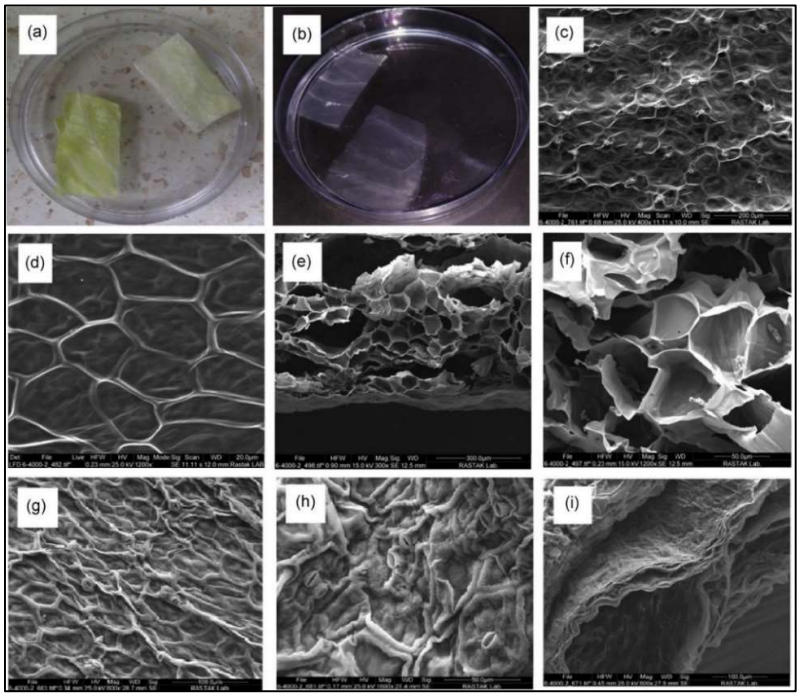


Figure 3. The camera images show the leaves of Cabbage (a) and decellularized Cb (b). c,d) SEM images of decellularized Cb. e,f) Cross-sectional SEM images of decellularized Cb. g,h) SEM images of decellularized Cb cultured with BM-MSCs. i) Cross-sectional SEM image of decellularized Cb cultured with BM-MSCs [22].

Benefitting from the structural similarities of *Bambusa vulgaris* with native bone tissues, Mohan et al. [year]prepared an oxidized decellularized Bamboo stem and assessed its ability to act as a bone scaffold. The plant stem was treated with different ratios of SDS, Triton X-100, and sodium hypochlorite for 24h, 48h, and 72h in a shaker, followed by washing with distilled water and a lyophilization process. The decellularized bamboo stem was oxidized using sodium periodate to improve its biodegradability and biocompatibility.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2022 doi:10.20944/preprints202202.0176.v1

Despite a lower compressive strength of the oxidized bamboo stem, it still remains within the range of cancellous bone's (0.5 to 20 MPa). Therefore, this material can be used for restoration of the damaged maxillo-facial and cranial bones that are exposed to non-significat load levels. In terms of protein adsorption, it was demonstrated that the protein and aldehyde groups bonded covalently between their amino groups on the fiber's surface. The oxidized bamboo fiber was notably coated with sericin and, as a result, cell adhesion increased compared to the non-oxidized one. In vitro studies revealed that filopodial extensions, cell viability, and the ALP activity were higher on the oxidized groups than non-oxidized ones. For the in vivo studies, the oxidized scaffold was implanted subcutaneously in a rat model. The results confirmed improved angiogenesis, biocompatibility, and biodegradation of the scaffold, thus its promising potential as a bone scaffold for non-load-bearing applications [23]. In another investigation by Salehi et al., the outermost skin of onion was assessed as a tissue engineering scaffold. After a decellularization process similar to their other study, the material's mechanical and structural characteristics were analyzed. The decellularized onion skin was shown to possess an orederd rectangular geometry, interconnected pores, moderate roughness, and a high tensile strength. Moreover, the water contact angle test indicated that the decellularized scaffold was amphiphilic with both hydrophilic and hydrophobic behavior. The in vitro tests based on BM-MSCs confirmed that the decellularized onion skin provides a biocompatible and non-toxic environment for cell growth up to five days. Furthermore, ALP activity and calcium content were shown to be higher in the cells present on the scaffold than those in the control group. Also, ALP, Runx-2, Osteocalcin, and Col-I were expressed in the BM-MSCs cultured on the decellularized onion skin superbly higher than the control group. All the results validated the positive influence of the decellularized onion scaffold on osteogenic differentiation of stem cells [24].

