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Sustainable Scaffolds-based Strategies in Tissue Engineering and Regenerative Medicine

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There is an urgent need to address the current paradigm shifts in bioengineering for Human Health aiming the creation of breakthrough tissue engineered products, manufacturing technologies and effective regenerative treatments for tackling different diseases/disorders in a personalized manner. Yet, the excessive costs and wastes related to the development and production of biomaterials and advanced therapy medicinal products, and the increasing use of plastics in cell culture methods and animal derived reagents has recognized the importance of decreasing the direct carbon footprint and thus, implement sustainable principles and solutions in the innovation ecosystem. The main goal of the volumes in Biomaterials, Bioengineering and Sustainability series is to catapult and consolidate new concepts and solutions towards the development of the next-generation of sustainable and eco-friendly biomaterials and tissue engineering and regenerative medicine approaches. Each volume will focus on the latest developments dealing with the identification of new sources of sustainable or recycled biomaterials, providing ideas for green technologies and methods that can be applied for biomaterials advanced processing and scaffolding strategies, and applications in biofabrication, tissue engineering, regenerative medicine, and drug delivery systems. It also aims to include the exploitation of renewable and sustainable source of human cells applied for cell therapies or in combination with sustainable biomaterials. The develop complex in vitro 3D/4D models and dynamic cell culture systems will be other subjects to be further explored from a sustainable perspective. This series aims to attract the contributions of leading experts in bioengineering, cell biology, materials engineering, and environmental sciences.

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Preface

Scaffold-based technologies and approaches play a pivotal role in modulation of cellular functions, extracellular matrix production, and in tissue regeneration and remodeling. The increasing use of scaffolds (3D) as alternative substrates to 2D cell culture methods may pose different challenges in respect to their sustainable application in *in vitro* models, tissue engineering and regenerative medicine (TERM), in a near future.

In this book, we will provide a concise overview on the relevant research dealing with the design and fabrication methods of sustainable scaffolds (conventional and advanced), considering not only the technological hurdles but also the increasingly important sustainability issues. In this context, the vast options of sustainable biomaterials and eco-friendly methods for biomaterials synthesis and scaffold's processing techniques are discussed. Importantly, a thorough discussion on the latest developments in the field of scaffolds-based strategies for TERM applications is provided. This book is divided into three main sections comprising 15 chapters, as follows: (i) Part I: Sustainable Scaffold Techniques and Designs in Tissue Engineering (five chapters); (ii) Part II: Natural Materials and Eco-Wastes Used as Sacrificial Templates (four chapters); and (iii) Part III: Biomedical Applications (six chapters). In brief, relevant topics on the types of biomaterials and its sustainable sources, and functionalization and processing methods, including green technologies and additive manufacturing, and different applications of sustainable scaffolds that promise to revolutionize preclinical research and clinics, are overviewed in depth.

This updated book includes the contribution of leading and multidisciplinary researchers, which provide an expert discussion on the significant achievements dealing with sustainable scaffolds for biomedical applications, thus serving as a core reference for a new generation of "Hybrid" students and established TERM researchers who aim to consider the sustainability dimension in their research activities.

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Contents

Part I

Sustainable Scaffold Techniques and Designs in Tissue Engineering

1 Recent Advancement of Sustainable Scaffolds in Regenerative Medicine

Swati Sambita Mohanty, Sanghamitra Pati,
and Sangram Keshari Samal

Abstract

Sustainable scaffolds offer efficient temporary support for cellular growth that can continue tissue regeneration for the development of desired organs without any toxic impact on the system in the foreseeable future. Regenerative medicine offers a transformative approach to tissue repair, but conventional scaffolds often raise environmental concerns. In recent years, several approaches have been adopted to incorporate sustainability into the designing of tissue engineering scaffolds during the fabrication process, which is achieved by using biodegradable/biocompatible, natural/synthetic polymers, energy-efficient and solvent-free processes, etc. Recent advancements in designing sustainable scaffolds and their potential use in tissue engineering applications are discussed. This sustainable approach paves the way for a future where promoting human health by reducing morbidity and mortality, goes hand-in-hand with fostering a healthier planet.

Keywords

Sustainable Biomaterials · Biodegradable Scaffolds · Cell Adhesion & Differentiation · Extracellular Matrix · Targeted Tissue Regeneration

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1.1 Introduction

Sustainable scaffolds offer efficient temporary support for cellular growth that can continue tissue regeneration to develop desired organs without any toxic impact on the system in the foreseeable future. Regenerative medicine offers a transformative approach to tissue repair. However, conventional fabrication techniques for designing scaffolds often rely on non-renewable resources or involve harsh production processes, raising concerns for biocompatibility and biodegradability in human health. Sustainable scaffolds, crafted from eco-friendly materials and employing greener fabrication methods, are emerging as a critical advancement in this field. The health benefits of sustainable scaffolds cannot be avoided as it offers the potential to regenerate damaged tissues and restore functionality. The sustainable scaffolds have the potential to degrade into non-toxic components, creating a safer environment for cell growth during regeneration. This enhanced biocompatibility is crucial for successful tissue regeneration (Trucillo 2024). However, translating this potential into reality presents significant engineering challenges. Mimicking the complexity and functionality of natural tissues requires a multifaceted approach. Engineering researchers strive to develop biocompatible scaffolds that provide structural support and cues for cell growth, while selecting the right cell types and delivering essential growth factors to promote tissue formation. Additionally, vascularization of engineered tissues, crucial for oxygen and nutrient delivery, remains a hurdle. Overcoming these challenges will be essential to ensure the long-term survival and function of engineered tissues in the body (Pogorielov et al. 2017). The sustainable scaffold paradigm is a cornerstone of tissue engineering, providing a crucial temporary structure for cell growth and guiding tissue formation. These sustainable scaffolds act as three-dimensional matrices, typically porous and biodegradable, that mimic the natural extracellular matrix (ECM) found within tissues. The ECM plays a vital role not only in providing structural support but also in directing cell adhesion, migration, proliferation, and differentiation (Schmidt et al. 2021). By mimicking the ECM's properties, scaffolds offer a platform for cells to organize and function similarly to how they would in native tissues.

Developing ideal sustainable scaffolds for tissue engineering requires careful consideration of various factors. Biocompatibility is paramount, ensuring the scaffold does not elicit an adverse immune response within the body (Lee et al. 2014). Additionally, the scaffold's degradation rate needs to be tailored to the specific tissue being regenerated. For instance, bone scaffolds require a slower degradation rate to provide long-term mechanical support for new bone formation, while scaffolds for skin regeneration can degrade more rapidly as the new tissue takes over (Chan and Leong 2008). Designing scaffolds with controlled biodegradability is another important consideration. Ideally, the scaffold should degrade at a rate that coincides with tissue formation, providing temporary support while gradually being replaced by functional tissue (Chan and Leong 2008). Natural polymers like chitosan or polycaprolactone offer biodegradable alternatives to traditional synthetic materials, allowing for a more controlled degradation process (Bolívar-Monsalve et al. 2021).

