Chapter

Pectin-Based Scaffolds for Tissue Engineering Applications

Anna Lapomarda, Aurora De Acutis, Carmelo De Maria and Giovanni Vozzi

Abstract

Tissue engineering (TE) is an interdisciplinary field that was introduced from the necessity of finding alternative approaches to transplantation for the treatment of damaged and diseased organs or tissues. Unlike the conventional procedures, TE aims at inducing the regeneration of injured tissues through the implantation of customized and functional engineered tissues, built on the so-called 'scaffolds'. These provide structural support to cells and regulate the process of new tissue formation. The properties of the scaffold are essentials, and they can be controlled by varying the biomaterial formulation and the fabrication technology used to its production. Pectin is emerging as an alternative biomaterial to non-degradable and high-cost petroleum-based biopolymers commonly used in this field. It shows several promising properties including biocompatibility, biodegradability, non-toxicity and gelling capability. Pectin-based formulations can be processed through different fabrication approaches into bidimensional and three-dimensional scaffolds. This chapter aims at highlighting the potentiality in using pectin as biomaterial in the field of tissue engineering. The most representative applications of pectin in preparing scaffolds for wound healing and tissue regeneration are discussed.

Keywords: pectin, tissue engineering, scaffolds, bioprinting, biofabrication, tissue regeneration

1. Introduction

Tissue engineering (TE) is an interdisciplinary field whose first definition dates back to 1987. It combines the knowledge from different research areas including medicine, material science and engineering to develop engineered biological substitutes able to restore, maintain or improve tissue functions [1]. TE was introduced from the necessity of finding alternative methodologies to organ transplantations due to their increasing demand in clinical medicine. Furthermore, TE emerged as a promising approach to overcome the limitations of the conventional surgical approaches for the treatment of tissue damages caused by injuries, diseases and congenital disorders [2, 3]. These surgical procedures are based on replacing the injured tissues or organs with a healthy one harvested from the same patient (autograft), or a compatible donor (allograft). Although these approaches have been revolutionary and lifesaving, there are still some drawbacks that need to be addressed. The surgical procedures used to harvest both autografts and allografts are often invasive and painful. The risk of post-surgical limitations in

the donor's body due, for example, to infections and hematomas is, in fact, quite high. Moreover, when allografts are transplanted, the chance of inflammatory and immune responses in the patient body together with the transmission of diseases from the donor to the patient is significant [4].

TE aims at overcoming the complications associated with the conventional techniques used during organ transplantation by inducing the complete regeneration of the damaged tissues instead of replacing them [2, 3]. Several approaches to promote *de novo* tissue formation have been implemented in TE so far. These are mainly based on the use of biodegradable and biocompatible engineered tissues, based on the so-called '*scaffolds*'. A scaffold is a structure that provides temporary mechanical support and a guiding template to cells during the synthesis of new tissue. With the desired shape, architecture and functions. Concurrently, the scaffold biodegrades leaving space for new tissue in-growth. Notably, the biodegradability of the scaffold is what differentiates it from permanent implants. The complete biodegradation of the scaffolds prevents, the need for additional surgical interventions to remove it or, eventually, substitute it. The scaffold can be directly implanted into the injured site to induce the regeneration of the tissues *in vivo.* Otherwise, prior to implantation, the scaffold can be initially cellularized with cells isolated from the patient, subsequently cultured *in vitro* to synthesize tissues that will finally be transplanted into the defect to restore its functions (**Figure 1**). In this case, scaffolds can be further

