Chapter 7

Exploring Innovative Approaches for Tissue Engineering and Regenerative Medicine

Mesude Bicer[1]

Abstract

Regenerative therapies represent a promising avenue for addressing the limitations inherent in conventional two-dimensional (2D) methods. While substantial strides have been made in the development of diverse biomaterials, including autografts, allografts metallic implants, and ceramics, the realization of the desired clinical outcomes remains an ongoing exploration. Among these biomaterials, natural and synthetic polymeric scaffolds, particularly bioactive hydrogels, emerge as highly promising candidates for advancing tissue engineering. This chapter aims to provide an insightful exploration of the advantages and disadvantages associated with state-of-the-art bioactive hydrogels, elucidating their intricate designs when integrated with other components to facilitate bone self-healing. Furthermore, the chapter will delve into the applications of these coveted hydrogels, shedding light on the intricacies of their fabrication techniques. It is posited that a deeper comprehension of the intricate interplay between hydrogels and the specific homing mechanisms of cells holds the potential to serve as a guiding paradigm for the development of novel therapeutic modalities that champion the cause of tissue engineering.

1. Introduction

Regenerative medicine represents a cutting-edge field of science and healthcare that aims to harness the body's innate capacity for self-repair and healing. At the forefront of this field is tissue engineering, a multidisciplinary approach that combines biology, engineering, and materials science to create functional replacement tissues and organs. Tissue engineering holds the potential to revolutionize healthcare by offering novel solutions for treating injuries, degenerative diseases, and organ failure. In this chapter, we will

1 Dr., Abdullah Gül University, Faculty of Life and Natural Sciences, Bioengineering Department, Email: mesude.bicer@agu.edu.tr, ORCID ID: 0000-0001-7089-5661

https://doi.org/10.58830/ozgur.pub250.c1206

explore the principles, techniques, and applications of tissue engineering in regenerative medicine (Alaribe et al., 2016; Baraniak and McDevitt, 2012; Engler et al., 2006; Frohbergh et al., 2012).

Tissue engineering is guided by several key principles, each of which plays a crucial role in the creation of functional replacement tissues: Cell Source: The foundation of tissue engineering is the use of living cells. These cells can be obtained from various sources, including the patient's own body (autologous), donors (allogeneic), or through induced pluripotent stem cell (iPSC) technology, where adult cells are reprogrammed into stem cells. Scaffolds: Scaffolds serve as the structural framework for tissue engineering. They are typically made from biocompatible and biodegradable materials and are designed to mimic the extracellular matrix (ECM), providing a suitable microenvironment for cell attachment, proliferation, and differentiation (Hersel et al., 2003; Hubbell, 2003, Huebsch et al., 2010).

Several techniques and approaches are employed in tissue engineering to create functional replacement tissues and organs: Cell Culture: Isolated cells are cultured in vitro under controlled conditions, allowing them to proliferate and differentiate into specific cell types. This is the foundational step in tissue engineering. Scaffold Design: Engineers design and fabricate scaffolds using a variety of methods, including 3D printing, electro spinning, and decellularization of natural tissues. These scaffolds provide the physical structure for tissue development (Kim et al., 2013; Karaehenbuehl et al., 2009; Liu et al., 2016a,b; Rustad et al., 2012; Wei et al., 2014; Wichterle and Lim, 1960).

2. Strategies for Biocompatible and Biodegradable Biomaterial

2.1. The superiority of three-dimensional (3D) cell culture techniques

Stem cell therapy is of a pivotal role in various scientific domains, particularly tissue engineering and regenerative medicine, owing to the inherent attributes of stem cells, which encompass self-renewal and differentiation into specialised cell types. In the context of delivering effective clinical treatments, there exists a fundamental necessity for large-scale cell expansion and homogenous differentiation. Typically, two primary methods have been employed for the maintenance and amplification of these cells: 1) Traditional methods, including two-dimensional (2D) cell culture techniques, conducted on plastic culture plates 2) Modern three-dimensional (3D) cell culture designed to mimic the *in vivo* physiological environment (Agarwal et al, 2005;(McKee and Chaudhry, 2017).

In the conventional 2D culture, the generation of stem cells as a monolayer might entail the utilization of xenogeneic materials such as cytokines and growth factors. However, it is worth noting that xenogeneic media could potentially pose risks related to the transmission of pathogens and may curtail the reproducibility of experimental outcomes (McKee and Chaudhry, 2017). Furthermore, the cultivation of stem cells and their subsequent transformation in 2D culture may elevate the risk of chromosomal abnormalities, a diminishment in multipotency, and cellular senescence. Additionally, stem cell expansion within 2D culture can lead to phenotypic alterations, including a transition from a spindle-shaped morphology to a broader, flattened form. These inherent limitations associated with 2D culture techniques have spurred the development of 3D methods, aimed at mitigating the challenges observed in stem cell culture (McKee and Chaudhry, 2017).

The utilization of 3D scaffolds to improve stem cell expansion and differentiation aligns with the principles of tissue engineering. A variety of tissue engineering techniques have gained substantial attention for their innovative biomedical applications (del Valle et al., 2017). A diverse array of techniques is employed in the fabrication of biomaterials, all aimed at promoting self-renewal and differentiation capabilities. These techniques include electrospinning, gas foaming solvent casting, 3D printing and self-assembly, resulting in materials characterized by distinct properties such as pore size, tensile strength, elasticity, and adhesion (McKee and Chaudhry, 2017). The introduction of tissue-specific cells into these 3D scaffolds fosters the replication of the natural extracellular matrix (ECM) found in targeted tissues (El-Sherbiny and Yacoub, 2013). The meticulous design of these scaffolds not only aids in the understanding of the delivery of seeded cells within the body, but also elucidates the interactions between cells and biomaterials, enhances cell proliferation and differentiation, and augments anti-inflammatory and cytotoxicity properties *in vivo* (Langer and Tirrell, 2004).