Despite its success, the sustainable scaffold paradigm is not without limitations. Engineering scaffolds with the intricate architecture and biochemical cues found in natural ECM remains a challenge. Additionally, vascularization of engineered tissues, essential for nutrient and oxygen delivery, can be hindered by the presence of scaffolds (De Pieri et al. 2021). Researchers are actively exploring strategies to address these limitations, such as incorporating bioactive molecules within scaffolds or developing techniques for scaffold-free tissue engineering approaches. Figure 1.1 illustrates the overcoming limitations in designing polymeric scaffolds for tissue engineering, such as mimicking the complexity of natural tissues or achieving optimal biocompatibility and biodegradability. The scaffold provides support and guidance for cell growth and new tissue formation. The pores allow for cell infiltration, nutrient transport, and waste removal, mimicking the natural ECM (Tarun 2012).

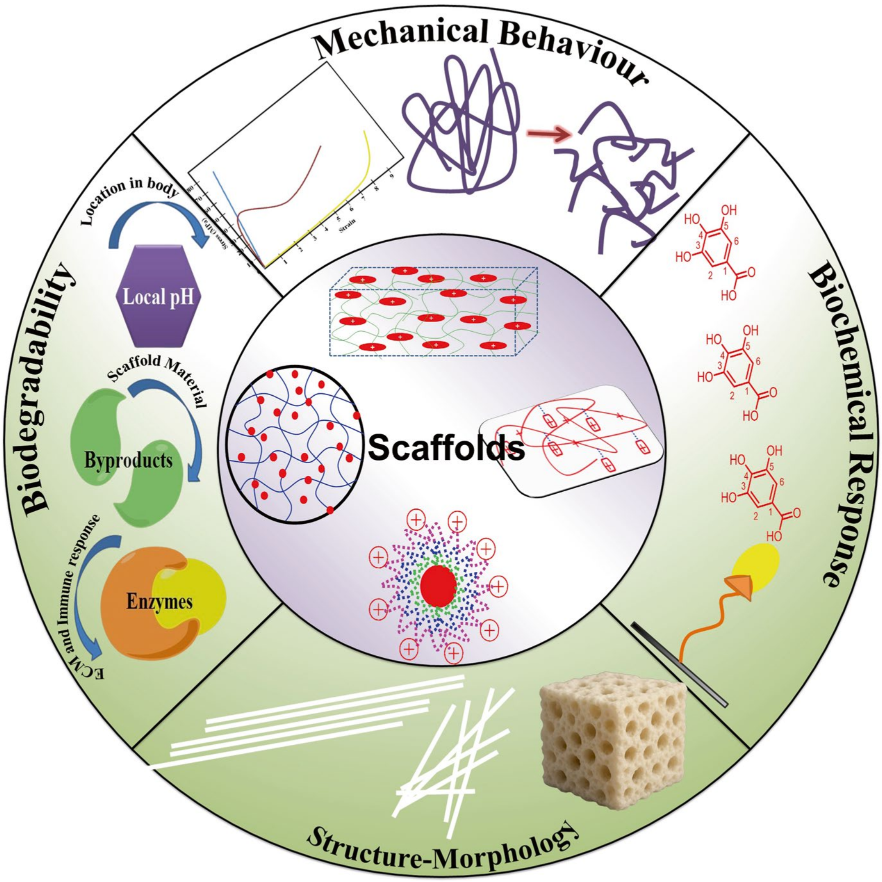

Fig. 1.1 Design challenges in scaffolds for Tissue Engineering (Garg Tarun et al. 2012)

1.1.1 Limitations of Conventional Scaffolds and the Need for Sustainability

While the sustainable scaffold paradigm has been instrumental in tissue engineering advancements, conventional scaffolds present limitations that hinder their effectiveness and raise sustainability concerns. A major challenge lies in mimicking the complex architecture and heterogeneity of natural tissues. Most scaffolds lack the intricate network of pores and channels found within the ECM, potentially hindering cell migration, nutrient diffusion, and waste removal within the engineered tissue (Ikada 2006). Additionally, the materials used to fabricate conventional scaffolds often rely on non-degradable synthetic polymers. These materials can persist in the body long after tissue regeneration is complete, potentially leading to chronic inflammation and foreign body reactions (Park 2015). Furthermore, the production of these synthetic polymers often involves energy-intensive processes and raises concerns about their environmental impact.

1.1.2 Seeking Sustainable Alternatives: Biodegradable and Biocompatible Scaffolds

The limitations of conventional scaffolds highlight the need for developing more sustainable and biocompatible alternatives. Researchers are actively exploring the use of naturally-derived materials for scaffold fabrication. These materials, such as collagen, chitosan, and silk fibroin, offer inherent biocompatibility and often degrade at a rate more closely aligned with tissue formation (Gomes et al. 2008). Additionally, advancements in bio-fabrication techniques allow for the creation of scaffolds with tailored architectures that better mimic the structure of natural tissues, potentially improving cell function and tissue integration (Henkel and Hutmacher 2013). Furthermore, these naturally-derived materials often hold advantages in terms of sustainability. They can be sourced from renewable resources, and their production processes typically require less energy compared to synthetic polymers. The limitations associated with scaffolds have also driven research toward exploring scaffold-free tissue engineering approaches. This strategy leverages the inherent ability of cells to self-assemble and organize into functional tissues. By utilizing cell-cell interactions and bioprinting technologies, researchers aim to create three-dimensional structures without the need for external scaffolds (De Pieri et al. 2021). Scaffold-free approaches offer several potential benefits, including improved cell viability and distribution within the engineered tissue, potentially leading to enhanced functionality. Additionally, this approach eliminates concerns associated with the long-term biocompatibility and environmental impact of synthetic scaffolds. Ultimately, achieving successful and sustainable tissue regeneration will likely require a multifaceted approach that combines advancements in scaffold design, material selection, and scaffold-free techniques. By fostering collaboration between engineers, material scientists, and biologists, researchers can develop innovative solutions that address the limitations of conventional scaffolds while prioritizing biocompatibility and environmental sustainability. This combined effort holds the potential to unlock the full potential of tissue engineering for improving human health and well-being in an environmentally responsible manner.

1.2 Sustainable Natural Polymers Scaffolds: Abundance and Biocompatibility

1.2.1 Natural Polymers Scaffolds

Natural polymers, unlike their synthetic counterparts, boast a unique combination of abundance and biocompatibility. Cellulose, for instance, is the most abundant biopolymer on Earth, with plants being the major producer (Balaji et al. 2018). These readily available materials are also attractive due to their minimal negative impact on the human body. Their biocompatible nature stems from their structural similarity to naturally occurring components found in living organisms (Mallik et al. 2019). This makes them ideal candidates for a variety of biomedical applications, ranging from drug delivery systems to tissue engineering scaffolds (Rajeswari 2017). Beyond just abundance and general biocompatibility, natural polymers offer a spectrum of advantages due to their diverse origins and chemical structures. Some, like silk fibroin, possess excellent mechanical strength, making them suitable for sutures, ligaments, and other implantable devices (Balaji et al. 2018). Others, like chitosan derived from crustacean shells, boast inherent antibacterial properties, reducing the risk of infection associated with medical implants (Troy et al. 2021). This biocompatibility extends beyond human applications. The natural degradation of these materials by microorganisms minimizes environmental impact, making them attractive for sustainable packaging materials and agricultural products (Ponnusamy and Mani 2022).