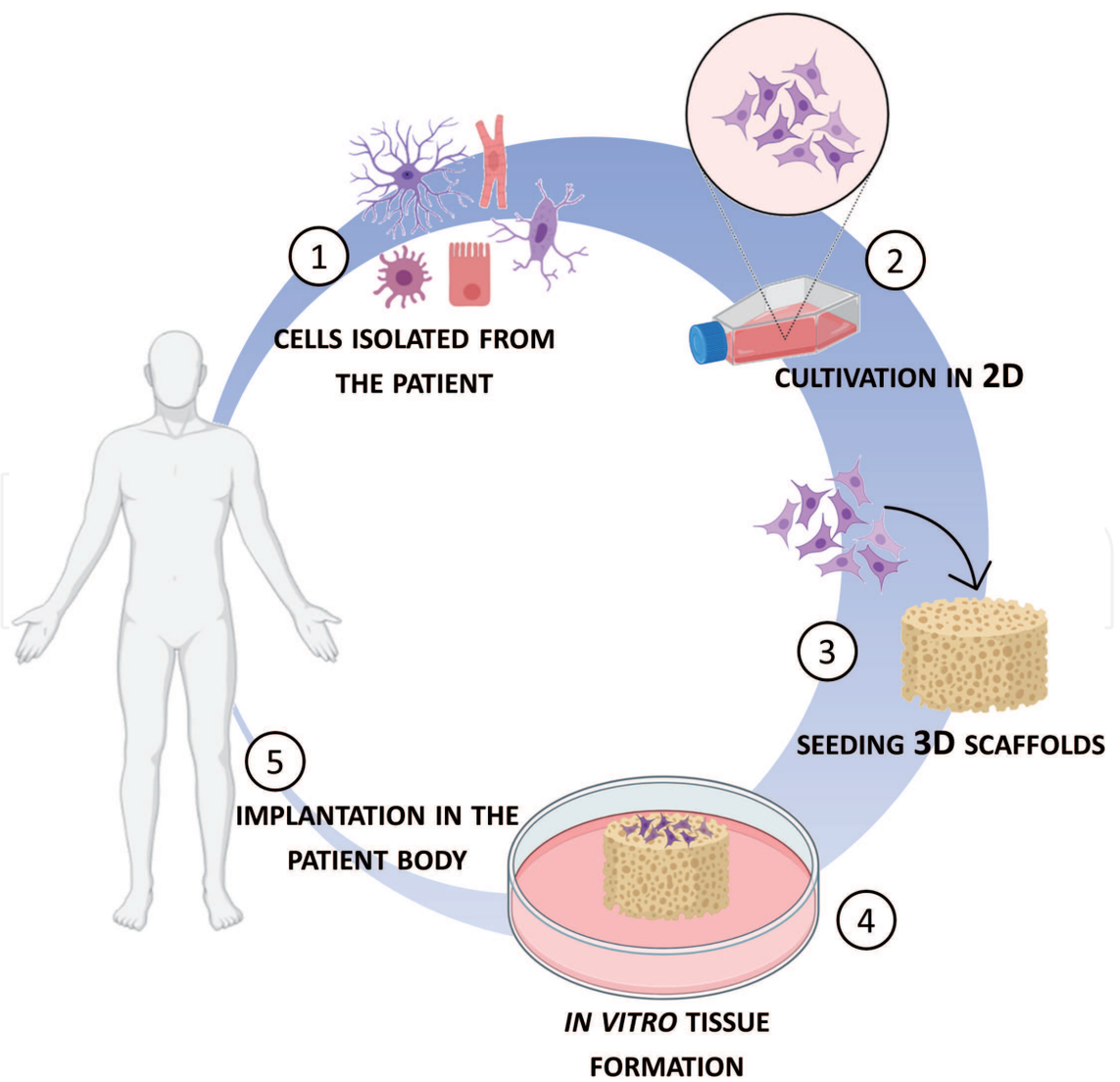

Figure 1.
Illustration of TE paradigm (figure created with BioRender.com).

cultivated in bioreactors, namely, devices able to apply biophysical stimuli to cells (e.g., mechanical or chemical) to better mimic the dynamic physiological conditions. In both approaches, the scaffold can be loaded with drugs, growth factors, micro- and/or nano-particles to further facilitate the recovering capabilities of tissues [5, 6].