3. Classification of Hydrogel Scaffolds in the Field of Tissue Engineering

3.1. Polymeric hydrogel matrices in tissue engineering

Hydrogel scaffolds have garnered attention for their utility as hydrophilic biomaterials, primarily due to their ability to mimic the hydrated ECM found in soft tissues (Fisher et al., 2010). They are classified into various categories, including natural, synthetic and self-assembly peptide-based

hydrogels, depending on their origin and composition (Table 1). Synthetic hydrogels are typically constructed from vinyl-activated monomers, whereas natural hydrogels are synthesized using naturally occurring polymers like polynucleotides, polypeptides and polysaccharides. (Alaribe et al., 2016). In addition to their origin, hydrogels are further categorized into five groups based on factors such as durability, response to environmental stimuli, charge-ability, structure and composition (Figure 1) (El-Sherbiny and Yacoub, 2013). There has been a notable research focus on the development of biodegradable hydrogels, as degradable polymers facilitate the formation of low molecular weight-oligomers that are rapidly eliminated through degradation processes (El-Sherbiny and Yacoub, 2013).

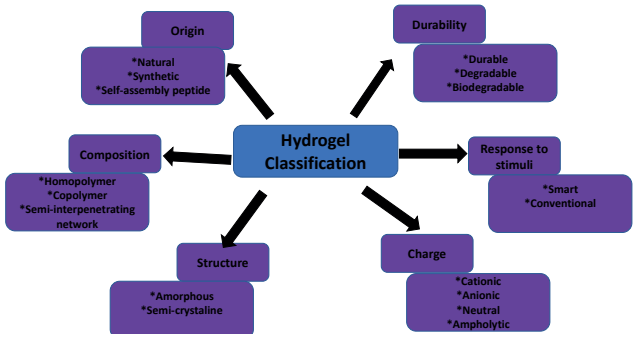


Figure 1. The diagram of hydrogel groups mostly used in tissue engineering.

Recent investigations into hydrogels have led to the development of stimuli-responsive hydrogels, which serve as smart materials with wide range of biomedical applications (Buwalda et al., 2014). These smart hydrogels offer advantages over conventional hydrogels, as they can alter their mechanical properties and swelling capacity in response to environmental stimuli such as pH, temperature, electric field, and ionic strength (Gutowska et al., 1992). Importantly, smart hydrogels can revert to their original state when the stimulating environmental conditions are removed (El-Sherbiny and Yacoub, 2013). Among the natural polymers-based hydrogels, collagen hydrogel stands out as a common choice due to its ability to promote the formation of tropocollagen triple helixes through self-aggregation and crosslinking. Other natural polymer sources for hydrogels include cellulose and chitosan (del Valle et al., 2017). In contrast, synthetic polymers-based hydrogels offer precise control over material structure and tissue responses, addressing some of the challenges associated with natural polymers (El-Sherbiny and Yacoub, 2013).

3.1.1. Collagen and gelatine hydrogels

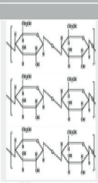
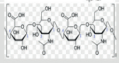
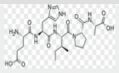
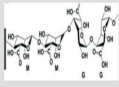
Collagen, an indispensable protein component integral to the ECM, is sourced from skin and other tissues through enzymatic-acidic treatments following the neutralization of acidic solutions (del Valle et al., 2017). Gelatine, composed from collagen, results from the breakdown of the collagen triple-helix structure into smaller molecules. The amalgamation of collagen and gelatine holds considerable promise in the context of biocompatibility within tissue engineering applications (del Valle et al., 2017). Martinelli and co-workers propose the reinforcement of natural hydrogels, obtained from gelatine and collagen, with gold nanostructures and carbon nanotubes (CNTs) when cultivating cardiac myocytes. This choice is underpinned by several advantageous properties exhibited by these materials, including high electrical conductivity, enhanced modifiability and fabrication capacities, reduced cytotoxicity, and the capability to design various architectural configurations such as nanowires, nanorods, and nanoparticles (Martinelli et al., 2012). Although these materials appear to hold substantial potential for biomedical purposes, ongoing research efforts are focused on developing new materials. This is driven by concerns related to their limited degradation rates and the necessity for enzymatic intervention for cell retrieval processes.