However, natural polymers also have limitations. Their inherent variability, compared to precisely controlled synthetic polymers, can present challenges in maintaining consistent material properties for large-scale applications (Ponnusamy and Mani 2022). Additionally, some natural polymers may trigger allergic reactions in a small percentage of the population, requiring careful selection and potential prescreening for specific uses (Balaji et al. 2018). Despite these limitations, research continues to refine processing techniques and explore modifications to natural polymers, enhancing their performance and expanding their potential applications. The inherent advantages of abundance, biocompatibility, and environmental friendliness make natural polymers a promising class of materials for the future of biomedicine and sustainable technologies.

Natural polymers, like collagen, chitosan, and silk, offer a unique set of advantages over synthetic alternatives with their excellent biocompatibility and biodegradability properties. These materials are readily accepted by the human body, minimizing the risk of rejection when used in medical devices or implants (Jiang and Loos 2016). Collagen, for instance, is the main structural protein in our skin and bones, making it an ideal candidate for tissue engineering applications

(Parenteau-Bareil et al. 2010). Similarly, chitosan, derived from shellfish, shows promise in wound dressings and other tissue engineering applications due to its biocompatibility, antibacterial properties, and versatility in scaffold fabrication (Dash et al. 2017; Rajinikanth et al. 2024; Sultankulov et al. 2019). Unlike some synthetic polymers that persist for extended periods in the environment, these materials can break down naturally by microorganisms. This characteristic makes them environment friendly and reduces the risk of long-term pollution (Jiang and Loos 2016). For example, silk-based sutures dissolve after the wound heals, eliminating the need for a second surgery for removal (Altman et al. 2003). The several possible applications of chitosan scaffolds in tissue engineering are depicted in Fig. 1.2.

Finally, natural polymers often possess inherent biofunctional properties that make them well-suited for specific applications. Collagen's structure provides excellent strength and elasticity, while chitosan's positive charge allows it to interact with negatively charged cells and tissues (Rajinikanth et al. 2024; Parenteau-Bareil et al. 2010). Silk fibers boast remarkable tensile strength, making them a valuable asset for textiles and biomaterials field (Altman et al. 2003). These inherent properties can be further tailored through processing techniques, expanding the potential uses of natural polymers. In conclusion, natural polymers offer a compelling combination of biocompatibility, degradability, and functional properties. As research continues to explore their potential, these materials hold promise for a wide range of applications in medicine, engineering, and other fields.

Biomaterials form the fundamental building blocks of scaffolds in regenerative medicine. These scaffolds are crucial for repairing, replacing, or improving tissue

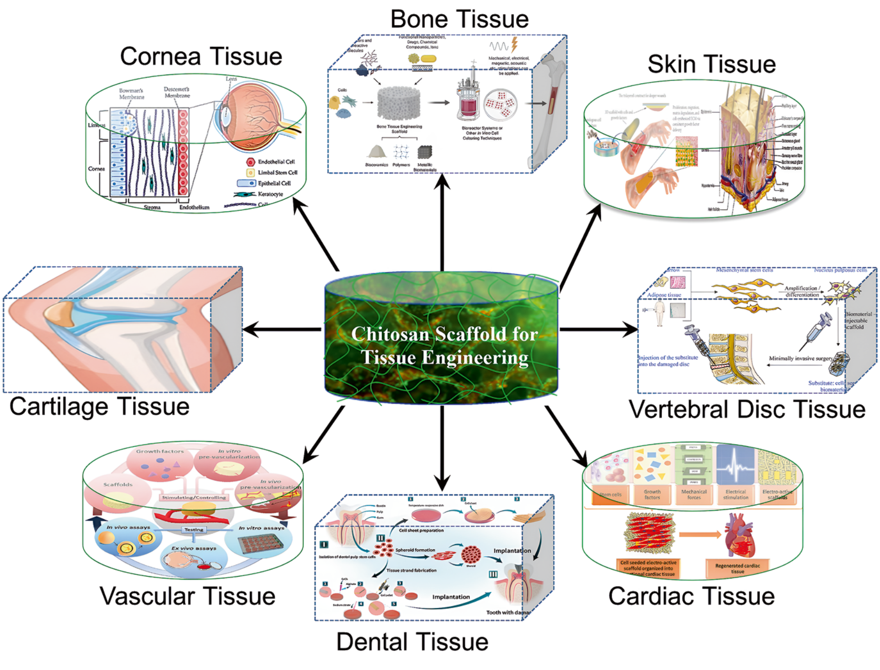

Fig. 1.2 Applications of chitosan scaffolds in tissue engineering (Liu et al. 2023)

function. Williams has extensively detailed the nature and function of these biomaterials (Williams 2009). Despite their numerous benefits, achieving true sustainability with natural polymers remains a significant challenge with major concern in sourcing. Large-scale production of some natural polymers, like chitosan obtained from shrimp shells, can put pressure on marine ecosystems if not managed responsibly (Rinaudo 2006). Sustainable practices for harvesting raw materials and utilizing by-products from existing industries are crucial. Another challenge lies in processing. Traditional methods for extracting and processing natural polymers can be energy-intensive and involve harsh chemicals, impacting the overall environmental footprint (Chemat et al. 2012). Research on greener extraction techniques using enzymes or less harmful solvents is ongoing. Additionally, optimizing processing steps to minimize waste and energy consumption is essential. Life cycle assessment (LCA) plays a vital role in developing sustainable strategies. LCA allows for a comprehensive evaluation of the environmental impact of a natural polymer throughout its entire life cycle, from sourcing to disposal (Fonseca et al. 2023). By identifying hotspots within the production chain, researchers and manufacturers can target areas for improvement. Beyond the challenges of sourcing and processing, ensuring the overall sustainability of natural polymers requires addressing issues like land use and potential competition with food production. For instance, large-scale cultivation of plants for biopolymers could lead to deforestation or depletion of arable land (La Rosa 2016). Strategies such as utilizing waste biomass or non-food crops can help mitigate this concern. Furthermore, promoting the circular economy principles for natural polymers is crucial. This involves designing products with end-of-life in mind, facilitating efficient collection and recycling of bio-based materials (Brandão et al. 2021). Additionally, exploring possibilities for biodegradation in industrial composting facilities can further enhance the sustainability of these materials. Finally, fostering consumer awareness plays a significant role. Educating consumers about the benefits and limitations of natural polymers can encourage responsible choices and support the development of a sustainable market for these materials (Anquez et al. 2022). Transparency throughout the supply chain, including clear labelling and certification programs, can build trust and encourage consumer adoption.

1.2.2 Synthetic Biodegradable Polymers: Tailoring Properties

Synthetic biodegradable polymers offer a powerful advantage—the ability to precisely control their properties for specific applications. Unlike their naturally derived counterparts, these materials can be meticulously designed at the molecular level to achieve desired characteristics. This tailored design approach unlocks a vast potential for various fields, including medicine, tissue engineering, and environmental solutions. One key aspect of tailoring synthetic biodegradable polymers is manipulating their degradation rate. By altering the chemical structure of the polymer backbone or incorporating specific additives, scientists can control how quickly the material breaks down in the body or environment (Rejinold et al. 2021). For instance, polylactic acid (PLA) have relatively fast degradation, making it ideal for short-term implants or drug delivery systems. Conversely, polycaprolactone (PCL) degrades at a slower pace, finding use in long-term implants or controlled release applications (Thakur and Thakur 2015).