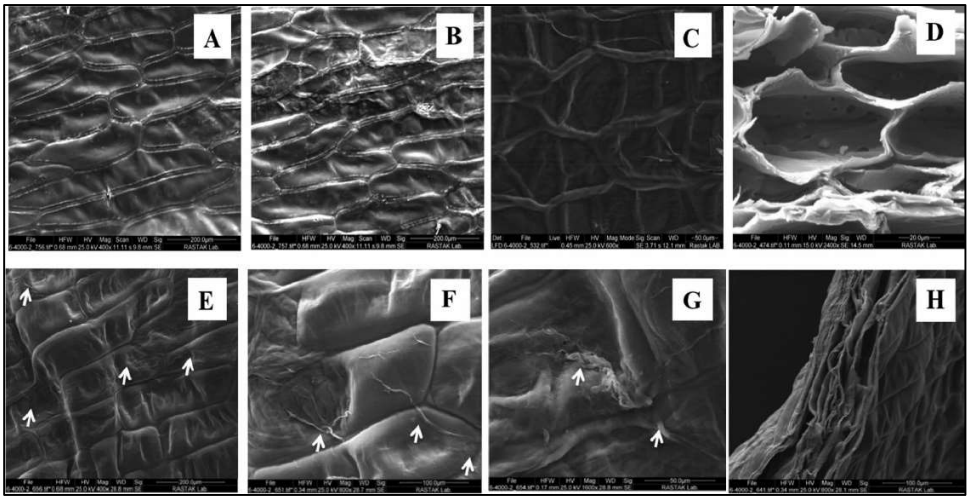


Figure 4. SEM images of the onion skin before (a) and after decellularization (b and c). d) Cross-sectional SEM image of the decellulaized onion skin after decellularization. SEM images of the surface (e-g) and cross-section (h) of the decellularized onion skin after 18 days BM-MSCs culture (the arrows mark ….) [24].

5. Natural Cellulose for Skin Tissue Engineering

Skin, the largest organ of the human body, is composed of the dermis, epidermis, and subcutaneous tissue. The external layer is epidermis that is without blood vessels and contains only keratinocytes. This layer is divided into five sublayers, stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2022 doi:10.20944/preprints202202.0176.v1

respectively, from the basal layer to the surface. Blood vessels and nerves pass through a thicker skin layer in the middle called dermis, including the papillary and reticular dermis [25]. The reconstruction of epidermis and dermis as two constituent layers of skin is the main challenge in skin tissue engineering [10]. The subcutaneous tissue, composed of adipocytes and collagen, is the deepest layer of skin [25]. Clinically, treatment of surface skin defects is difficult to achieve and requires more sophisticated strategies. In this regard, skin tissue engineering offers new opportunities for scaffolding, wound dressing, and skin substitution [10].

Several biomaterials such as chitosan [26], silk fibroin [27], collagen [28], and hyaluronic acid [29], have been proposed for wound healing and skin regeneration. Cellulose, as the most abundant natural polymer, has also shown promsing wound haling potentials. For instance, Wahid et al. prepared a BC-based wound dressing containing ε-polylysine (ε-PL). BC was crosslinked by polydopamine (PDA) through a 24h self-polymerization process, followed by the ε-polylysine treatment (1, 2, 3, and 5%). The zone inhibition and colony-forming unit (CFU) assays along with a Live/Dead fluorescent staining method revealed a profound antibacterial activity for the wound dressing. Additionally, the functionalized dressing material exhibited proper cytocompatibility and hemocompatibility in vitro. In vivo testing based on the Sprague–Dawley rats with full-thickness wounds infected with S.aureus, indicated improved wound closure and skin regeneration compared to the control groups. Therefore, the functionalized BC-based wound dressing is able to properly disinfect skin wounds and induce their healing in clinical applications [30].