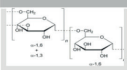
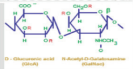
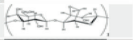
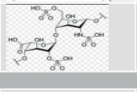
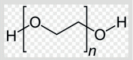
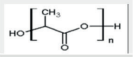
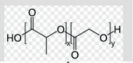
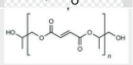
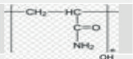
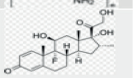
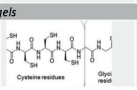
3.1.2. Chitosan hydrogels

Chitin, a natural polymer consisting of poly-(1-4)-N-acetyl-glucosamine, plays a crucial role in the formation of microfibrils found in the cell walls of fungi and the exoskeletons of invertebrates. This polysaccharide shares structural similarities with cellulose, such as the presence of ß-(1-4) glycosidic bond. The conversion of chitin into water-soluble chitosan (CS) is achieved through deacetylation using strong alkaline solutions (del Valle et al., 2017). CS molecules display notable cationic properties, enabling the formation of gel particles through electrostatic interactions, often employing sodium sulphate as a precipitant (Joye and McClements, 2014). As demystified by del Valle and co-workers, CS molecules possess the capacities for self-assembly and encapsulation due to their interactions with hydrophobic components. This feature is effective for targeted drug delivery applications (del Valle et al., 2017). Chitosan's appeal as a hydrogel material is further reinforced by its biodegradability, biocompatibility, and exceptional water-absorption ability. Furthermore, its capability to absorb both anionic and cationic molecules through hydrogen-bond interactions finds utility in water purification and protein encapsulation (Boardman et al., 2017).

In addition, chitosan has found approach in bone regeneration, facilitated by the incorporation of crosslinking agents such as glycerophosphate salt, genipin, and hydroxyapatite. These agents enhance osteogenic proliferation, thereby promoting bone tissue regeneration (Frohbergh et al., 2012). The use of sodium bicarbonate ($NaHCO_{3)}$) as a gelling agent in combination with hydroxyapatite is favourable for cell encapsulation due to its non-cytotoxic nature and rapid gelation effects. Gunathilake and his team have explored the reinforcement of pH-sensitive CS hydrogels with cellulose nanocrystals (CNCs) using glutaraldehyde as a cross-linking agent. This application gives rise to a composite material characterized by a combination of amorphous and crystalline phases, with increased mechanical strength. When CNC content was at 2.5 wt%, the composite displayed enhanced toughness, with the cross-linker forming strong bonds with chitosan amino groups. This innovative combination of CS with CNC hydrogel holds promise for drug delivery approaches (Sampath et al., 2017).

Table 1. The categorization of hydrogel scaffoldings with their origins, advantages, and limitations

Hydrogels		*Origin*	*Chemical structure*	*Used cells*	*Developed tissue*	*Advantages*	*Limitations*	*Combination*	*Reference*
Natural polymers-based hydrogels									
Cellulose	**Nanocrystal (CNC)**	polysaccharide		human dental follicle cells (hDFC)	bone, chondrocytes,	biocompatibility, nontoxicity, biodegradability, high chemical stability, humidity tolerance, good mechanical properties, high degree of conformability, cost effective, wound healing	cost expensive purification to prevent endotoxin contamination, low yield	Hydroxyapatite, Collagen, bFGF, alginate, gelatin	Dumanli et al., 2017' Lin, 2014
	Cellulose nanofibers (NFC)			fibroblast, hESCs, hiPSCs, MSC	bone, chondrocytes, neuron				Lin, 2014
	Bacterial cellulose (BC)			fibroblast, keratinocytes	bone, chondrocytes,			HA, gelatin, chitosan, chondroitin sulfate, Hydroxyapatite, nanoparticles (Ag, Cu, Au)	Azoidis, 2017 Lin, 2014
Chitosan		amino-polysaccharide		COS-7 cells	Bone chondrocytes	biodegradability, biocompatibility, structural stability, nontoxicity, heavy metal ions chelation, ease of chemical modification, high affinity to proteins	water insolubility, the decreased antimicrobial properties by acetylation	Hydroxyapatite genipin, glycerophospate salt, gelatine	del Valle, 2017, Krajewska, 2005,
Collagen		protein		chondrocytes	cartilage, skin, neuron	high conductivity, easy modification and fabrication, low cytotoxicity, good cell adhesion	using enzymes for retrieved cells	alginate, HA, gold nanostructures	Azoidis, 2017
Gelatin		protein		chondrocytes	cartilage, vascular	bioresorbable, biocompatible, rapid degradation, better solubility and less antigenicity to collagen	poor mechanical properties, short degradation rates, lack of termal stability	Agar, HA	Tsang et al., 2015
Peptide amphiphile		protein combined with fatty acid tail		hESCs, fibroblast, Schwann cells	vascular, bone, ECM, peripheral nerve cells	biocompatible, degrade over time, support angiogenesis without causing inflammation	-	Ti composite, HA, Gelatin, Chondroitin sulfate	Black et al., 2015
Hyaluronic acid (HA)		polysaccharide		Fibroblasts, chondrocytes, hepatocytes	connective tissue, eye, skin, bone, cartilage, synovial fluid, cardiovascular	biocompatible, nonimmunogenic properties, toughness.	-	Alginate, collagen, gelatine, carboxymethylcellulose, synthetic polymers	Fisher et al., 2015
Fibrin		protein		chondrocytes, bone marrow cells	cartilage, skin, cardiovascular, nerves, bone and muscular tissue	higher proliferation, higher self-renewal capacity,	using enzymes for retrieved cells	-	Azoidis, 2017, Greiner et al., 2011
Alginate		polysaccharide		chondrocytes	cartilage, bone, vascular	biocompatible biodegradable without lowering pH, gentile gelation, hydrophilicity, low cost, easy handling, encourage cell viability, prolonged shelf life.	unstable mechanical properties, lack of the specific cell-recognition signals, limited absorption ability of serum proteins	HA, Collagen, carboxymethylcellulose	Azoidis, 2017