Another crucial property under control is the mechanical strength of the polymer. Depending on the intended use, the material can be engineered to be rigid and supportive, like bone scaffolds, or flexible and elastic, mimicking soft tissues (Hutmacher 2001). This control over mechanical properties is achieved through various techniques, including copolymerization with different monomers or modifying the polymer architecture through branching or crosslinking (Colquhoun and Tanner 2015). By harnessing the power of synthetic chemistry, scientists can create a diverse range of biodegradable polymers with precisely tailored properties. This ability to fine-tune material characteristics paves the way for the development of innovative solutions that address specific needs across various sectors. The ability to tailor properties extends far beyond just the degradation rate in synthetic biodegradable polymers. Scientists can introduce various functionalities to these materials, creating a new level of control over their behavior within the body or environment. One exciting area is the incorporation of bioactive molecules. These molecules can be physically blended, chemically attached, or encapsulated within the polymer matrix (Hutmacher et al. 2023). For instance, antibiotics loaded onto synthetic scaffolds can provide localized infection control for bone implants, while growth factors can promote cell adhesion and proliferation for tissue engineering applications (Yao et al. 2020). Another approach involves tailoring the surface properties of the polymer. By introducing specific chemical groups, scientists can design hydrophilic (water-loving) or hydrophobic (water-repelling) surfaces (Katti et al. 2008). This control over surface chemistry influences factors like cell attachment, protein adsorption, and drug release kinetics. For example, hydrophilic surfaces can promote cell adhesion, while hydrophobic surfaces may be better suited for controlled drug delivery applications. The concept of biocompatibility also comes into play when tailoring synthetic biodegradable polymers. By incorporating specific functionalities, researchers can modulate the immune response to the implanted material (Ma et al. 2007). This can be crucial for minimizing inflammation and rejection, especially for long-term implants or devices in contact with sensitive tissues.

In brief, tailoring properties in synthetic biodegradable polymers goes beyond just basic biodegradation. The ability to introduce functionalities and control surface chemistry unlocks a new realm of possibilities. This allows scientists to design biomaterials that actively interact with the biological environment, leading to the development of more advanced and targeted solutions in areas like medicine, drug delivery, and tissue engineering.

Synthetic polymers also offer greater flexibility in mechanical property design. Depending on the intended use, the material can be engineered to possess specific strength, elasticity, or stiffness. This control over mechanical properties is achieved through techniques like copolymerization or modifying the polymer architecture. For example, PLA can be blended with other polymers to create stronger and more rigid materials, while PCL can be modified to be more flexible for applications mimicking soft tissues (Capuana et al. 2021; Zheng and Pan 2020). Furthermore, synthetic polymers can be readily functionalized with specific molecules to enhance their performance. These functionalities can range from bioactive molecules like growth factors for tissue engineering to antimicrobial agents for medical devices. This ability to introduce desired functionalities allows for the creation of targeted biomaterials with specific therapeutic or diagnostic capabilities (Martinez-Robinson 2020).

Finally, synthetic polymers offer the advantage of being readily functionalized with specific molecules to enhance their performance. These functionalities can range from bioactive molecules like growth factors for tissue engineering to antimicrobial agents for medical devices. This ability to introduce desired functionalities allows for the creation of targeted biomaterials with specific therapeutic or diagnostic capabilities (Martinez-Robinson 2020). Figure 1.3 represents the synthetic polymers for tissue scaffolds. In conclusion, synthetic biodegradable polymers offer a powerful advantage for controlled design due to their consistency, tunable degradation rates, tailorable mechanical properties, and ease of functionalization. These features empower scientists and engineers to create innovative solutions across various fields, from medicine and drug delivery to tissue engineering and environmental applications.

Synthetic polymers, a cornerstone of modern life, present a complex challenge in terms of sustainability. Their undeniable benefits in applications ranging from medical devices to food packaging are often overshadowed by their environmental impact. Herein, we explore two key considerations for a more sustainable future of synthetic polymers: biodegradability and resource utilization. Biodegradability refers to a polymer's ability to decompose naturally by microorganisms into harmless substances. Conventional synthetic polymers, derived from fossil fuels, are notoriously resistant to degradation, leading to massive plastic pollution in landfills and ecosystems (Martinez-Robinson 2020). Research on biodegradable polymers

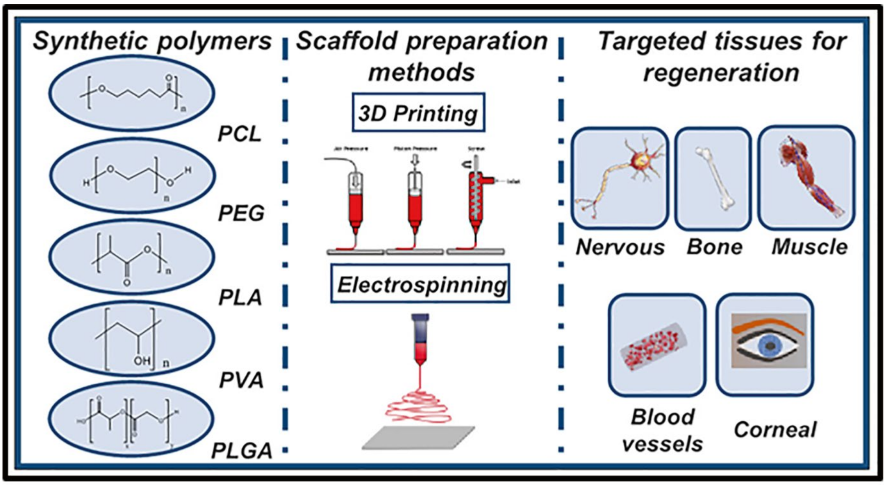

Fig. 1.3 Biocompatible synthetic polymers for tissue engineering purposes (Perez-Puyana et al. 2020)

derived from renewable resources like plant starches or microorganisms offers a promising solution (Park 2015). These materials can significantly reduce plastic waste accumulation, mitigating environmental harm (Zhu et al. 2016b).

While biodegradability is a crucial aspect of sustainable polymers, it's not the only factor. Polymers are commonly engineered for strength and durability, but not for efficient breakdown. Developing polymers that can be easily broken down into their parts at the end of their useful life facilitates efficient recycling and reduces reliance on virgin materials (Kuhl et al. 2015). Not all biodegradable polymers are suitable for composting. Some require industrial composting facilities with specific temperature and pressure conditions, limiting their environmental benefit. Developing polymers that can biodegrade in home or backyard compost piles offers a more accessible and sustainable end-of-life solution (Ghosh and Jones 2021). Biodegradable polymers themselves, or the additives used in their production, can potentially introduce new toxins into the environment during degradation. Careful selection of materials and thorough toxicity assessments are essential to ensure biodegradability doesn't come at the expense of environmental or human health (Adekomaya et al. 2021).