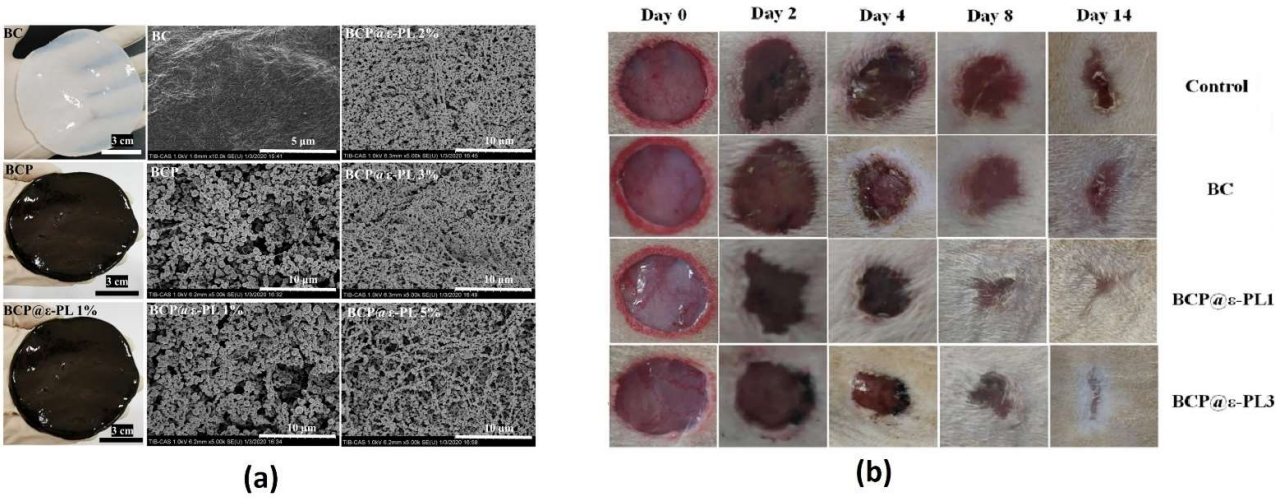


Figure 5. a) Camera images of the wound dressings made of BC, BC/PDA (BCP), and BC/PDA with 1% ε-PL and their corresponding SEM micrographs (note that the last (right) column contains SEM images of BCP@ε-PL dressings with higher additive concentrations). b) In vivo wound healing potential of the BC and BCP@ ε-PL1&3 dressings compared to the control imaged at different time points up to two weeks [30].

To exclude the necessity to use cytotoxic crosslinking agents, Li et al. prepared an all-natural injectable hydrogel composed of dialdehyde bacterial cellulose (DABC) reinforced chitosan for wound dressing. In this composition, DABC was meant to perform as a non-toxic crosslinker. The as-developed dressing material showed optimum mechanical properties, injectability, a promising drug sustained-release, and antibacterial activity. Moreover, in vitro tests using L929 fibroblast cells implied biocompatibility of the CS-DABC hydrogel [31]. The byproduct of symbiotic culture of yeast and bacteria in kombucha is the bacterial cellulose pellicle. In a recent study, Pilliai et al. partially acid hydrolyzed the bacterial cellulose-based sustainable kombucha (KBC) sheet and assessed its ability to

 doi:10.20944/preprints202202.0176.v1

serve as a 3D printed scaffold. In this regard, two acidic solutions including sulphuric acid and hydrochloric acid were employed to partially hydrolyze the obtained bacterial cellulose. The better extrusion conditions were achived when 30% sulphuric acid was used. The acid hydrolyzed KBC showed controllable mechanical properties and was successfully extruded to a multi-layered 3D structure, implying its proper formability and printability. The seeding of human adult dermal fibroblast and MC3T3-E1 cells on the scaffolds certified their biocompatibility and non-toxicity in vitro. As a result, this 3D printed gel scaffold has a remarkable potential to be used in soft tissue engineering applications such as wound healing or skin regeneration [32].

