Scaffolds for Tissue Engineering: A State-of-the-Art Review Concerning Types, Properties, Materials, Processing, and Characterization

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Abstract Given the constant lack of donors for organ transplantation, tissue engineering has been considered a very important tool for regenerative medicine to overcome the limitations of conventional treatments. Tissue engineering is mainly based on obtaining biodegradable three-dimensional (3D) scaffolds. Based on a bibliometric study covering the last three decades of scientific research in scaffolds, this review will address the existing types of scaffolds (solid and fluid); the necessary scaffold properties for adequate tissue regeneration, such as biocompatibility and adequate mechanical properties; the materials that can be used to manufacture the scaffold, from metals to natural and synthetic polymers; scaffold fabrication techniques, considering their advantages and disadvantages and which are the main selection criteria; and finally, the methods of scaffold characterization, such as chemical, morphological, mechanical, and biological.

Keywords Scaffold · Tissue engineering · Biomaterial · Biopolymer Biodegradable · Tissue regeneration

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Introduction

Tissue engineering (TE) is a relatively new research line within the field of regenerative medicine, which has the aim of restoring, keeping, or improving the function of a tissue or group of organs through a specific combination of cells, scaffolds, and bioactive factors, such as growth factors and cytokines [1, 2]. The main goal of TE is to overcome the limitations of conventional treatments based on organ transplants.

Currently, the major obstacle for the clinical transplant of organs is the lack of donors. Based on OPTN (Organ Procurement and Transplantation Network) data, in 2018, only in the United States of America, 36,529 people received organ transplants, while more than 113,000 are still on the waiting list.

Furthermore, the increase of life expectancy and the malfunction and/or loss of tissue caused by injury or diseases have led to the reduction of the quality of life for many patients and an increase in socioeconomic costs associated with improving health around the world. In this context, TE has become a promising and important research field, once it can offer viable and less invasive alternatives for the repair and regeneration of tissues and damaged organs.

TE is based on obtaining three-dimensional (3D) biodegradable scaffolds where specific cells can proliferate and differentiate in a structure similar to tissues or organs.

Scaffolds are temporary 3D matrices that work as an extracellular matrix, organizing cells three-dimensionally and stimulating the growth and formation of the desired tissue. Besides allowing for the adherence and migration of cells inside the scaffold and promoting cell proliferation and differentiation, the scaffold must provide an environment where the cells can keep their phenotype and synthesize proteins and/or other necessary biomolecules. A scaffold must yet promote vascularization and nutrient migration, and possess degradation rates and mechanical properties suitable to support new tissue formation [1]. The scaffolds can also work as carriers of cells, growth factors, and/or other bioactive molecules [3].

In regenerative medicine, scaffolds represent the conductive capacity inside the cell proliferation triangle—which also includes undifferentiated cells and growth factors or other bioactive molecules (Fig. 1)—and can be used to carry cells before

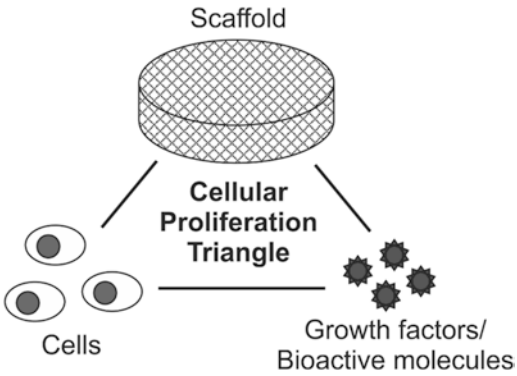

Fig. 1 Cell proliferation triangle

their in vivo implantation, or work only as a bioactive material attracting cells on the tissue where they are implanted [2, 4].

Scaffolds from different materials, manufactured by different technologies, have been used for hard and soft tissues regeneration, such as bones, cartilage, tendons, ligaments, skin, blood vessels, and muscles [5].

Many 3D matrices have been used as scaffolds to promote the proliferation and differentiation of various progenitor cells, including adult mesenchymal stem cells. However, most of these matrices do not provide a suitable biological environment so that cells can proliferate and differentiate in the same way as in the in vivo systems.