The scaffold plays an essential role in regulating the process of new tissue formation. An ideal scaffold should be biocompatible and should degrade with kinetics compatible with the rate of tissue regeneration. It should be highly porous (< 75% [7]) with adequate pore size to promote cell migration/scaffold colonization and nutrient transfer throughout the scaffold. A scaffold should mimic the features of biological tissues in terms of topological properties (e.g., shape, size), mechanical properties (e.g., stiffness), and the biochemical processes that control and regulate the functionalities of the tissues. Moreover, it should not alter the normal functions of cells, which should adhere, migrate and proliferate within the scaffold before producing new tissue [5, 6, 8, 9]. Depending on their applications, scaffolds with different shapes, compositions and properties have been developed so far.

The biomaterial formulations used to produce the scaffold strongly affect its properties [10, 11]. Thus, the selection of the proper biomaterial formulation is pivotal for inducing the regeneration of the tissue in a controlled manner avoiding any undesired side-effects (e.g., cytotoxicity, apoptosis, carcinogenicity). The most used biomaterial formulations in TE are mainly based on synthetic biopolymers, natural biopolymers and composites [12, 13]. Synthetic biopolymers, like polycaprolactone, can be produced on a large scale under controlled conditions with predictable and reproducible physicochemical properties (e.g., mechanical properties, biodegradability) [6, 14, 15]. However, many synthetic biopolymers that have been developed so far are mainly derived from petroleum and coal, which make them not compatible with the environment [16]. Natural biopolymers include animal-derived proteins (e.g., gelatin, hyaluronic acid, collagen, silk) and animal- and vegetal-derived polysaccharides (e.g., cellulose alginate, chitosan). One of the advantages of this class of biopolymers is their biological similarity to native tissues which is beneficial for supporting cell functionalities (e.g., cell adhesion). Nonetheless, the use of animal-derived biopolymers may be associated with a high risk of transmission of diseases from animal to patient [10, 17, 18]. Therefore, the use of naturally occurring biopolymers from vegetal sources represents an attractive alternative to overcome these limitations. Moreover, they represent an ecological alternative to synthetic biopolymers in the preparation of sustainable and green scaffolds.

In recent years particular attention has been paid to the adoption of methodologies to derive biopolymers from renewable sources, such as industrial by-products, such as pectin from fruit pomace produced from the fruit processing industry [19] and cellulose nanofibers obtained from paper waste [20]. The application of more ecologically viable biomaterials in TE may, in fact, strongly contribute to reduce the polluting impact of producing and using un-recyclable synthetic biopolymers. Among the renewable and natural biopolymers, pectin is gaining particular attention in TE for its advantageous properties including biocompatibility, biodegradability and non-toxicity [21, 22]. In addition, the versatility in processing pectin-based formulations allows to produce scaffolds with diverse properties and for different applications (Section 2).

This chapter aims at highlighting the applications of pectin as the building block of bidimensional (2D) and three-dimensional (3D) scaffolds for TE applications. With this aim, in Section 2 the properties of pectin as biomaterial are provided. Section 3 reports the most representative applications of pectin-based formulations for producing scaffolds for tissue regeneration in the shape of 2D films for wound healing and 3D scaffolds for tissue regeneration.

2. Properties of pectin as a biomaterial for TE applications

Pectin shows several remarkable properties as a biomaterial. It is biocompatible and biodegradable, and it is soluble in cytocompatible and non-toxic solvents (such as water). Pectin is a versatile biomaterial as its physical properties can be facilely tuned due to the presence of several functional groups (e.g., carboxylic groups) that can serve as binding sites for other functional groups, biomolecules and drugs [21–23]. It is a low-cost biomaterial due to its ubiquity in nature, and this can strongly reduce the costs associated with the development of engineered tissues.

Pectin can form hydrogel due to the ability of its macromolecules to absorb and retain large volumes of water. This unique property makes pectin a suitable candidate to produce a natural extracellular matrix, which naturally surrounds cells. Furthermore, due to the possibility to be processed under sterile and physiological conditions (i.e., the aqueous environment at 37°C), pectin enables to encapsulate cells within its matrix to produce cell-laden scaffolds [23, 24].