Phan et al. [X] introduced a technique for decellularizing tobacco BY-2 cells and rice cells, and tobacco root tissues using deoxyribonuclease I (DNase I) without surfactants. The THP-1-derived macrophage activation experiment indicated that the BY-2 cell-derived matrices did not stimulate TNF-α secretion. Thus, implementing the decellularized matrices into scaffolds can potentially avoid inflammatory responses. Human foreskin fibroblasts (hFFs) were used for in vitro assessment of decellularized BY-2 cell-derived matrices. The hFFs were shown to properly attach and grow on the plant cells and tissue-derived matrice. Thus, the cultured plant-derived cellulose matrices were confirmed to be a promising platform for tissue engineering applications [33]. In another study, Mehendiran et al. [X] decellularized Borassus flabellifer endosperms to mimic ECM for the purpose of tissue engineering. After decellularization, cellulose-chitosan (CS/CHI) hybrid scaffolds were obtained by treating decellularized endosperms. The as-prepared scaffolds showed improved thermal stability and compressive strength but a reduction in biodegradability compared to the control group. Furthermore, in vitro investigations using fibroblasts indicated that a better microenvironment is provided by the scaffolds enabling enhanced cell adhesion and colonization. Taking into account such merits, the CS/CHI scaffold can be regarded as a promising candidate for tissue engineering and 3D cell culture [34].

5. Natural Cellulose for Dental Tissue Engineering

Dental complications including cavities, periodontitis, apical periodontitis, and pulpitis are among the most costly healthcare issues. In addition to all economical burdens both patients and the healthcare system encounter, many of these difficulties lead to tooth loss. As an alternative method to traditional tooth removal treatments and hard restorative mimetic materials, the progressive scaffold-based tissue engineering is being developed as a safe and practical regenerative therapy [35].

Voicu et al. [X] synthesized a mineral binder powder and bacterial cellulose-based composite for application in endodontics. After turning BC into a white-beige powder, it was mixed with silicate cement powder synthesized via a sol-gel method. The acquired hydrocompounds showed an increased structuring degree and favorable adhesion to the surface of teeth. Moreover, the in vivo studies revealed the ability of this structure to promote cell viability and proliferation while showing no cytotoxicity. Above all, it caused a significant mineralization process, indicating the high potential of this material for dental tissue engineering [36]. In another study by An et al. [X] bacterial cellulose membranes were irradiated at 100 kGy and 300 kGy and used for guided bone regeneration for dental applications. In vitro and in vivo studies showed higher cell viability and biodegradebility [37].

6. Natural Cellulose for Other Tissue Engineering Applications

The applications of plant-based and bacterial cellulose in tissue engineering are not limited to bone and skin regeneration. For instance, in a recent study, Guo et al. [X] fabricated a bacterial cellulose-graphene foam (3D-BC/G) for neural stem cell (NSC)-based therapy. The 3D-BC/G foam was fabricated through culturing A. xylinumon on the multilayer 3D-

 doi:10.20944/preprints202202.0176.v1

graphene foams made by interfacial polymerization on the skeleton of synthetic foams. The structural and morphological analyses showed the improved nano-sized structures, which allow for enhanced cell-scaffold interactions. In vitro studies validated the biocompatibility of 3D-BC/G scaffold when NSCs were cultured on them for three days. Furthe in vitro experiments indicated that the scaffold promotes the growth, proliferation, and adhesion of NSCs and maintains their stemness. Altogether, the 3D-BC/G scaffold can serve as a promising conductive structure for neural tissue engineering [38].

To provide a structure for maintaining the patency of vascular networks, Zhang et al. [X] synthesized a bacterial cellulose (RMBC) membrane decorated with RGD peptide-grafted magnetic nanoparticles. Sequentially, a bacterial cellulose membrane was cultured by fermentation. Then, PEG-Coated iron oxide nanoparticles were deposited on it through the Steglich esterification reaction, and finally, the magnetic bacterial cellulose was conjugated with RGD peptides. Culturing murine endothelial C166 cells on the as-fabricated membrane demonstrated better cell adhesion and proliferation than stand-alone bacterial cellulose (BC) and magnetic bacterial cellulose (MBC). Meanwhile, applying slow frequency by an external oscillating magnetic field promoted cell attachment so that the endothelialization was achieved after culture for four days. As suggested by the authors, the developed method is a non-invasive and convenient way to regulate endothelialization and can be used for vascular tissue engineering applications [39].

In another study by Zhang et al. [X], in vitro and in vivo biocompatibility of bacterial cellulose was assessed for corneal stroma replacement. Rabbit corneal epithelial and stromal cells were employed for in vitro study that confirmed the biocompatibility and non-cytotoxicity of BC plus a favorable environment for the growth and adhesion of both cells. Slit-lamp examination and HE staining showed that BC preserved its transparency with no neovascularization and inflammation. Furthermore, the in vivo investigation showed no edema or inflammation in the cornea during the 90 days post-operation. Zhang et al. concluded that BC is a fascinating biomaterial for corneal stroma tissue engineering [40].