Sustainability goes beyond just environmental impact. For synthetic polymers to be truly sustainable, social and economic factors must also be considered. Sustainable alternatives often have higher upfront costs. Life cycle cost analysis, which considers the total cost of a polymer throughout its lifetime, including disposal or recycling, can provide a more complete picture and incentivize the adoption of sustainable options (Kneifel and Webb 2020). Effective recycling and composting infrastructure is crucial for maximizing the sustainability of synthetic polymers. Investment in building and maintaining this infrastructure is essential to support the transition towards a circular economy for these materials (Epps et al. 2022).

Resource utilization during polymer production is another critical factor. The traditional reliance on non-renewable resources like petroleum for polymer feedstocks is unsustainable. Developing methods for utilizing recycled materials or renewable feedstocks like biomass can significantly reduce the environmental footprint of synthetic polymer production (Epps et al. 2022). Life cycle assessments, which consider the environmental impact throughout a polymer's life cycle from extraction to disposal, are crucial tools in guiding the development of more sustainable production processes (Amarakoon et al. 2022). By focusing on biodegradability and efficient resource utilization, the future of synthetic polymers can be more sustainable. Continuous research and development efforts are essential to create new materials and production processes that minimize environmental impact while retaining the valuable properties that synthetic polymers offer. By addressing these diverse considerations, we can create a future for synthetic polymers that benefit the environment, society, and the economy.

1.2.3 Decellularized Extracellular Matrix (dECM): Mimicking the Native Niche

The human body is a complex ecosystem, with each tissue harboring a unique microenvironment essential for proper cell function. This microenvironment, known as the ECM, provides structural support, biochemical cues, and mechanical signals that guide cellular behavior. In regenerative medicine, replicating this intricate niche is crucial for promoting successful cell transplantation and tissue engineering. dECM emerges as a promising solution, offering a natural scaffold that mimics the native tissue environment and has also shown promise in hemostatic applications (Cai and Weng 2023). dECM is derived from various tissues by removing the cellular component while preserving the intricate three-dimensional structure and biochemical composition of the ECM. This process retains vital components like collagen, glycosaminoglycans, and growth factors, which orchestrate cellular processes like adhesion, proliferation, and differentiation (Gilpin and Yang 2017). By providing a familiar biological blueprint, dECM scaffolds can significantly enhance cell function compared to traditional synthetic biomaterials (Yue 2014).

The ability of dECM to mimic the native niche extends beyond structural similarity. Studies have shown that dECM derived from specific tissues can promote the growth and differentiation of corresponding cell types. For instance, cardiac dECM effectively supports the growth and maturation of cardiomyocytes, while lung dECM promotes the development of lung epithelial cells (Golebiowska et al. 2024; Stabler et al. 2015). This tissue-specific biomimicry holds immense potential for regenerative medicine applications targeting damaged or diseased tissues.

However, challenges remain in optimizing dECM technology. Decellularization techniques need to be carefully tailored to ensure complete cell removal while preserving the delicate ECM structure and composition. Additionally, sourcing dECM from various tissues and ensuring consistent quality across batches requires further development (Yi et al. 2017). Despite these challenges, dECM offers a revolutionary approach for mimicking the native cellular niche, paving the way for advancements in regenerative medicine. The utilization of dECM scaffolds for bone regeneration: a cell-based approach is illustrated in Fig. 1.4.

The tissue-specific advantage of dECM goes beyond structural similarity. Studies have shown remarkable success in directing cell fate using dECM. For example, cardiac dECM scaffolds effectively promote the growth and maturation of cardiomyocytes, the muscle cells of the heart (Barbulescu et al. 2022). Similarly, lung dECM has been shown to support the development of functional lung epithelial cells, which are crucial for gas exchange in the lungs (Gilpin and Wagner 2018). This inherent ability of dECM to guide cell differentiation towards specific lineages holds immense potential for regenerative medicine applications targeting damaged or diseased tissues. Furthermore, dECM scaffolds offer inherent biological cues that synthetic materials often lack. The retained growth factors and signaling molecules within the dECM can directly influence cellular behavior. These signals guide

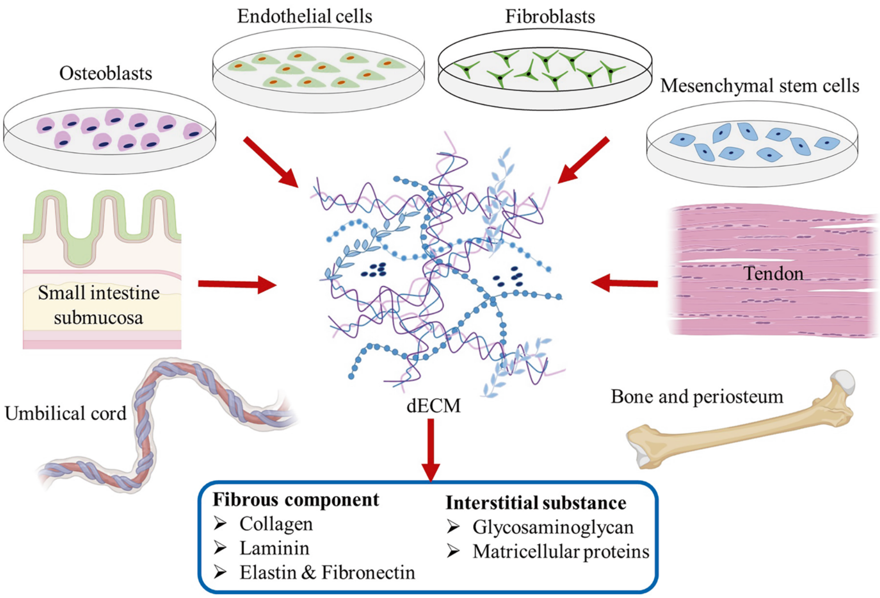

Fig. 1.4 Decellularized extracellular matrices in bone tissue engineering: from cells to tissues (Gilpin and Yang 2017)

processes like migration, proliferation, and differentiation, creating a microenvironment conducive to tissue regeneration (Zhang et al. 2022b). Additionally, dECM scaffolds possess inherent vascularization potential, promoting the formation of blood vessels within the scaffold, which is essential for nutrient delivery and waste removal in newly formed tissues (McInnes et al. 2022). In conclusion, dECM scaffolds offer a revolutionary approach for tissue-specific applications in regenerative medicine. By mimicking the native tissue environment and providing essential biological cues, dECM promotes targeted cell growth and differentiation. As research continues to optimize decellularization techniques and explore dECM applications for various tissues, these scaffolds hold promising potential for the regeneration of damaged or diseased tissues across the body.

dECM holds immense promise for regenerative medicine due to its ability to mimic the native tissue environment. However, the processing of dECM presents several sustainability challenges that need to be addressed for its widespread adoption. Herein, we explore some key challenges and potential strategies for optimizing dECM processing towards a more sustainable future. One major challenge lies in the resource intensity of decellularization techniques. Conventional methods often rely on harsh chemicals and detergents to remove cells, raising concerns about environmental impact and potential toxicity. Disposing of these chemicals after use can pollute waterways and require significant energy for wastewater treatment