Therefore, the development of scaffolds that specifically address cell culture and can mimic the extracellular matrix and be mechanically stable, biocompatible, and biodegradable, still represents a big challenge to TE. In this review, a bibliometric analysis of the last 30 years was done to summarize the current state of the art in TE scaffold design, manufacturing, and use, as well as the advances made to overcome limitations of traditional techniques. Posteriorly, the main topics related to TE and scaffolds are extensively reviewed.

Methods

Database and Search Strategy

The data for this study were collected from the SciVerse Scopus database on May 17, 2019. The search term selected was "scaffold AND technique." The duration of this study was set from 1990 to 2019. The types of documents were limited to "articles" and "reviews." Documents within the subject areas of "arts and humanities," "social sciences," "psychology," "business, management and accounting," "decision sciences," and "economics, econometrics and finance" were excluded.

Bibliometric Mapping

To verify the trends in this research field over time, data downloaded from Scopus were imported into VOSViewer software (Leiden University, Leiden, Netherlands). This software can be used to create networks based on keywords extracted from publications [6]. A minimum of two occurrences was set to filter the keywords and the most relevant ones were extracted by the VOSViewer built-in mining text function [6]. All of the terms extracted and presented by the software were filtered manually to 30 relevant keywords (see Appendix), chosen to represent the scaffolds types, materials, properties, and fabrication techniques, and are reviewed and discussed throughout this work. These scaffold-related terms were then selected to

generate the co-occurrence map. This map represents the frequency of occurrence of each keyword in the retrieved documents using the size of the circles and portrays its co-occurrences through colors (clusters). For the keyword analysis, the maps were divided in three parts, each one representing 10 years from the last three decades, to study the historical developments on scaffolds for TE.

Results

The total retrieved publications, using the described search methodology, were 19,934 studies from 1990 to 2019. The growth in scientific studies involving scaffold techniques over the years was verified through the annual publications obtained on Scopus until 2018 (Fig. 2). The results show an increase in publications over the last decade which was most expressive, consisting of 71.6% (13,426 papers) of all publications, although there was a decrease in publications in 2018, probably because of the emphasis on commercialization, considering that, on 2018 only at the United States, there was 49 public companies operating in TE and regenerative medicine sector, undergoing clinical trials or commercial stages [7].

Three network maps were created (Fig. 3), each one representing 10 years of scientific production related to scaffolds over the last three decades: from 1990 to 1999 (Fig. 3a), from 2000 to 2009 (Fig. 3b), and from 2010 to 2019 (Fig. 3c).

Comparing the three scientific landscapes, until 1999, the terms “scaffolds” and “tissue engineering” were new and beginning to show some occurrence and links with different applications (for example, cartilage, bone, and nerve regeneration) and with important scaffold properties (for example, biodegradation, mor-

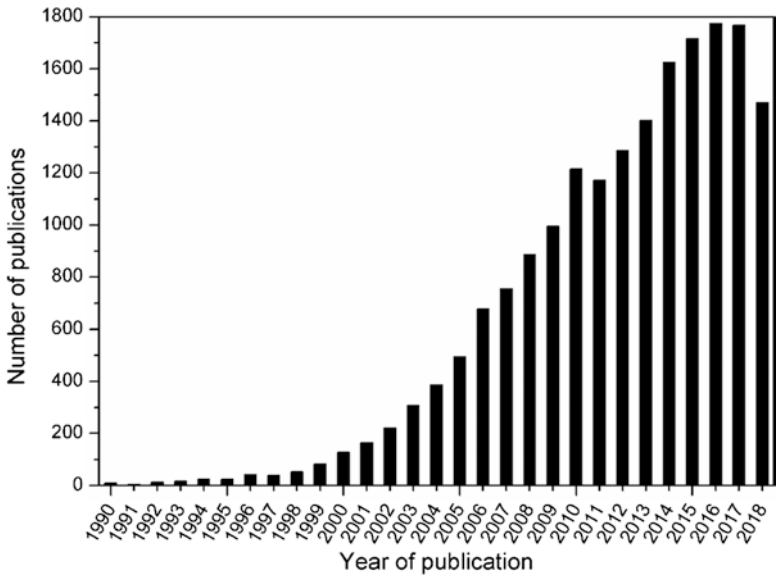

Fig. 2 Annual growth of publications in TE scaffolds (1990–2018)