Binnetoglu et al. [X] conducted in vivo studies on 40 female Sprague Dawley rats to assess the potential of bacterial cellulose as a tubularization biomaterial for repairing transected facial nerves. The results of whisker movements and electrophysiological parameters tests confirmed that despite no improvement in facial nerve function in the presence of the BC tubes, the regenerating axons were significantly higher in number than other control methods. This observation suggests that the BC hallow structure guides the fibers from proximal to distal nerve stump, enhances axonal regeneration, and provides a nerve conduit for neural regeneration [41].

In a recent study, Bai et al. [X] investigated the applicability of decellularized leaf and onion skin as vascular patches. They also assessed the drug delivery potential of these structures. First, the leaf and onion skin were incubated in 10% sodium dodecyl sulfate (SDS) and then washed with PBS. The leaf was then bleached in a 10% sodium chlorite solution for 12h, then washed with PBS to remove the bleaching agent. A polylactic-co-glycolic acid (PLGA)-based rapamycin nanoparticles were fabricated, later perfused in the leaf vasculature, and coated to the onion cellulose. The as-prepared scaffolds were examined in a rat inferior vena cava patch venoplasty model, which resulted in a rapidly formed and thickness-decreased neointimal after implantation as a patch. As a venous neointimal hyperplasia inhibitor, the administered nanoparticles caused thinner neointima for both decellularized scaffolds. Based on this study, Bai et al. recommended plant grafts as potential scaffolds for human vascular implantations [42].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2022 doi:10.20944/preprints202202.0176.v1