Matrigel™	*protein*		-	-	*high cell attachment high reorganization, more physiological relevant for soluble growth factor and hormones in vivo, higher versatility for 3D culture*	*batch-to-batch variability, cross-species immunogenicity*	-	*Azoidis, 2017, Fang and Eglen, 2017*
Dextran-based	polysaccharide		-	-	nontoxic, biocompatible, used as coating material to improve biocompatibility,	do not support cell attachment due to having only hydroxyl group rather than various functional groups (amine and amide)	-	Rodríguez-Velazquez et al., 2015
Glycosaminoglycans	polysaccharide		-	-	biocompatible, nonimmunogenic properties.	-	collagen	Lam et al., 2014
Chondroitin sulphate (CS)	polysaccharide		-	skin, cartilage, bone, connective tissue	biocompatible, high water absorption, multifunctionality and biodegradability		chitosan, gelatin, hyaluronan, collagen, alginate, poly(vinyl alcohol)	Oprea et al., 2009
Agarose carbomer	polysaccharide		glial cells	neural	permeability and strength	-	-	Rahfoth et al., 1998)
Heparin	polysaccharide		fibroblast	liver-specific differentiation stem cell	higher cell adhesion, conductivity for hepatic phenotypes	-	poly (ethylene glycol) (PEG)	You et al., 2014
Synthetic polymers-based hydrogels								
PEG (Polyethylene glycol)	polymer		fibroblast, chondrocytes, ESCs, MSCs, osteoblasts, smooth muscle cells, endothelial cell, embryonic carcinoma	bone, cardiovascular, cartilage, intraperitoneal, pancreatic	biocompatibility, water solubility and low cost	do not support cell adhesion and proliferation, lack of endogenous factors, less oxygen availability.	PLA, PVA, PEG/PLA	Lee et al., 2015
PLA (poly lactic acid)	polymer		chondrocytes,	cartilage	osteoconductivity, controllable biodegradation rate, biocompatibility, good mechanical strength.	low biodegradability , may include toxic substances	PEG	Lee et al., 2011
PLGA (poly-lactic-glycolic acid)	polymer		osteoarthritic chondrocytes	cartilage	controllable degradation rate, supporting osteoblast attachment, growth, differentiation	poor mechanical properties, low osteoconductivity	composite material like ceramics, bioglass	Liu and Ma, 2004
PPF (Polypropylene fumarate)	polymer		-	craniofacial bone	biodegradable, biocompatible, osteoconductive, injectable and sufficiently strength	-	PLA, PGA or PLGA	Henslee et al., 2012
Polyacrylamide (PAM)-based	polymer		-	-	bioinert and hydrophilic properties (contact lens), low toxicity	poor cell adhesion	Alginate, collagen, peptides like RGD	Wang et al., 2014)
Dex-MA-LA&Gel-MA	polymer		ECs&SMCs	vascular	bicompatible, biodegradable, noncytotoxic, stable at 37C, nonimmunogenic, long term cell-viability	-	-	Becker et al.,2015
Self-assembled peptides (SAPs)-based hydrogels								
SAPs	protein		fibroblast, MSCs, endothelial cells, murine embryonic pluripotent stem cells	chondrocytes	compatibility with water, low cytotoxicity	-	dimethylsulfoxide	del Valle, 2017

3.1.3. Cellulose hydrogels

The utilization of cellulose-based materials in tissue engineering has garnered remarkable attention due to their enhanced sustainability, biocompatibility, biodegradability, and reduced cytotoxicity. From a chemical perspective, their molecular structure exhibits similarities to stiff, rod-like conformations, akin to those seen in crystalline fibrous materials. These cellulose-based materials display strong intra- and intermolecular bonding within hydrogels, characterized by different crystal structures originating from various sources (e.g., cellulose Iα produced by bacteria and cellulose Iß produced by plants) (del Valle et al., 2017). The application of cellulose in the development of hydrogels within innovative biomedical applications relies on two distinct approaches: cellulose-based matrices and the incorporation of nanocelluloses. Although cellulose-based-hydrogel matrices possess intrinsic qualities that render them excellent materials for

such purposes, nanocelluloses have garnered more attention due to their enhanced biodegradation potency (del Valle et al., 2017). Nanocellulose hydrogels encompass three fundamental groups: cellulose nanocrystals (CNC), nanofibrillar cellulose (NFC), and bacterial cellulose (BC), as presented in Table 1. Various types of nanocellulose exists, including nanocrystals, nanowhiskers, and nanofibres.

Nanofibrillar cellulose (NFC) can be produced through mechanical treatments such as high-pressure homogenization or grinding. Compared to CNC, NFC is of a remarkable progress because of its combination of amorphous and crystalline regions, allowing the improvement of moisture absorption and mechanical strength (Dumanli, 2017). BC, produced by Acetobacter xylinum, has a significative role in retaining water, and this feature affords it a high level of conformability. This unique property is underpinned by the hydrogen bonds coherent to fibrillar units of BC, which participate in the equilibrium of cellulose structure, with notable mechanical strength and a high degree of crystallinity (Dumanli, 2017). An additional intriguing facet of nanocellulose hydrogels lies in their chemical composition, which distinguishes them from plant-based cellulose counterparts, such as hemicellulose and lignin (Dumanli, 2017). Hydrophilic cellulose displays a strong affinity for water, rendering it suitable for hydrogel formation. Of particular importance, CNCs possess unique magnetic features, including anisotropic magnetic susceptibility, compared to NFC and BC, which lack of behaviour to magnetic fields (Dumanli, 2017). The gelation mechanism of CNCs plays an attention-grabbing role in enhancing dimensional stability, mechanical properties, and drug absorption. For instance, formulations incorporating CNC at a concentration of 50 wt% in conjunction with sodium alginate and gelatin have been explored as a beneficial strategy for cartilage regeneration (Domingues et al., 2014). A variety of hemicelluloses such as galactoglucan, xyloglucan, and xylan have cross-linking abilities, influencing hydrogel effects on cell proliferation and mechanical properties in therapeutic approaches (Liu et al., 2016b).