Pectin tends to dissolve under physiological conditions, therefore physicochemical approaches are required to stabilize pectin-based scaffolds. These are mainly based on the use of physicochemical crosslinking approaches which consist of the formation of a stable network of links among the pectin molecules. This network reduces the interactions of pectin molecules with water and prevents the disruption of pectin-based scaffolds. For example, the most employed approach to form water-insoluble scaffolds of low-methoxyl pectin is based on the use of divalent cations (e.g., Ca^{2+}) that interact with the carboxylic groups of pectin forming the so-called 'egg box' structure [21]. Notably, the crosslinking treatments should also be cytocompatible (under specific conditions/concentrations), and should not interfere with the capability of pectin to encapsulate cells [25].

One of the major drawbacks that limit the application of pectin as a biomaterial for TE applications is its low cell adhesivity due to the lack of sites for cell adhesion (such as arg-gly-asp (RGD) sequences). Therefore, pectin is often combined/blended with other biopolymers or biomolecules to enhance its bioactivity [21, 26].

3. Applications of pectin in TE

Pectin-based formulations have been processed through different fabrication approaches into scaffolds with various shapes for different applications. In particular, pectin has been mainly used for the production of 2D films for wound healing, and 3D scaffolds for tissue regeneration. **Figure 2** provides a graphical overview of the main applications of pectin in TE.

3.1 2D patches for tissue regeneration

One of the applications of pectin-based formulations is the preparation of 2D hydrogel patches for the treatment of wounds. These patches provide mechanical support to cells during the process of new tissue formation, and an antibacterial barrier preventing eventual infections. Moreover, the hydrophilic pectin molecules in the film can react with the fluids of the wound forming a soft gel. The presence of a gel allows to maintain a moist environment in the wound. This helps to remove or control secretions from the wounded tissue and in turn facilitates the healing process. The regeneration of the damaged tissue can be further promoted by the incorporation of bioactive molecules such as drugs (e.g., antibiotics) and/or growth factors within the pectin patches [21]. The controlled and prolonged release

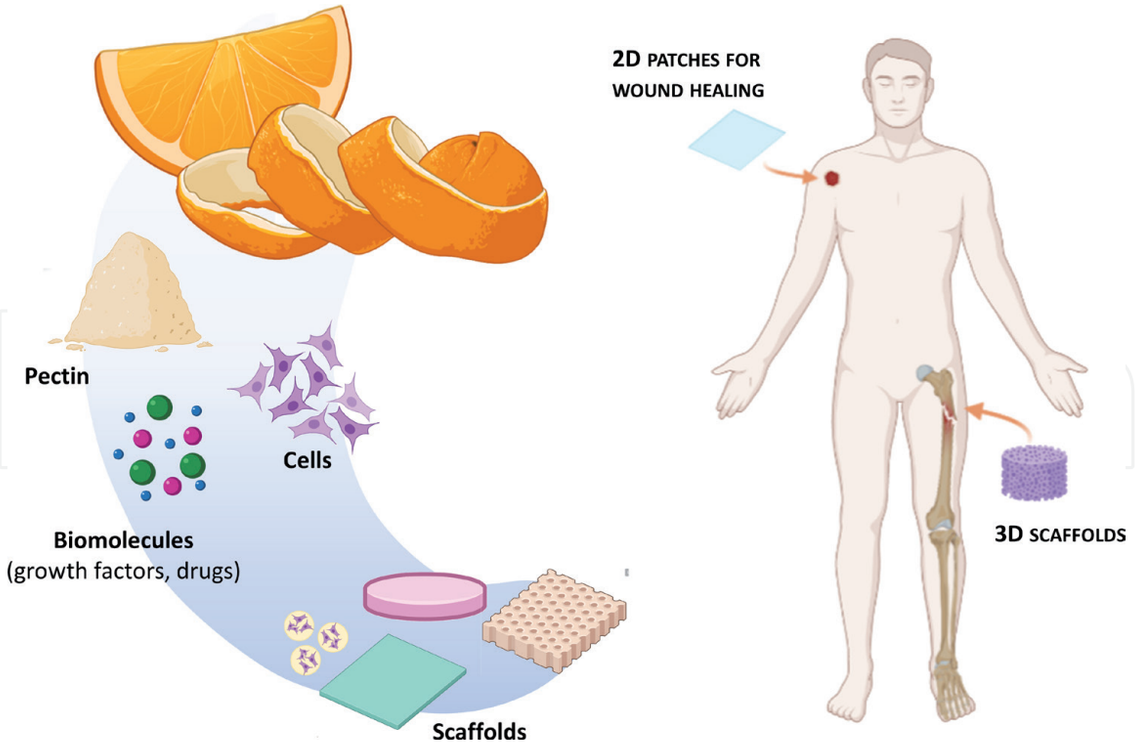

Figure 2.
Illustration of the application of pectin (derived from citrus fruits) for the production of scaffolds for TE applications (created with BioRender.com).