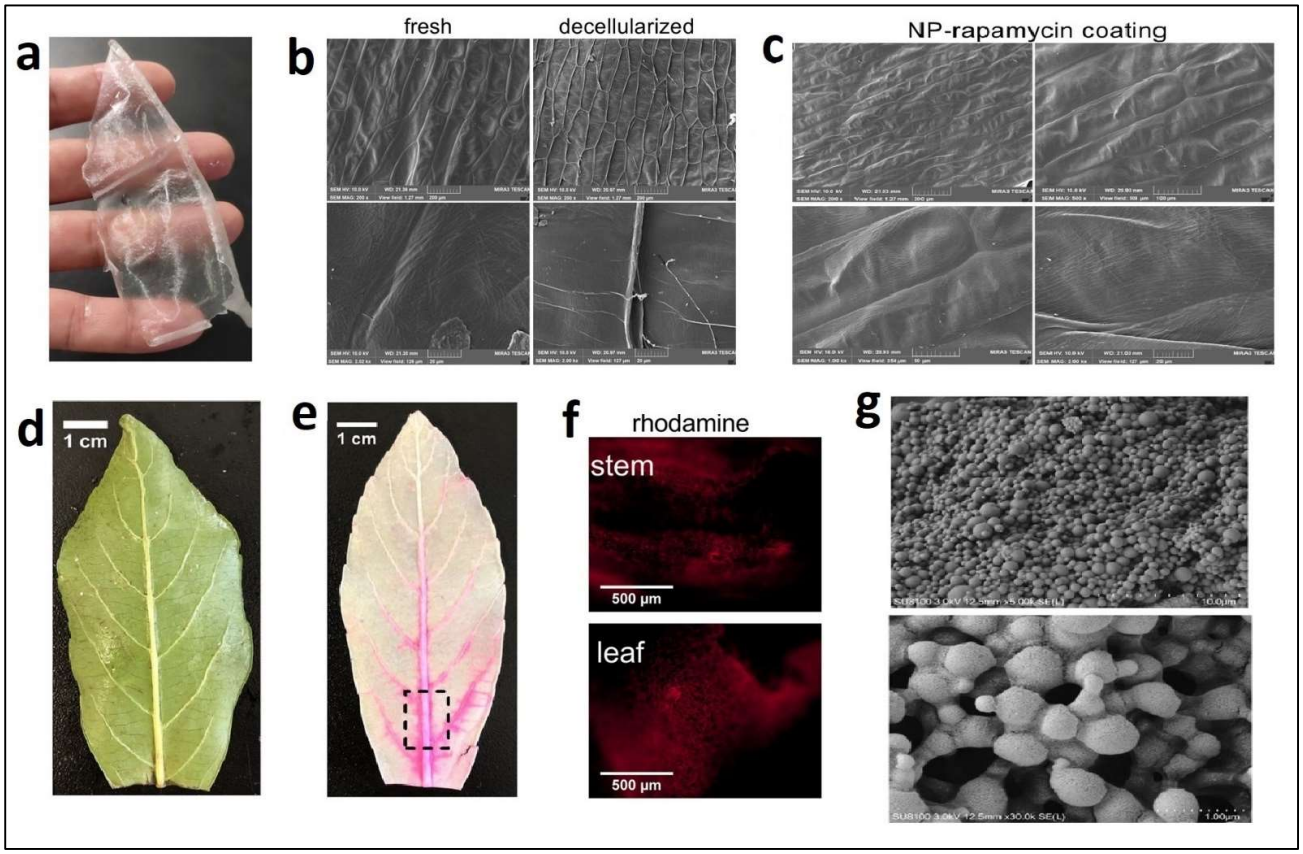


Figure 6. a) Camera image of a decellularized onion skin. b) SEM images of an onion skin before and after decellularization at different magnifications. c) SEM images of the decellularized onion skin after coating with the rapamycin nanoparticles. d&e) The camera image of a leaf before and after decellularizaion, respectivelt. f) Immunofluorescence images of stem and leaf after rhodamine water perfusion. g) SEM images of PLGA-based rapamycin nanoparticles at different magnifications [42].

Robbins et al. [X] carried out a comparative study between the cardiac patches made of non-coated and collagen IV or fibronectin-coated decellularized spinach aiming to address inadequate vascularization typically found in tissue-engineered cardiac patches. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) were used for in vitro studies on the decellularized scaffolds. The collected results, comparing protein-coated scaffolds with non-coated ones, confirmed no improvement in cardiomyocyte cell adhesion, behavior, and contractility when scaffolds were coated with ECM proteins. The decellularized leaf patches proved to serve as a potential candidate to refunctionalize diseased cardiac tissue [43].

In a multi-sectional study by Contessi Negrini et al. [X], decellularized apple and celery-derived scaffolds were assessed to regenerate adipose tissue and tendons, respectively. After decellularization and washing using SDS, CaCl2, and distilled water, decellularized plants' mechanical and morphological properties were examined. Apple-derived scaffolds possessing large and homogenous porosity were shown to be adequately fit for adipose tissue engineering applications. For in vitro study, pre-adipogenic cell line (3T3-L1) were cultured on the decellularized apple scaffold for 14 days. The results showed an increase in the metabolic activity of the cells as a proof for the scaffold-supported pre-adipocyte cells growth and proliferation followed by an induced differentiation after seven days of culture. The obtained results demonstrated the applicability of the decellularized apple-derived scaffolds for adipose tissue regeneration. Furthermore, the differentiated

 doi:10.20944/preprints202202.0176.v1

adipocytes were more prominent in size and rounder in shape than those cultured in the pre-adipogenic medium. The celery-derived scaffolds were characterized as morphologically oriented structures with arranged parallel pores, suitable for mimicking native anisotropic connective tissue of tendons. The mechanical and biological tests indicated the potential of decellularized celery-derived scaffolds with regards to anisotropic tissue regeneration [44].

6. Conclusions

Various approaches have been studied to apply bacterial and plant-based cellulose as the two main natural cellulose sources for tissue regeneration. Nevertheless, not thoroughly investigated, from bone and skin to cardiac and even corneal stroma, both PC and BC have shown promising characteristics to adapt and mimic the native tissue behaviors. The native tissue-like structure of PC and the tunable BC features have made natural cellulose an interesting subject for the fabrication of novel scaffolds. While recently, it has been tended more increasingly to use BC and PC in tissue engineering, the main studies have focused on bone regeneration or skin tissue engineering. However, the full potential of both BC and PC will likely be explored in the future.

Author Contributions: Writing an initial draft of the manuscript, MA, SD and PA; Editing ,SH; Conceptualization, revising the manuscript and funding acquisition, HS and AA. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Shahid Beheshti University, grant number 1400 To HS.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.
Data Availability Statement: No new data were created or analyzed in this study.