(Neishabouri et al. 2022). Additionally, the high volume of detergents needed can be resource-intensive and contribute to the depletion of non-renewable resources. Strategies to overcome this challenge involve exploring alternative decellularization methods. Research is ongoing to develop techniques using enzymes, sonication, or even supercritical fluids, which offer a more environment friendly approach (Allu et al. 2023; Duarte et al. 2009). Enzymes, for instance, can specifically target cellular components while leaving the ECM intact, reducing the need for harsh chemicals. Similarly, sonication and supercritical fluids utilize physical forces to disrupt cellular membranes, offering a potentially more sustainable alternative. Another challenge is the variability associated with dECM derived from different sources. Decellularization efficiency can be impacted by factors like tissue type, donor health, and processing protocols. This variability can lead to inconsistencies in the resulting dECM scaffolds, affecting their performance and ultimately requiring the use of more tissue samples to achieve consistent results (Badylak 2002). Strategies mitigating this challenge involve standardization of decellularization protocols. Developing well-defined protocols for specific tissue types, considering factors like decellularization time, detergent concentration, and temperature control, can help ensure consistent quality across dECM batches. Additionally, implementing quality control measures throughout the processing pipeline can further minimize variability in the final product.

Finally, the scalability of dECM production for broader clinical applications needs to be addressed. Currently, obtaining sufficient tissue for dECM production can be challenging, especially for tissues with limited availability. Strategies for overcoming this hurdle include exploring the use of xenogeneic (animal-derived) dECM sources or utilizing smaller tissue biopsies with optimized decellularization protocols (Massaro et al. 2021). Furthermore, research into bioprinting dECM-like structures using biocompatible inks holds promise for large-scale production of tissue-specific scaffolds in the future. By addressing these challenges and adopting sustainable processing strategies, dECM can truly fulfill its potential as a revolutionary tool for regenerative medicine. Continuous research and development efforts focused on environment friendly decellularization methods, standardized protocols, and scalable production techniques are crucial for realizing the full potential of dECM in a sustainable manner.

1.3 Fabrication Techniques for Sustainable Scaffolds

Tissue engineering relies heavily on scaffolds to provide structural support and biochemical cues for cell growth and differentiation. However, traditional scaffold materials often raise concerns about sustainability due to factors like reliance on non-renewable resources or challenges with biodegradability. To address these issues, research is focusing on developing sustainable fabrication techniques for scaffolds used in tissue engineering. One promising approach involves utilizing natural biomaterials, derived from sources like collagen, chitosan, or cellulose that are often biodegradable and can be processed using environment friendly methods. Techniques like solvent casting, freeze-drying, or electrospinning can be employed to create scaffolds with desired pore sizes and architectures from these natural polymers (Brovold et al. 2018; Zhao et al. 2023). Additionally, advancements in 3D printing allow for precise control over scaffold design and the incorporation of bioactive molecules for enhanced cell function (Tajik et al. 2023). Another strategy for sustainable scaffold fabrication focuses on utilizing waste materials. For instance, recycled plastics or discarded agricultural byproducts can be processed into biocompatible scaffolds. Techniques like melt extrusion or solvent casting can be adapted to create scaffolds from these recycled materials, offering a solution for waste management while creating valuable tools for tissue engineering applications (Balla et al. 2021; Šafarič et al. 2020).

Beyond the materials themselves, sustainable fabrication techniques consider the environmental impact of the processing methods. Techniques with lower energy consumption or reduced reliance on harsh chemicals are preferred. For example, supercritical fluid processing offers an environment friendly alternative to traditional solvent-based methods for scaffold fabrication (Manjare and Dhingra 2019). In addition, research is ongoing to develop biofabrication techniques that utilize living cells to create scaffolds, potentially eliminating the need for harsh processing conditions (Vesvoranan et al. 2022). There are various conventional methods of sustainable scaffold fabrication (Fig. 1.5) that have been developed for drug delivery, 3D cell culture and tissue engineering applications. The choice of fabrication technique ultimately depends on the desired properties of the scaffold and the specific tissue engineering application. However, by focusing on natural biomaterials, recycled materials, and environment friendly processing methods, researchers are paving the way for a more sustainable future for scaffold fabrication in tissue engineering.

Tissue engineering relies on the development of scaffolds that mimic the natural ECM to promote cell growth and differentiation. Solvent casting/particulate leaching (SCPL) emerges as a simple and versatile technique for creating porous scaffolds for various tissue engineering applications. This approach offers several advantages, making it a valuable tool for researchers and scientists.

The core principle of SCPL is straightforward. A polymer is first dissolved in a suitable solvent to create a homogeneous solution. Next, well-defined particles, often salts like sodium chloride (table salt), are incorporated into the polymer solution. This mixture is then cast into a mold with the desired shape for the final scaffold. As the solvent evaporates, the polymer forms a solid matrix around the embedded particles (Hutmacher 2001). Finally, the scaffold is immersed in a solvent that dissolves the sacrificial particles, leaving behind a network of interconnected pores within the polymer matrix. The pore size and distribution can be controlled by the size and shape of the leached particles (Lyons et al. 2020).

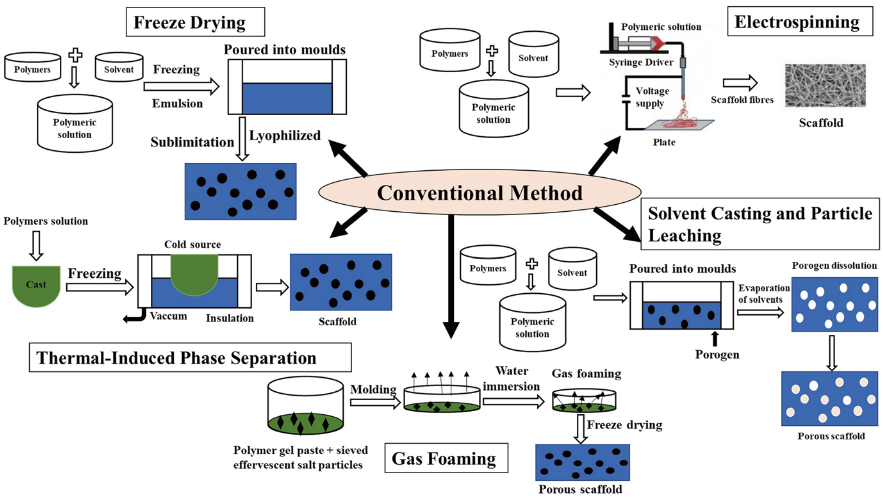

Fig. 1.5 Sustainable techniques in scaffold fabrication process for tissue engineering applications (Song et al. 2018; Zeng et al. 2017)

The simplicity of SCPL is one of its key advantages. This technique requires minimal specialized equipment and can be readily implemented in various laboratory settings. Additionally, SCPL offers a high degree of versatility. A wide range of polymers can be utilized, allowing researchers to tailor the scaffold properties to specific tissue engineering needs. For instance, biodegradable polymers like PLA can be used for scaffolds that degrade over time, mimicking the natural remodeling process in tissues (Langer and Vacanti 1993).