of these molecules directly in the damaged site can actively contribute to decreasing the risk of infections and accelerating the formation of new tissue. As mentioned in Section 2, pectin is often combined with other biopolymers to enhance its bioactivity and also to modulate the physical properties (e.g., tensile strength) of the final patch.

Pectin-based patches for wound healing reported in the literature so far are principally obtained in the shape of non-porous films and porous membranes, as detailed described in the following Sections 3.1.1 and 3.1.2, respectively.

3.1.1 Pectin-based films

Pectin-based films are generally 2D, non-porous and flexible substrates able to retain large volumes of water within their matrix. One of the approaches used to produce these films is the so-called 'solvent casting'. In this approach, a pectin-based solution is initially poured into a mold, and the solvent is subsequently let to evaporate leaving a 2D non-porous film (**Figure 3**).

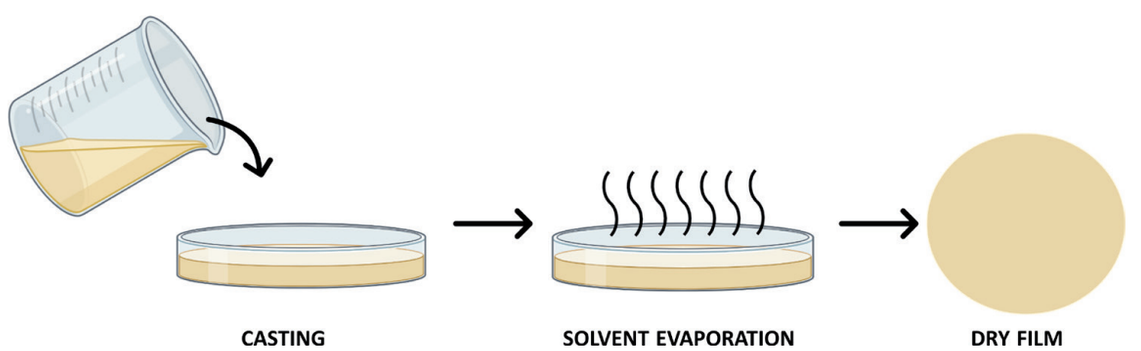

Figure 3.
Illustration of the solvent casting approach (created with BioRender.com).

Pectin-based patches produced with this approach support cell adhesion and proliferation and accelerate the processes occurring during the formation of new tissue [27–30]. Moreover, films with high toughness and stretchability can be produced with solvent casting, and these can be potentially used as pectin-based patches for load-bearing tissues (e.g., cartilage, tendon) [28]. In addition, pectin-based patches for a controlled drug into the targeted tissue were also produced by incorporating drugs in the pectin matrix [30, 31].

3.1.2 Pectin nanoporous membranes

Nanoporous membranes based on pectin have been mainly obtained through electrospinning. This approach allows to produce highly porous and flexible patches starting from pectin-based/polymer solutions subjected to an external electric field. A standard electrospinning apparatus is illustrated in **Figure 4**. It generally consists of (i) a syringe pump containing the polymer solution, (ii) a metallic needle through which the polymer solution is ejected, (iii) a high voltage power supply (in the range of tens of kVolts), and (iv) a grounded collector (usually a metal plate). When a drop of the polymer solution is extruded through the needle, the high electric forces in the space between the needle and the collector induce its stretching and the formation of fibers from a few nanometers to microns in diameters [32]. These fibers are therefore deposited and collected on the collector forming a non-woven fibrous membrane after complete evaporation of the solvent (**Figure 4**).