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

References

1. Ding, X., et al., *Synthetic peptide hydrogels as 3D scaffolds for tissue engineering.* Advanced Drug Delivery Reviews, 2020.
2. Zheng, X., et al., *Applications of nanomaterials in tissue engineering.* RSC Advances, 2021. **11**(31): p. 19041-19058.
3. Einchhorn, S., et al., *Review: current international research into cellulose nanofibres and composites.* J Mater Sci, 2010. **45**: p. 1-33.
4. Shojaeiarani, J., D.S. Bajwa, and S. Chanda, *Cellulose Nanocrystal Based Composites: A Review.* Composites Part C: Open Access, 2021: p. 100164.
5. Li, Q., L. Ma, and C. Gao, *Biomaterials for in situ tissue regeneration: development and perspectives.* Journal of Materials Chemistry B, 2015. **3**(46): p. 8921-8938.
6. Hickey, R.J. and A.E. Pelling, *Cellulose biomaterials for tissue engineering.* Frontiers in bioengineering and biotechnology, 2019. **7**: p. 45.
7. Bar-Shai, N., et al., *Seaweed cellulose scaffolds derived from green macroalgae for tissue engineering.* Scientific reports, 2021. **11**(1): p. 1-17.
8. Naomi, R., R. Bt Hj Idrus, and M.B. Fauzi, *Plant-vs. Bacterial-derived cellulose for wound healing: A review.* International journal of environmental research and public health, 2020. **17**(18): p. 6803.
9. Bilirgen, A.C., et al., *Plant-based scaffolds in tissue engineering.* ACS Biomaterials Science & Engineering, 2021. **7**(3): p. 926-938.
10. Sionkowska, A., O. Mężykowska, and J. Piątek, *Bacterial nanocelullose in biomedical applications: a review.* Polymer International, 2019. **68**(11): p. 1841-1847.

11. Roberts, A.W. and E. Roberts, *Evolution of the cellulose synthase (CesA) gene family: insights from green algae and seedless plants*, in *Cellulose: molecular and structural biology*. 2007, Springer. p. 17-34.
12. Zhong, C., *Industrial-Scale production and applications of bacterial cellulose.* Frontiers in Bioengineering and Biotechnology, 2020. **8**.
13. Peter, Z., *Order in cellulosics: Historical review of crystal structure research on cellulose.* Carbohydrate Polymers, 2020: p. 117417.
14. Ye, G., et al., *Nanomaterial-based scaffolds for bone tissue engineering and regeneration.* Nanomedicine, 2020. **15**(20): p. 1995-2017.
15. Shang, F., et al., *Advancing application of mesenchymal stem cell-based bone tissue regeneration.* Bioactive materials, 2021. **6**(3): p. 666-683.
16. Pang, M., et al., *Application of bacterial cellulose in skin and bone tissue engineering.* European Polymer Journal, 2020. **122**: p. 109365.
17. Dubey, S., et al., *3-D macro/microporous-nanofibrous bacterial cellulose scaffolds seeded with BMP-2 preconditioned mesenchymal stem cells exhibit remarkable potential for bone tissue engineering*. International Journal of Biological Macromolecules, 2021. **167**: p. 934-946.
18. Cakmak, A.M., et al., *3D printed polycaprolactone/gelatin/bacterial cellulose/hydroxyapatite composite scaffold for bone tissue engineering.* Polymers, 2020. **12**(9): p. 1962.
19. Aki, D., et al., *3D printing of PVA/hexagonal boron nitride/bacterial cellulose composite scaffolds for bone tissue engineering.* Materials & Design, 2020. **196**: p. 109094.
20. Zhang, W., et al., *A 3D porous microsphere with multistage structure and component based on bacterial cellulose and collagen for bone tissue engineering*. Carbohydrate polymers, 2020. **236**: p. 116043.
21. Codreanu, A., et al., *Bacterial cellulose-modified polyhydroxyalkanoates scaffolds promotes bone formation in critical size calvarial defects in mice*. Materials, 2020. **13**(6): p. 1433.
22. Salehi, A., et al., *Cabbage‐derived three‐dimensional cellulose scaffold‐induced osteogenic differentiation of stem cells.* Journal of Cellular Physiology, 2021. **236**(7): p. 5306-5316.
23. Mohan, C.C., et al., *Decellularization and oxidation process of bamboo stem enhance biodegradation and osteogenic differentiation.* Materials Science and Engineering: C, 2021. **119**: p. 111500.
24. Salehi, A., et al., *Natural cellulose-based scaffold for improvement of stem cell osteogenic differentiation.* Journal of Drug Delivery Science and Technology, 2021. **63**: p. 102453.
25. Weng, T., et al., *3D bioprinting for skin tissue engineering: Current status and perspectives.* Journal of Tissue Engineering, 2021. **12**: p. 20417314211028574.
26. Islam, M.M., et al., *Chitosan based bioactive materials in tissue engineering applications-A review.* Bioactive materials, 2020. **5**(1): p. 164-183.
27. Sun, W., et al., *Silk fibroin as a functional biomaterial for tissue engineering*. International Journal of Molecular Sciences, 2021. **22**(3): p. 1499.
28. Hernández‐Rangel, A. and E.S. Martin‐Martinez, *Collagen based electrospun materials for skin wounds treatment.* Journal of Biomedical Materials Research Part A, 2021.
29. Graça, M.F., et al., *Hyaluronic acid—Based wound dressings: A review.* Carbohydrate Polymers, 2020. **241**: p. 116364.
30. Wahid, F., et al., *Fabrication of Bacterial Cellulose-Based Dressings for Promoting Infected Wound Healing.* ACS Applied Materials & Interfaces, 2021.
31. Li, W., et al., *All-natural injectable hydrogel with self-healing and antibacterial properties for wound dressing*. Cellulose, 2020. **27**(5): p. 2637-2650.
32. Pillai, M.M., et al., *Symbiotic culture of nanocellulose pellicle: A potential matrix for 3D bioprinting.* Materials Science and Engineering: C, 2021. **119**: p. 111552.