Despite its advantages, SCPL also has limitations to consider. The choice of solvent can impact the final properties of the scaffold, and residual solvent traces may require additional processing steps. Additionally, the selection of suitable porogens (leachable particles) is crucial for achieving the desired pore morphology. Finally, SCPL may not be suitable for creating highly complex scaffold architectures due to limitations in mold design (Kaliaraj et al. 2023). SCPL offers a valuable approach for fabricating porous scaffolds for tissue engineering applications. Its simplicity, versatility, and compatibility with various materials make it a widely used technique. While limitations exist regarding solvent selection, porogen choice, and scaffold complexity, ongoing research is focused on optimizing SCPL protocols for diverse tissue engineering needs.

1.3.1 Electrospinning: Nanofiber Scaffolds for Enhanced Cell-Material Interactions

Tissue engineering strives to create functional tissues by mimicking the natural cellular environment. A key element in achieving this is providing the cells with a scaffold that offers not only structural support but also crucial cues for growth and differentiation. Electrospinning emerges as a powerful fabrication technique for generating nanofiber scaffolds that promote enhanced cell-material interactions. Electrospinning utilizes an electric field to draw a polymer solution into ultrathin fibers, typically ranging in diameter from tens to hundreds of nanometers. These nanofibers mimic the ECM, the natural network of proteins and polysaccharides that surrounds cells in tissues (Flores-Rojas et al. 2023). The high surface area-to-volume ratio inherent to nanofibers provides a multitude of attachment sites for cells, promoting cell adhesion and proliferation (Bhardwaj and Kundu 2010). Additionally, the interconnected porosity of electro-spun scaffolds allows for efficient nutrient and waste transport, which is crucial for maintaining cell viability within the scaffold. Beyond mimicking the ECM structure, electrospinning offers versatility in tailoring the scaffold properties for specific cell types. By manipulating factors like polymer choice, fiber diameter, and alignment, researchers can create scaffolds with specific mechanical properties, degradation rates, and biological functionalities (Hu et al. 2014). For instance, incorporating bioactive molecules like growth factors or adhesion peptides into the nanofibers can further enhance cell-material interactions and promote specific cellular responses (Flores-Rojas et al. 2023). The ability of electrospinning to create complex architectures further expands its potential in tissue engineering. By controlling the electric field and collector design, researchers can fabricate scaffolds with specific pore sizes, gradients, and even multilayered structures. These intricate architectures can mimic the native tissue microenvironment in greater detail, providing even more sophisticated cues for cell behavior (Xing et al. 2023).

Hence, electrospinning offers a robust and versatile technique for creating nanofiber scaffolds that enhance cell-material interactions. By mimicking the ECM structure, offering a high surface area for cell attachment, and allowing for tailored properties and architectures, electro-spun scaffolds provide a valuable tool for researchers in tissue engineering. Figure 1.6 outlines the nanofiber scaffolds fabricated by electrospinning for wound healing. As research continues to refine electrospinning techniques and explore novel material combinations, its impact on developing functional tissue replacements is likely to grow even stronger.

1.3.2 3D Printing: Designing Complex Architectures for Functional Tissues

A critical challenge in tissue engineeris still lies in replicating the intricate three-dimensional (3D) structures of native tissues. 3D printing emerges as a revolutionary technology, allowing researchers to design and fabricate scaffolds with complex architectures that mimic the natural environment of various tissues. Unlike traditional scaffold fabrication techniques with limited control over structure, 3D

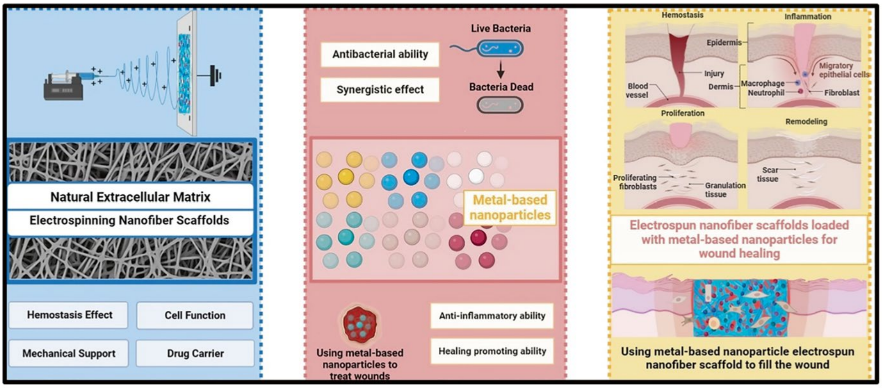

Fig. 1.6 Electrospun sustainable nanofiber scaffolds for wound healing applications (Dang et al. 2023)

printing offers unparalleled freedom in designing. Using computer-aided design (CAD) software, researchers can create digital models of scaffolds with intricate internal features, channels, and gradients. These models can then be translated into instructions for the 3D printer, enabling the precise deposition of biocompatible materials layer-by-layer to create the desired scaffold architecture (Fang et al. 2023). This level of control allows for the creation of scaffolds that closely resemble the complex geometries of natural tissues, such as the branching networks of blood vessels or the porous structure of bone.

The ability to design complex architectures with 3D printing extends beyond mere shape replication. By incorporating channels and gradients within the scaffold, researchers can control factors like nutrient and oxygen diffusion, crucial for cell survival and functionality within the scaffold (Bose et al. 2012). Additionally, 3D printing allows for the precise patterning of bioactive molecules or cells within the scaffold. For instance, specific growth factors can be deposited in designated regions to promote the differentiation of desired cell types, fostering the development of functional tissues (Gu et al. 2016). The versatility of 3D printing extends to the materials used. A wide range of biocompatible materials, including natural polymers, synthetic biomaterials, and even bioinks containing living cells, can be utilized for 3D printing. This allows researchers to tailor the scaffold properties to match the specific needs of different tissues (Zieliński et al. 2023). For example, soft hydrogels can be used to mimic brain tissue, while stronger polymers may be suitable for bone or cartilage regeneration. 3D printing offers immense potential for tissue engineering, however, there are some specific challenges remain. Optimizing printing parameters for various biomaterials and ensuring the fidelity of complex structures during printing are ongoing areas of research. Additionally, the development of robust vascularization strategies within 3D-printed scaffolds is crucial for the survival of cells within thick tissues (Chan and Leong 2008). 3D printing revolutionizes tissue engineering by enabling the design and fabrication of scaffolds with intricate architectures that mimic native tissues. The ability to control structure, incorporate functional cues, and utilize diverse biomaterials makes 3D printing a powerful tool for creating functional tissue replacements. As research continues to address current challenges and explore new possibilities, 3D printing is poised to play a transformative role in regenerative medicine.

1.3.3 Bioprinting: Biomimetic Fabrication with Cellular Control

Bioprinting emerges as a ground-breaking technology in tissue engineering, offering unprecedented control over cell placement and organization within scaffolds. It builds upon traditional 3D printing principles, but utilizes biocompatible materials called bio inks to precisely deposit living cells in a layer-by-layer fashion. This allows researchers to create intricate, biomimetic structures that closely resemble natural tissues, fostering enhanced cell function and tissue regeneration. A key strength of bioprinting lies in its ability to mimic the cellular microenvironment. Bio inks can be formulated using natural biomaterials like collagen, alginate, or hyaluronic acid, which provide essential structural support and biochemical cues for cells (Waidi et al. 2024). These biomaterials mimic the natural ECM, the complex network of proteins and polysaccharides that surrounds cells in tissues. Furthermore, bio inks can be enriched with bioactive molecules like growth factors or adhesion peptides (Wang et al. 2021). These act as signals that guide cellular behaviour and promote differentiation towards specific lineages, such as muscle cells or bone cells. This biomimetic approach allows for the creation of scaffolds that not only offer structural support but also actively influence cell fate, leading to the development of functional tissues. Figure 1.7 illustrates the “Bioprinting Functional Scaffolds for Tissue Regeneration” (Bioprinting emphasizes the 3D printing aspect specifically for biological applications).