3.2. Self-assembled peptide (SAPs) hydrogels

Self-assembled peptides (SAPs)-based hydrogels represent a class of materials composed of hydrophilic and amphiphilic molecules. These molecules have the ability to be functionalized with cell adhesion ligands, such as RGD, and are coupled with hydrophobic alkyl tail (El-Sherbiny and Yacoub, 2013). SAPs stand out as a particularly promising medium for mimicking the properties of natural fibrillar proteins within the ECM. This emulation is achieved through the formation of a porous network that

facilitates vital processes including cell growth, cell differentiation, and cell transplantation. Diverse peptide sequences capable of assembling into various nanostructures, such as nanofibers, nanotubes, and nanoparticles, are likely to be generated by altering pH, temperature, or introducing different external cations (del Valle et al., 2017). Several distinct categories of self-assembled peptides have been identified, each exhibiting characteristic structural features. These include RADA-like SAPs, complementary co-assembling peptides (CAPs), peptide amphiphiles, cyclo-SAPs, and functionalized SAPs. Among these sequences, RADA-like SAPs have garnered remarkable attention for applications in 3D cell culture and wound-amelioration due to their structural similarity to the well-recognized RGD motif involved in cell adhesion (del Valle et al., 2017). Despite the impressive advantages offered by SAPs, such as their capability to self-assemble and mimic natural ECM proteins, they do have certain limitations. Notably, the lack of crosslinking agents and the ease of functionalization contribute to the instability of their mechanical characteristics. This drawback necessitates further research and development to enhance the overall stability and structural integrity of SAP-based hydrogels for broader biomedical approaches.

4. Material characteristics and biocompatibility of nanocellulose composites in biomedical applications

A comprehensive understanding of material characteristics is of paramount importance in the realm of biomedical research, as the selected materials, including metals, alloys, polymers, ceramics and composites, must exhibit *in situ* cohesion to avert various complications that could otherwise result in severe heath issues (Dumanli, 2017). Notably, particular emphasis has been placed on comprehending the attributes of NFC concerning its biomedical applications. A core objective has been the assessment of its biocompatibility and its interactive compatibility with living tissues, with a keen focus on mitigating cytotoxic effects. A variety of studies have delved into the cytotoxicity and immunogenicity of NFC (Lopes et al., 2017, Pereira et al., 2013). These studies have consistently reported that NFC emerges as a non-cytotoxic biomaterial, even when employed at high fibre concentrations, thereby establishing its biocompatible nature. Moreover, chemical modifications of nanocellulose composites have been explored, revealing NFC's adaptability to facile and effective chemical alterations to yield well-defined characteristics (Habibi, 2014). Significantly, these chemical modifications have been found not to compromise the material's toxicological profile, as affirmed by Harper et al. (Harper et al., 2016). It is worth noting that in the context of medical implants, metal and alloys used

within the body can be susceptible to corrosion, particularly in the lack of appropriate chemical compositions, such as oxygenated saline electrolyte at pH 7.4 and a temperature of 37C. With respect to this, research by Zhong and co-workers has underscored cellulose and its derivatives as effective corrosion inhibitors for stainless steels (Zhong et al., 2015).

Collectively, when considering its biocompatibility, versatile chemical modification abilities, cost-effectiveness, ready availability, and intriguing mechanical properties, NFC emerges as an exceedingly promising candidate for applications in the biomedical and pharmaceutical domains. These attributes position cellulose-based biomaterials as highly attractive options for advancing biomedical research and approaches.

4.1. Nanocellulose-based wound dressings: Advancements and potential in biomedical approaches

Wound healing is a multifaceted process comprising distinct stages essential for the effective repair of damaged skin and blood vessels (Singer and Clark, 1999). These stages are initiated following injury, with platelets initiating the release of chemotactic factors to stimulate fibrin formation and macrophage activation. The ensuing inflammatory response recruits macrophages to facilitate the removal of damaged cells via phagocytosis. Subsequently, stages involve neovascularization, epithelization and the formation of granulation tissue, which contribute to the restoration of new tissue. The final phage involves the remodelling of the ECM until normal cellular conditions are reestablished. Traditionally, cotton has served as the substrate for wound dressings, but contemporary hydrogel-based wound dressings are emerging as viable alternatives due to their reduced adherence to wounds (Madaghiele et al., 2014), suitable swelling properties, high moisture content, and efficient removal of damaged tissue (Chen et al., 2016). Dumanli and her co-workers have proposed BC-based wound dressing such as commercially available products like Bioprocess®, XCell®, Biofill®, as an alternative with intriguing features such as pain reduction, exudate retention, enhanced epithelization, and shorter recovery times (Dumanli, 2017).