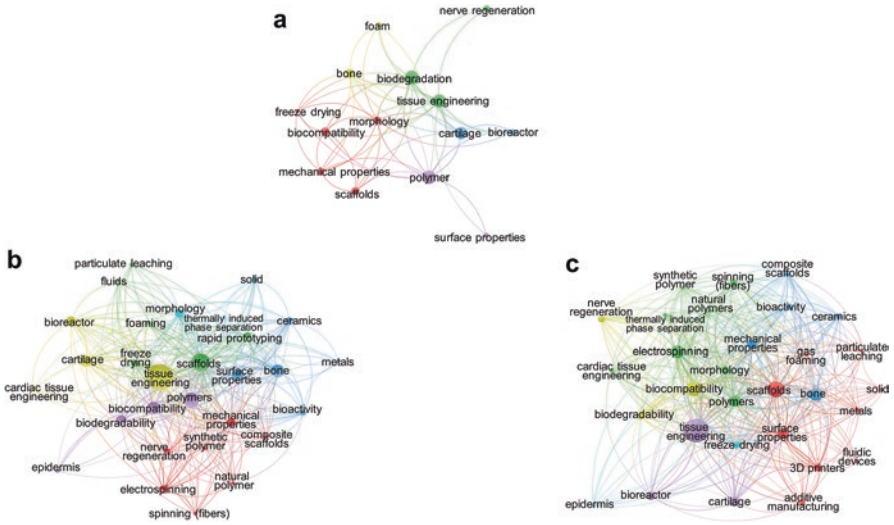

Fig. 3 Network visualization maps of keywords in scaffolds from the last three decades. (**a**) 1990–1999; (**b**) 2000–2009; (**c**) 2010–2019

phology, biocompatibility, mechanical properties, and surface property). However, only polymers, as materials, and a few fabrication techniques, such as freeze-drying, foaming, and bioreactors, significantly appeared during this period (Fig. 3a).

From 2000 to 2009, this scenario changed (Fig. 3b), with an increase of 9000% occurrences of the term "scaffolds" when compared to the previous decade, and with more links with many clusters. In this period, the studies gained force for many previous applications (for example, bone, cartilage, and nerve regeneration) and new applications (for example, cardiac and epidermis). In addition to the properties already studied, bioactivity also began to be explored. Besides that, scaffolds were now being produced with numerous different materials (natural and synthetic polymers, ceramics, metals, and composites) and fabrication techniques (electrospinning, particulate leaching, rapid prototyping, spinning, and thermally induced phase separation). The material with more occurrences remained to be polymers and the most cited techniques in this period were electrospinning, rapid prototyping, and freeze-drying.

Furthermore, on the last decade, the network map (Fig. 3c) shows a higher density of links between all the clusters. All of the applications, materials, techniques, and properties of scaffolds remained the same as the previous decade, but the occurrence of the terms increased considerably. The term "rapid prototyping" begin to change to "additive manufacturing" or "3D printing." Electrospinning became the technique with more occurrences, followed by additive manufacturing (or 3D printing or rapid prototyping). Although polymers still occurred more than other materials, a growth was observed for metals, ceramics, and composites. Moreover, a tendency in the evaluation of the biocompatibility and mechanical properties of scaffolds was observed during this time period.

Discussion and Literature Review

Scaffolds and tissue engineering are relatively new terms to the scientific community and started to appear in the early 1990s. As can be seen from the annual growth of publications graph, only a few works about scaffolds were published to 1999 and then, the research on this field began to grow exponentially (Fig. 2). The same can be verified on the network visualization maps of each decade (Fig. 3), in which the terms related to scaffolds increased as well as the occurrences and the amount of links between them. The terms presented on the network maps will be fully discussed on the following sections, separated into types, materials, properties, fabrication techniques, and characterization of scaffolds.