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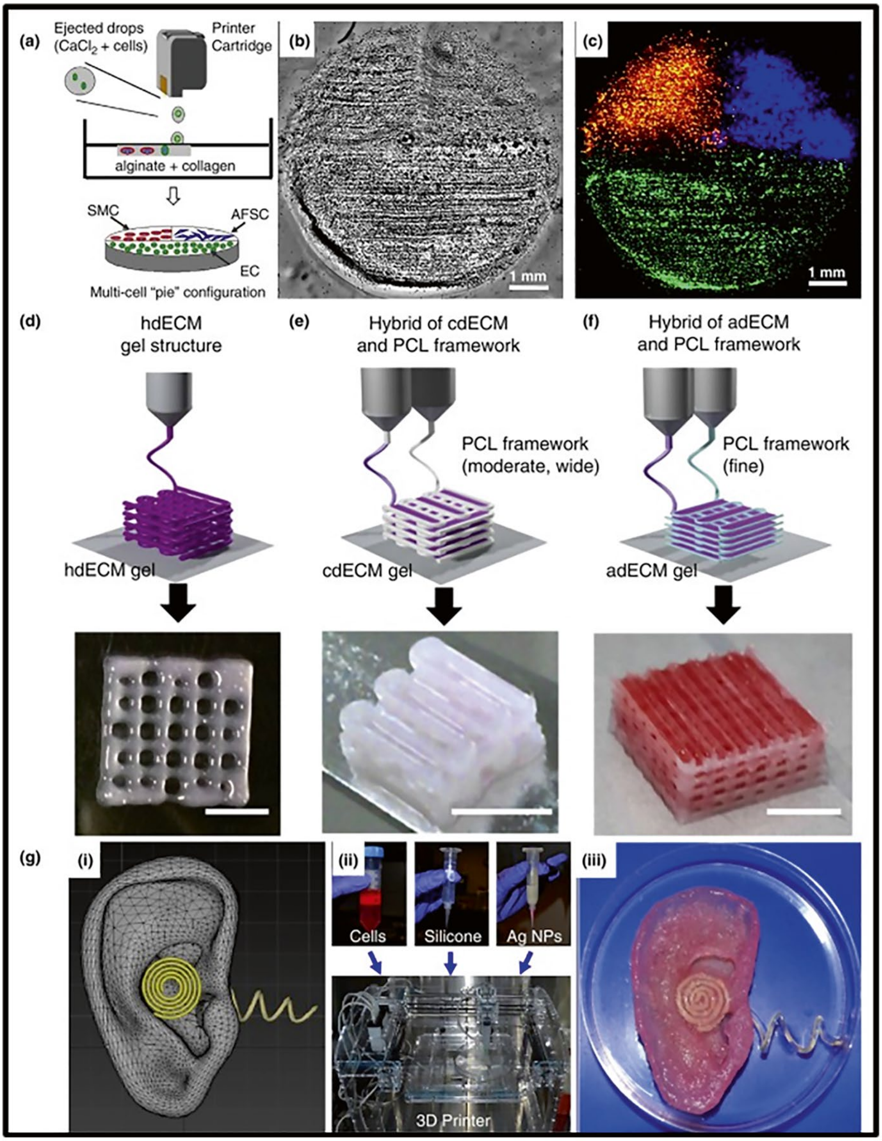

Fig. 1.7 3D printing of functional biomaterials for tissue engineering (Zhu et al. 2016a): (**a**) Schematic showing multi-cellular "pie"-like configuration created by bioprinting $CaCl_2$ + cell droplets onto an alginate + collagen layer, organizing different cell types—smooth muscle cells (SMC), amniotic fluid stem cells (AFSC), and endothelial cells (EC)—into distinct regions. (**b**) Optical image of the printed construct showing the layered deposition pattern. (**c**) Fluorescently labeled image illustrating distinct regions corresponding to different cell types in the printed structure (orange, blue, green), demonstrating spatial organization. (**d**) Printing of human-derived ECM (hdECM) gel using extrusion-based bioprinting, yielding a soft grid-like gel structure. (**e**) Hybrid structure combining cartilage-derived ECM (cdECM) gel and polycaprolactone (PCL) framework, with moderate and wide spacing to provide mechanical support and biological compatibility. (**f**) Hybrid structure with adipose-derived ECM (adECM) gel and finely spaced PCL framework to optimize tissue integration and stability. (**g**) 3D bioprinting of a functional bionic ear: (i) Computer model of the ear integrated with an electronic coil antenna, (ii) Multimaterial bioprinting process using cells, silicone, and silver nanoparticles (Ag NPs), (iii) Final printed bionic ear construct exhibiting integrated electronics and biomaterials

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However, traditional bio inks often rely on synthetic polymers or harsh processing techniques, raising concerns about sustainability. Research efforts are now focused on developing sustainable alternatives. One promising approach involves utilizing dECM as a bio ink source (Zhang and Zhang 2015). dECM is derived from natural tissues and retains the inherent biological cues needed for cell function. Additionally, researchers are exploring the use of minimally processed biomaterials like plant-derived hydrogels (Nikolova and Chavali 2019). These sustainable bio inks offer comparable performance to their synthetic counterparts while minimizing environmental impact. Sustainability considerations extend beyond the bio ink materials themselves. Bioprinting techniques that utilize cell spheroids or self-assembling biomaterials hold promise for reducing the overall bio ink volume required (Mandrycky et al. 2016). Cell spheroids are clusters of cells that can self-organize and mimic some aspects of tissue structure. Self-assembling biomaterials are designed to form specific structures when exposed to certain cues, reducing the need for precise bio ink deposition during the printing process. By minimizing bio ink usage and exploring sustainable materials, researchers are ensuring that bioprinting remains an environmentally responsible technology.

Bioprinting with cellular control offers a powerful tool for regenerative medicine. By combining biomimetic scaffold design, precise cell placement, and the use of sustainable bio inks, researchers are paving the way for creating functional tissue replacements for a variety of diseases and injuries. Examples include bioprinting skin grafts for burn victims, heart patches for damaged cardiac tissue, or even complex structures like bones or ears. As bioprinting technology continues to advance and integrate sustainability principles, its impact on regenerative medicine is poised to be transformative.

1.4 Strategies for Optimizing Scaffold Sustainability

Tissue engineering relies heavily on scaffolds to provide structural support and biochemical cues for cell growth and differentiation. However, traditional scaffold materials often raise sustainability concerns due to factors like reliance on non-renewable resources or challenges with biodegradability. To address these issues, researchers are actively exploring various strategies for optimizing scaffold