However, despite these advantages, BCs-based wound healing approaches face challenges associated with the cost-intensive purification process required to prevent endotoxin contamination. Jack and his team have suggested that plant-derived NFC-based wound dressings hold promise as cost-effective and effective alternatives for potential wound healing applications (Jack et al., 2017). In this study, NFC-based wound dressings displayed optimal

moisture content, desirable porosity, and surface roughness conducive to promoting wound healing. Bhattacharya and co-workers reported that NFC hydrogels facilitated cellular differentiation in human hepatic cell lines (HepaRG and HepG2) and induced spheroid formation, showcasing their versatility (Bhattacharya et al., 2012). Lin's team emphasized that NFC-based hydrogels offer advantages for cell growth and gas diffusion, further endorsing their potential in wound healing (Lin et al., 2014). Furthermore, NFC wound dressings have been shown to promote fibroblast proliferation and viability, crucial factors in wound healing (Liu et al., 2016a). Taken together, the biocompatibility, non-toxic nature, and cost-effectiveness of NFC render it a unique and highly promising material for wound healing research (Liu et al., 2016a).

4.2. Cellulose-based biomaterials for tissue engineering and *in situ* implantation

The utilization of cellulose-based scaffolds within a 3D-network represents a promising approach for tissue engineering applications due to their notable mechanical properties and high porosity. However, while cellulosic materials exhibit a commendable level of biocompatibility, they present challenges related to cell adhesion on their surfaces during *in situ* implantation procedures (Dumanli, 2017). Thus, addressing these issues becomes imperative. To mitigate these limitations, the initial step involves the creation of an anti-thrombogenic environment to inhibit blood clot formation by regulating inflammatory responses and blood coagulation. The endothelium emerges as a pivotal tissue for establishing a non-thrombogenic interface between the scaffold material and circulating blood. Bodin and co-workers have illustrated the successful incorporation of xyloglucans, a reinforcing molecule, into cellulose-based scaffolds, in combination with the cell adhesion peptide RGD. This innovative modification has resulted in enhanced adhesion of endothelial cells, particularly beneficial in the context of cardiovascular surgery (Bodin et al., 2007).

In vivo transplantation via injections of NFC hydrogels is also an option. However, the external environmental conditions influence the gelation process of these hydrogels, rendering them unsuitable for immediate clinical applications (Priya James et al., 2014). To achieve *in situ* implantations, shear-thinning techniques are employed to induce rapid gel structure formation immediately following the injection process. It is worth noting that despite the challenges associated with NFC-based hydrogels, some studies have emphasized their potential for *in vivo* implantation, even though BC remains a predominant choice (Abeer et al., 2014). The efficacy of BCs

has garnered the increased attention in the field of biomedical implantations (Halib et al., 2017).

4.3. Bioadhesion in drug delivery: Potential of nanocellulose-based materials and polymers

Bioadhesion serves as a crucial mediator in the context of drug administration, facilitating the interaction between a biological layer and the surface to which it is applied. Recent research by Meneguin et al. (2017) has explored the utilization of NFC embedded into starch/pectin (RS/P) films for targeted drug delivery (Meneguin et al., 2017). Notably, in comparison to BC, NFC-based materials exhibited superior bioadhesive properties, enhanced mechanical characteristics, and improved interactions within the RS/P matrix during the release of methotrexate. Brako and collaborators (2015) conducted a comprehensive investigation into various nanofibrous materials supported by different polymers to assess their suitability for bioadhesive applications (Brako et al., 2015). Their findings underscored the significant bioadhesive potential of carboxymethylcellulose (CMC) and polyethylene glycol (PEG) in enhancing bioadhesion capabilities (Brako et al., 2015). Moreover, in a separate study regarding to PEG-alginate systems, cellulose nanofiber aerogels were identified as promising biomaterials for drug delivery due to their remarkable bioadhesive properties (Bhandari et al., 2017). These studies collectively emphasize the critical role of bioadhesion in drug delivery and highlight the potential of NFC-based materials, CMC, PEG, and cellulose nanofiber aerogels as key components in this domain.

5. Concluding Remarks

Regenerative therapies stand as a promising frontier in addressing the challenges posed by conventional 2D approaches to tissue engineering. While various biomaterials have been explored, including autografts, allografts, metallic implants, and ceramics, realizing the desired clinical outcomes remains an ongoing journey. Among these biomaterials, natural and synthetic polymeric scaffolds, with a particular focus on bioactive hydrogels, have emerged as highly promising candidates for advancing the field of tissue engineering. This chapter has provided an insightful exploration of the strengths and limitations associated with state-of-the-art bioactive hydrogels. It has elucidated the intricate designs that involve integrating these hydrogels with other components to promote bone self-healing. In addition, we have delved into the practical applications of these coveted hydrogels, shedding light on the intricacies of their fabrication techniques. Crucially, it is worth

highlighting that a deeper understanding of the complex interplay between hydrogels and the specific homing mechanisms of cells holds the potential to guide the development of novel therapeutic approaches in the realm of tissue engineering. These approaches have the capacity to revolutionize the field and bring us closer to realizing the full potential of regenerative medicine. As we continue to unravel the mysteries of cellular interactions within bioactive hydrogels, we move one step closer to the development of innovative and effective therapies that can truly transform the landscape of regenerative medicine.

REFERENCES

ABEER, M., MOHD AMIN, M. C. I. & MARTIN, C. 2014. *A review of bacterial cellulose-based drug delivery systems: Their biochemistry, current approaches and future prospects*.

AGGARWAL, S. & PITTENGER, M. F. 2005. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood,* 105**,** 1815-1822.

ALARIBE, F., MANOTO, S. & MOTAUNG, S. 2016. *Scaffolds from biomaterials: Advantages and limitations in bone and tissue engineering*.