Pectin-based patches obtained by this approach show several advantageous properties for TE applications. The random organization of electrospun pectin fibers together with the hydrogel nature of pectin enables to mimic the nanoscale organization of the native extracellular matrix. Furthermore, the high porosity and high surface-to-volume ratio typical of electrospun patches promote cell migration and nutrient diffusion within the scaffold, which is beneficial for the process of new tissue formation [33]. Nevertheless, it is quite challenging to produce electrospun structures from pristine pectin due to some intrinsic molecular properties of pectin (such as insufficient chain entanglement) that disable the fiber formation [34]. Thus, to improve its electrospinning ability, pectin is often chemically modified [35, 36] and/or combined with other biodegradable biopolymers such as poly(ethylene oxide) [34], polyhydroxybutyrate [37] that work as carrier polymer to induce the formation of stable fibers.

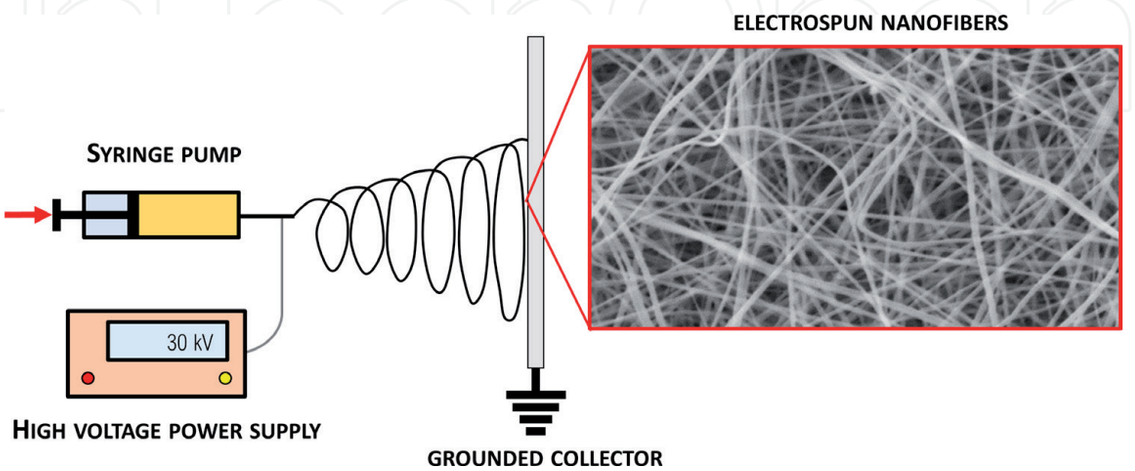

Figure 4.
Illustration of an electrospinning setup with a magnification of the electrospun nanofibers on the collector (image obtained with scanning electron microscopy).

Pectin-based nano-fibers find application for the preparation of films/structures that can be potentially used as patches for wound healing of soft tissues [35–37] (e.g., vascular tissue [35], retinal tissue [37]). In addition, drugs (such as antibiotics [38, 39]) and particles (such as argentum ions for antibacterial purposes [38]) can be successfully loaded in these structures obtaining patches for a local and controlled release of drugs directly into the wound.

3.2 3D pectin scaffolds

Pectin-based formulations can be further processed to obtain 3D scaffolds able to mimic the complex architecture of biological tissues. 3D pectin-based scaffolds have been principally obtained in the shape of porous 3D sponges and 3D bio-printed scaffolds.

3.2.1 Pectin-based sponges

Sponges are comparable to foams with an interconnected network of pores. This type of architecture is beneficial for cell penetration and scaffold colonization, while ensuring adequate diffusion of nutrients to cells within the scaffold. Moreover, a highly porous scaffold with open and connected pores is of critical importance as it allows for the diffusion of nutrients and waste products through the scaffold [6, 7].

Pectin-based sponges are mainly obtained by freeze-drying, also known as lyophilization. This technique consists in freezing a polymer solution followed by the evaporation of the frozen solvent by sublimation. Thus, a solid polymer matrix with numerous and interconnected pores is obtained (**Figure 5**). Before freezing, polymer solutions are generally poured into molds to produce porous scaffolds with the desired shape.