Types of Scaffolds

Scaffolds are divided into solids and fluids (injectable) and can be manufactured into several shapes (Fig. 4), as sponges, hydrogels, fibers, membranes, micro- and nanoparticles, tubes, and spheres [8–14],which depends on their desired application and the fabrication process used.

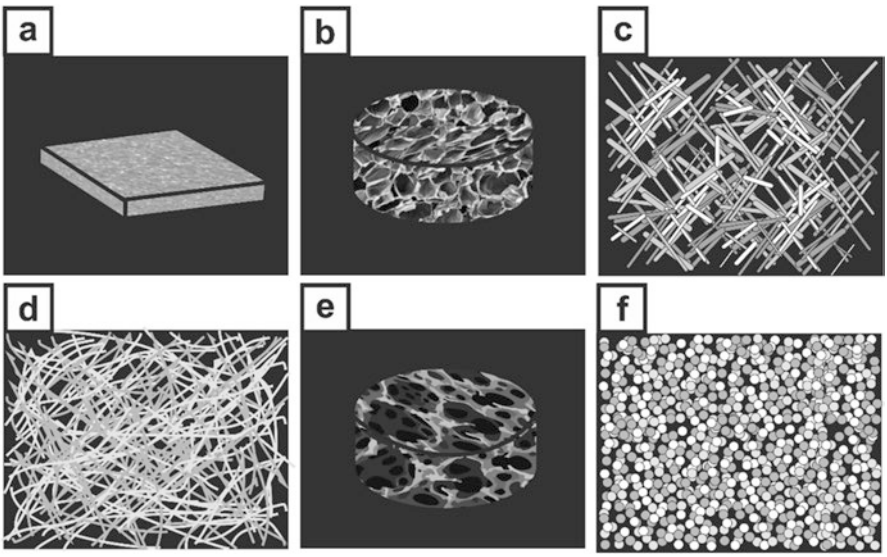

Fig. 4 Illustrative figure of different scaffold types: (**a**) membranes; (**b**) sponge; (**c**) tubes; (**d**) fibers; (**e**) foam; and (**f**) microparticles

Solid Scaffolds

In TE, solid scaffolds include sponges, foams, fibers, membranes, and tubes. These scaffolds present a stable and well-defined 3D porous structure. However, their application for the regeneration of different tissues is limited to morphology, pore dimensions along the structure, and mechanical properties of the scaffolds.

The materials used to fabricate these scaffolds must be able to create structures that will not collapse under the conditions of in vitro cell cultivation (inside an aqueous environment) or when implanted in vivo.

Although these scaffolds can be manufactured with a high control of its architecture, suitable nutrient transportation and effective adherence and cellular migration, the main disadvantage of conventional scaffolds is the need of surgical intervention (or high invasiveness) for their implantation.

Solid scaffolds can be used for various applications, especially those requiring a structural base capable of supporting their in vivo application, such as for the regeneration of bones, muscles, ligaments, and other tissues and organs [15, 16].

Fluid Scaffolds

Fluid scaffolds, in the hydrogel form, have been considered promising in the drug delivery area, as well as in TE, mostly due to their minimally invasive application [17]. Fluid scaffolds are usually flat hydrogels, micro- or nanoparticle hydrogels, or are formed by spheres.

From the clinical point of view, using fluid scaffolds is very interesting, because it minimizes patient discomfort, risk of infection, formation of scars, and treatment cost [16].

The fluid material can homogeneously fill the defect or the point of repair, incorporate many therapeutic agents and does not demand highly invasive surgical procedures for implantation. In addition, the high hydration of the hydrogels mimics the extracellular matrix, consequently being ideal for cell proliferation and differentiation.

Hydrogel provides an initial structural support that retains the cells on the damaged area for cellular growth and the synthesis of a new extracellular matrix, and is easily degradable when cells secrete the extracellular matrix. This strategy allows for cell transplantation and the combination with hydrogels with growth factors in a minimally invasive way.