BARANIAK, P. R. & MCDEVITT, T. C. 2012. Scaffold-free culture of mesenchymal stem cell spheroids in suspension preserves multilineage potential. *Cell and Tissue Research,* 347**,** 701-711.

BHANDARI, J., MISHRA, H., MISHRA, P., WIMMER, R., AHMAD, F. & TALEGAONKAR, S. 2017. *Cellulose nanofiber aerogel as a promising biomaterial for customized oral drug delivery*.

BOARDMAN, S. J., LAD, R., GREEN, D. C. & THORNTON, P. D. 2017. Chitosan hydrogels for targeted dye and protein adsorption. *Journal of Applied Polymer Science,* 134.

BODIN, A., AHRENSTEDT, L., FINK, H., BRUMER, H., RISBERG, B. & GATENHOLM, P. 2007. Modification of nanocellulose with a xyloglucan-RGD conjugate enhances adhesion and proliferation of endothelial cells: implications for tissue engineering. *Biomacromolecules,* 8**,** 3697-704.

BRAKO, F., RAIMI-ABRAHAM, B., MAHALINGAM, S., CRAIG, D. Q. M. & EDIRISINGHE, M. 2015. Making nanofibres of mucoadhesive polymer blends for vaginal therapies. *European Polymer Journal,* 70**,** 186-196.

BUWALDA, S. J., BOERE, K. W., DIJKSTRA, P. J., FEIJEN, J., VERMONDEN, T. & HENNINK, W. E. 2014. Hydrogels in a historical perspective: from simple networks to smart materials. *J Control Release,* 190**,** 254-73.

CHEN, Y., ZHANG, Y., WANG, F., MENG, W., YANG, X., LI, P., JIANG, J., TAN, H. & ZHENG, Y. 2016. Preparation of porous carboxymethyl chitosan grafted poly (acrylic acid) superabsorbent by solvent precipitation and its application as a hemostatic wound dressing. *Materials Science and Engineering: C,* 63**,** 18-29.

DEL VALLE, L., DÍAZ, A. & PUIGGALÍ, J. 2017. Hydrogels for Biomedical Applications: Cellulose, Chitosan, and Protein/Peptide Derivatives. *Gels,* 3**,** 27.

DOMINGUES, R. M., GOMES, M. E. & REIS, R. L. 2014. The potential of cellulose nanocrystals in tissue engineering strategies. *Biomacromolecules,* 15**,** 2327-46.

DUMANLI, A. G. 2017. Nanocellulose and its Composites for Biomedical Applications. *Curr Med Chem,* 24**,** 512-528.

EL-SHERBINY, I. M. & YACOUB, M. H. 2013. Hydrogel scaffolds for tissue engineering: Progress and challenges. *Glob Cardiol Sci Pract,* 2013**,** 316-42.

ENGLER, A. J., SEN, S., SWEENEY, H. L. & DISCHER, D. E. 2006. Matrix elasticity directs stem cell lineage specification. *Cell,* 126**,** 677-689.

FISHER, O. Z., KHADEMHOSSEINI, A., LANGER, R. & PEPPAS, N. A. 2010. Bioinspired Materials for Controlling Stem Cell Fate. *Accounts of Chemical Research,* 43**,** 419-428.

FROHBERGH, M. E., KATSMAN, A., BOTTA, G. R., LAZAROVICI, P., SCHAUER, C. L., WEGST, U. G. K. & LELKES, P. I. 2012. Electrospun hydroxyapatite-containing chitosan nanofibers crosslinked with genipin for bone tissue engineering. *Biomaterials,* 33**,** 9167-9178.

GUTOWSKA, A., BAE, Y. H., FEIJEN, J. & KIM, S. W. 1992. Heparin Release from Thermosensitive Hydrogels. *Journal of Controlled Release,* 22**,** 95-104.

HABIBI, Y. 2014. Key advances in the chemical modification of nanocelluloses. *Chemical Society Reviews,* 43**,** 1519-1542.

HALIB, N., PERRONE, F., CEMAZAR, M., DAPAS, B., FARRA, R., ABRAMI, M., CHIARAPPA, G., FORTE, G., ZANCONATI, F., POZZATO, G., MURENA, L., FIOTTI, N., LAPASIN, R., CANSOLINO, L., GRASSI, G. & GRASSI, M. 2017. *Potential Applications of Nanocellulose-Containing Materials in the Biomedical Field*.

HARPER, B. J., CLENDANIEL, A., SINCHE, F., WAY, D., HUGHES, M., SCHARDT, J., SIMONSEN, J., STEFANIAK, A. B. & HARPER, S. L. 2016. Impacts of chemical modification on the toxicity of diverse nanocellulose materials to developing zebrafish. *Cellulose,* 23**,** 1763-1775.

HERSEL, U., DAHMEN, C. & KESSLER, H. 2003. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials,* 24**,** 4385-4415.

HUBBELL, J. A. 2003. Materials as morphogenetic guides in tissue engineering. *Current Opinion in Biotechnology,* 14**,** 551-558.

HUEBSCH, N., ARANY, P. R., MAO, A. S., SHVARTSMAN, D., ALI, O. A., BENCHERIF, S. A., RIVERA-FELICIANO, J. & MOONEY, D. J. 2010. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nature Materials,* 9**,** 518-526.