Pectin-based sponges have been principally used to produce scaffolds for wound healing and tissue regeneration. For example, sponges obtained with pectin-based formulations have been used as scaffolds for different types of tissues including cartilage [40, 41], skin [42], and bone [43]. The high hydrophilicity of pectin molecules and the interconnected porosity enables these sponges to entrap a large volume of water creating a 3D hydrogel-based environment that can mimic the natural extracellular matrix [40, 41]. Furthermore, this provides and stabilizes a moist environment for wounds that strongly contributes to accelerating the healing of the wounds [44].

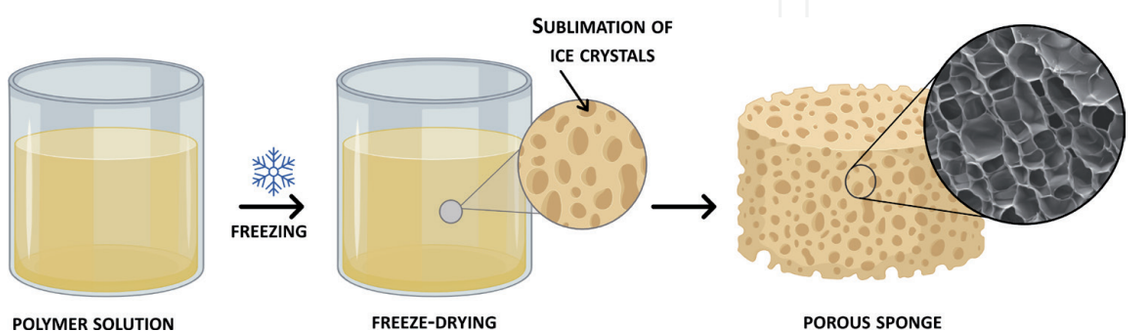

Figure 5.
Schematic of the process for obtaining cylindrical porous sponges was obtained by freeze-drying. Magnification of the porous sponges obtained by scanning electron microscopy (image created with BioRender.com).

3.2.2 Complex shaped pectin-based scaffolds

Producing scaffolds with a customized architecture and by automated and high reproducible approaches is one of the main challenges of TE. The development of pectin-based scaffolds with patient-specific architecture may boost their clinical applications.

Pectin-based scaffolds with complex shapes have been principally obtained by extrusion-based bioprinting so far. Extrusion-based bioprinting is one of the most widely used technology in TE due to its simplicity and versatility in processing a large variety of biomaterials, cells and biomolecules. An extrusion-based bioprinter usually consists of a movable cartridge containing the biomaterial formulation (called '*ink*') and of a movable deposition stage (**Figure 6**). Before bioprinting, the architecture of the scaffolds can be designed by a computer-aided design (CAD) software, or it can be derived from patient medical images acquired, for example, by computed tomography scans or magnetic resonance imaging. The 3D model of the scaffold is subsequently sliced by a computer-aided manufacturing (CAM) software in bioprinting paths and finally converted to a printable code file (called '*G-code*') [45, 46]. During the bioprinting process, the ink is extruded onto the deposition stage following the preprogrammed paths contained in the G-code, in a layer-by-layer process.

The application of pectin-based inks in extrusion-based bioprinting is relatively recent compared to the other fabrication approaches described in the previous sections. Pectin solutions are often not suitable to be processed through extrusion-based bioprinting and structures with poor shape fidelity are often obtained. The first application of pectin as ink for extrusion-based bioprinting dates back to 2017. In this case, pectin was combined with another biopolymer (Pluronic F-127), and complex-shaped scaffolds were bioprinted [47, 48]. Cells were successfully loaded within this formulation and 3D bioprinted to produce living 3D constructs [24]. From that moment, other pectin-based inks have been developed and optimized to produce 3D scaffolds with high shape fidelity [49–51]. For example, pectin-based scaffolds with more complex shapes such as a human ear and nose shape for cartilage tissue regeneration were successfully obtained (**Figure 6**) [41].