Usually, the cells are isolated through a small biopsy, expanded in vitro, and encapsulated in the hydrogel precursors, for in situ solidification, or in the hydrogel already formed. Subsequently, these materials are transplanted to the patient by injection, using appropriate needles.

Fluid scaffolds have been widely used, mainly in wound healing, treatment of cartilage lesions, regeneration of soft tissues and in drug delivery [18].

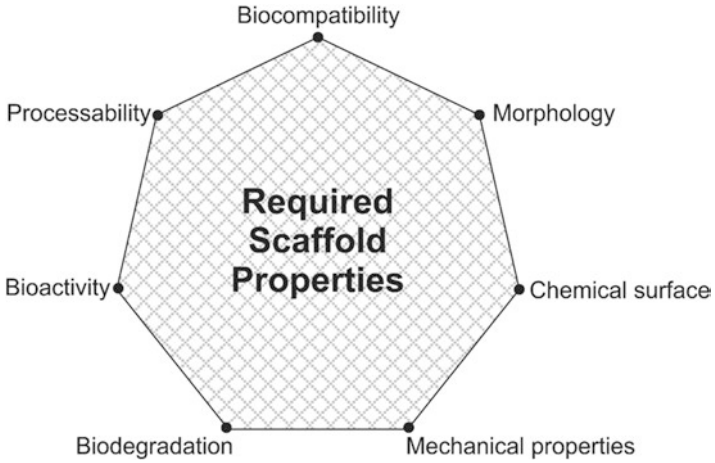

Fig. 5 Required scaffold properties

Required Scaffold Properties

Since the main function of scaffolds is to provide a suitable temporary support that allows for the cellular processes necessary for tissue regeneration, many requirements depending on the cell type and the tissue to be regenerated must be considered in the development of TE scaffolds. Among these requirements, the following can be highlighted (Fig. 5). The detailed description of each property is presented in Table 1.

Materials

Material selection for scaffold production is one of the most important steps in TE. Several different materials have been proposed for their manufacturing, among them metals, ceramic materials, natural and/or synthetic polymers, and composites. One of the main motivations for the study of biodegradable materials is the fact that these materials present some degree of degradation when exposed to physiological environments. This behavior has the advantages of the absence of a new surgical procedure for the removal of implanted materials [22].

Inorganic materials have still been used mostly for the production of scaffolds for bone TE and other mineralized tissues [23]. Currently, the main metallic materials used as scaffolds for implants are stainless steel and cobalt and titanium alloys. Among the ceramic materials, alumina, zirconia, hydroxyapatite (HA), calcium phosphate, and bioglass can be highlighted. However, these are not biodegradable and their processability is very limited. Besides that, their application is invasive, requiring surgical intervention.

Table 1 Detailed description of each scaffold's properties

Scaffold desirable properties	Description
Biocompatibility	Scaffolds must be biocompatible and demonstrate satisfactory performance in order to produce adequate response to the host tissue without producing cytotoxic or immune response. No by-product of its degradation can cause inflammatory or toxic reactions
Biodegradability	Scaffolds must have degradation rates compatible with the new tissue formation. The degradation of the scaffolds may occur by mechanisms involving physical (dissolution) or chemical (hydrolysis) processes and/or biological processes, such as enzymatic cleavage
Pore morphology	Scaffolds should exhibit high porosity with cell–scaffold interactions, in order to control the adequate diffusion of nutrients and oxygen to cells, metabolite dispersal, local pH stability, and cell signaling
Pore size	Pore size is an important feature due to cell penetration and tissue vascularization. The scaffolds must satisfy the condition of providing an empty volume of pores; according to Liu and Ma (2004), scaffolds with high porosity (>90%) allow the effective release of bioactive molecules and are appropriate substrates for nutrient exchange [19]
Chemicalproperties	A scaffold surface can control the effect of cell adhesion and proliferation, due to being the primary site of interaction between the cell and scaffold. The scaffold surface must present properties that allow cellular adhesion and promote proliferation and differentiation. These properties primarily comprise the chemical composition of the material surface with suitable functional groups, which influence its hydrophobicity and charge [20]
Mechanical resistance	Scaffolds must have adequate mechanical properties for manipulation in vitro and in vivo. Scaffolds directed to the regeneration of hard tissues must have a compression modulus around 10–1500 MPa, while scaffolds directed to soft tissues must have a modulus around 0.4–350 MPa [21]
Bioactivity	Scaffolds can be used as carriers or reservoirs for bioactive and/or signaling molecules that can accelerate tissue regeneration
Processability	In order to become clinically and commercially viable, scaffolds must be easily processed in a variety of shapes and sizes and present low fabrication costs. Besides, the fabrication process must be reproducible and scalable, and easily sterilizable and storable