JACK, A. A., NORDLI, H. R., POWELL, L. C., POWELL, K. A., KISHNANI, H., JOHNSEN, P. O., PUKSTAD, B., THOMAS, D. W., CHINGA-CARRASCO, G. & HILL, K. E. 2017. The interaction of wood

nanocellulose dressings and the wound pathogen P. aeruginosa. *Carbohydrate Polymers,* 157**,** 1955-1962.

JOYE, I. J. & MCCLEMENTS, D. J. 2014. Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application. *Current Opinion in Colloid & Interface Science,* 19**,** 417-427.

KIM, I. L., KHETAN, S., BAKER, B. M., CHEN, C. S. & BURDICK, J. A. 2013. Fibrous hyaluronic acid hydrogels that direct MSC chondrogenesis through mechanical and adhesive cues. *Biomaterials,* 34**,** 5571-5580.

KRAEHENBUEHL, T. P., FERREIRA, L. S., ZAMMARETTI, P., HUBBELL, J. A. & LANGER, R. 2009. Cell-responsive hydrogel for encapsulation of vascular cells. *Biomaterials,* 30**,** 4318-4324.

LANGER, R. & TIRRELL, D. A. 2004. Designing materials for biology and medicine. *Nature,* 428**,** 487-492.

LIU, J., CHINGA-CARRASCO, G., CHENG, F., XU, W., WILLFÖR, S., SYVERUD, K. & XU, C. 2016a. Hemicellulose-reinforced nanocellulose hydrogels for wound healing application. *Cellulose (London),* 23**,** 3129-3143.

LIU, J., CHINGA-CARRASCO, G., CHENG, F., XU, W. Y., WILLFOR, S., SYVERUD, K. & XU, C. L. 2016b. Hemicellulose-reinforced nanocellulose hydrogels for wound healing application. *Cellulose,* 23**,** 3129-3143.

LOPES, V. R., SANCHEZ-MARTINEZ, C., STRØMME, M. & FERRAZ, N. 2017. In vitro biological responses to nanofibrillated cellulose by human dermal, lung and immune cells: surface chemistry aspect. *Particle and Fibre Toxicology,* 14**,** 1.

MADAGHIELE, M., DEMITRI, C., SANNINO, A. & AMBROSIO, L. 2014. Polymeric hydrogels for burn wound care: Advanced skin wound dressings and regenerative templates. *Burns & Trauma,* 2**,** 153-161.

MARTINELLI, V., CELLOT, G., TOMA, F. M., LONG, C. S., CALDWELL, J. H., ZENTILIN, L., GIACCA, M., TURCO, A., PRATO, M., BALLERINI, L. & MESTRONI, L. 2012. Carbon nanotubes promote growth and spontaneous electrical activity in cultured cardiac myocytes. *Nano Lett,* 12**,** 1831-8.

MCKEE, C. & CHAUDHRY, G. R. 2017. Advances and challenges in stem cell culture. *Colloids Surf B Biointerfaces,* 159**,** 62-77.

MENEGUIN, A. B., FERREIRA CURY, B. S., DOS SANTOS, A. M., FRANCO, D. F., BARUD, H. S. & DA SILVA FILHO, E. C. 2017. Resistant starch/pectin free-standing films reinforced with nanocellulose intended for colonic methotrexate release. *Carbohydrate Polymers,* 157**,** 1013-1023.

PEREIRA, M. M., RAPOSO, N. R. B., BRAYNER, R., TEIXEIRA, E. M., OLIVEIRA, V., QUINTÃO, C. C. R., CAMARGO, L. S. A., MATTOSO, L. H. C. & BRANDÃO, H. M. 2013. Cytotoxicity and expression

of genes involved in the cellular stress response and apoptosis in mammalian fibroblast exposed to cotton cellulose nanofibers. *Nanotechnology,* 24**,** 075103.

PRIYA JAMES, H., JOHN, R., ALEX, A. & ANOOP, K. R. 2014. Smart polymers for the controlled delivery of drugs – a concise overview. *Acta Pharmaceutica Sinica B,* 4**,** 120-127.

RUSTAD, K. C., WONG, V. W., SORKIN, M., GLOTZBACH, J. P., MAJOR, M. R., RAJADAS, J., LONGAKER, M. T. & GURTNER, G. C. 2012. Enhancement of mesenchymal stem cell angiogenic capacity and stemness by a biomimetic hydrogel scaffold. *Biomaterials,* 33**,** 80-90.

SAMPATH, U. G. T. M., CHING, Y. C., CHUAH, C. H., SINGH, R. & LIN, P. C. 2017. Preparation and characterization of nanocellulose reinforced semi-interpenetrating polymer network of chitosan hydrogel. *Cellulose,* 24**,** 2215-2228.

SINGER, A. J. & CLARK, R. A. F. 1999. Cutaneous Wound Healing. *New England Journal of Medicine,* 341**,** 738-746.

WEI, J. S., HAN, J., ZHAO, Y. N., CUI, Y., WANG, B., XIAO, Z. F., CHEN, B. & DAI, J. W. 2014. The importance of three-dimensional scaffold structure on stemness maintenance of mouse embryonic stem cells. *Biomaterials,* 35**,** 7724-7733.

WICHTERLE, O. & LÍM, D. 1960. Hydrophilic Gels for Biological Use. *Nature,* 185**,** 117.

ZHONG, Z. Y., QIN, J. L. & MA, J. 2015. Cellulose acetate/hydroxyapatite/chitosan coatings for improved corrosion resistance and bioactivity. *Materials Science & Engineering C-Materials for Biological Applications,* 49**,** 251-255.