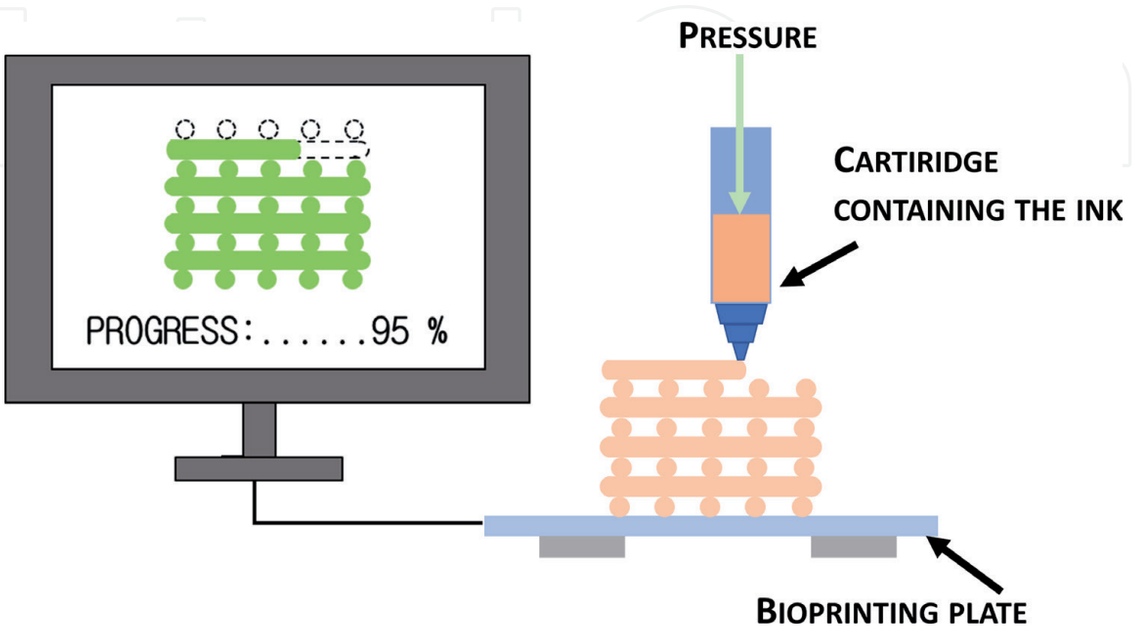

Figure 6.
Schematic of extrusion-based bioprinting.

4. Conclusions

TE represents an alternative approach to conventional surgical techniques used to treat damaged, injured or diseased tissues or organs. This approach is based on the use of tissue-mimicking and biodegradable constructs, based on the so-called 'scaffolds', able to restore, maintain or improve tissue functions. The physicochemical properties of the final scaffold play a key role in the process of new tissue formation. The selection of the proper biomaterial formulation is therefore essential. Recently, renewable biomaterials derived from industrial by-products are finding increasing application in TE as an alternative to petroleum-derived and unrecyclable polymers. In this regard, pectin, a polysaccharide commercially derived from citrus peel and apple pomace (both by-products of the food processing industry), is gaining attention in TE due to its biocompatibility, biodegradability and non-cytotoxicity. Diverse pectin-based formulations have been developed and employed for the fabrication of functional scaffolds for TE applications.

This chapter presented the most representative applications of pectin-based formulations for the fabrication of scaffolds for TE applications. In particular, by properly processing these formulations through specific fabrication techniques is possible to produce pectin-based scaffolds with different features: from 2D non-porous films (obtained by solvent casting) to 3D scaffolds with patient-specific shape (obtained by extrusion-based bioprinting). Although pectin shows diverse advantageous properties as biomaterial, its application in clinical practice is still under investigation. The increasing number of studies on the preparation of biocompatible pectin-based formulations may strongly boost the employment of this polysaccharide in the fabrication of sustainable scaffolds for future TE applications.

Acknowledgements

The authors wish to acknowledge the Crosslab Additive Manufacturing of the Department of Information Engineering of the University of Pisa.

Conflict of interest

The authors declare no conflict of interest.

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