33. Phan, N.V., et al., *In vitro biocompatibility of decellularized cultured plant cell-derived matrices.* ACS Biomaterials Science & Engineering, 2020. **6**(2): p. 822-832.
34. Mahendiran, B., et al., *Decellularized natural 3D cellulose scaffold derived from Borassus flabellifer (Linn.) as extracellular matrix for tissue engineering applications.* Carbohydrate Polymers, 2021: p. 118494.
35. Yazdanian, M., et al., *Decellularized and biological scaffolds in dental and craniofacial tissue engineering: a comprehensive overview.* Journal of Materials Research and Technology, 2021. **15**: p. 1217-1251.
36. Voicu, G., et al., *Improvement of silicate cement properties with bacterial cellulose powder addition for applications in dentistry.* Carbohydrate polymers, 2017. **174**: p. 160-170.
37. An, S.-J., et al., *Preparation and characterization of resorbable bacterial cellulose membranes treated by electron beam irradiation for guided bone regeneration.* International journal of molecular sciences, 2017. **18**(11): p. 2236.
38. Guo, R., et al., *Biomimetic 3D bacterial cellulose-graphene foam hybrid scaffold regulates neural stem cell proliferation and differentiation.* Colloids and Surfaces B: Biointerfaces, 2021. **200**: p. 111590.
39. Zhang, L., et al., *Oscillating Magnetic Field Regulates Cell Adherence and Endothelialization Based on Magnetic Nanoparticle-Modified Bacterial Cellulose.* ACS Applied Materials & Interfaces, 2020. **12**(47): p. 52467-52478.
40. Zhang, C., et al., *Biocompatibility evaluation of bacterial cellulose as a scaffold material for tissue-engineered corneal stroma.* Cellulose, 2020. **27**(5): p. 2775-2784.
41. Binnetoglu, A., et al., *Bacterial cellulose tubes as a nerve conduit for repairing complete facial nerve transection in a rat model.* European Archives of Oto-Rhino-Laryngology, 2020. **277**(1): p. 277-283.
42. Bai, H., et al., *Application of the Tissue-Engineered Plant Scaffold as a Vascular Patch.* ACS omega, 2021. **6**(17): p. 11595-11601.
43. Robbins, E.R., et al., *Creation of a contractile biomaterial from a decellularized spinach leaf without ECM protein coating: an in vitro study*. Journal of Biomedical Materials Research Part A, 2020. **108**(10): p. 2123-2132.
44. Contessi Negrini, N., et al., *Plant tissues as 3D natural scaffolds for adipose, bone and tendon tissue regeneration.* Frontiers in bioengineering and biotechnology, 2020. **8**: p. 723.