Magnesium and its alloys represent promising solutions in the field of biodegradable metal biomaterials and are being widely investigated for orthopedic applications [24]. From the physiological point of view, magnesium is an essential mineral for human nutrition and crucial for bone health [25]. Furthermore, the mechanical properties of magnesium alloys are similar to those found in human bone (elastic modulus 40 GPa) [22].

Although magnesium alloys have potential for biomedical applications, their processing is extremely challenging. Magnesium has a low boiling point, is combustible in the form of billets or plates and has an increased risk of explosion due to the increased surface area when presented as a powder.

Therefore, over the last few years, these non-degradable materials have been replaced by a variety of natural and/or synthetic polymeric materials manufactured with different microstructures, which mainly include hydrogels, porous matrices, and fibrous matrices [19, 26].

Synthetic polymers have the advantage of presenting a reproducible production process under large scales and higher control of mechanical properties, degradation rate, and microstructure.

Thus, biodegradable synthetic polymers including linear aliphatic polyesters, polyglycolic acid (PGA), polylactic acid (PLA), and polylactic-*co*-glycolic acid (PLGA) copolymers have been widely used as vehicles for cell transplantation and scaffolds for the engineering of different tissues mainly due to its relatively hydrophilic nature [27].

Beyond that, these polyesters have in vitro and in vivo controllable degradation rates and are among the synthetic polymers approved by the Food and Drug Administration (FDA) for certain human clinical applications.

Other linear aliphatic polyesters such as poly(ε-caprolactone) (PCL) have also been investigated in TE mostly for long-term implants because of its significantly slower degradation rate than PLA, PGA, and PLGA [28].

Polyethylene glycol (PEG) has been used as fluid scaffolds in the form of hydrogels. Nevertheless, the toxicity and the low degradability of this material limit its application considerably, requiring prior modification.

Over the past few years, polyurethanes (PU) have also been widely used mainly because of the easiness of controlling its mechanical and morphological properties [29].

Despite the advantages of biodegradable synthetic polymers, natural polymers have been considered attractive materials for scaffold manufacturing, due to their similarities with the extracellular matrix, chemical versatility, biological performance, and specific cellular interactions.

Besides, they are susceptible to the enzymes of the organism, being inherently biodegradable. However, they are often immunogenic, may contain pathological impurities, have laborious manipulation and/or processing and exhibit variability from batch to batch. The most commonly used natural polymers in TE include fibrin, collagen, gelatin, chitosan, alginate, and hyaluronic acid.

Fibrin, one of the major constituents of blood clots, has been used in mixtures with thrombin to produce fluid scaffolds composed of fibrin gels (mesh) [30]. Since it is an autologous product, fibrin is completely biocompatible, thus it has desirable non-immunogenic responses. Furthermore, it is completely biodegradable and can be applied in the injured site using a non-invasive procedure. Fibrin scaffolds can be formed in situ or used as cell carriers associated to scaffolds of other materials. However, inadequate mechanical properties limit its application in TE considerably, especially in hard tissues [31].

Type I collagen extracted from animal tissues and gelatin prepared from collagen denaturation have been widely used as scaffolds for the regeneration of various tissues, especially soft tissues. However, these biomaterials can potentially transmit

pathogens and immunological reactions, also showing handling difficulties and inadequate mechanical properties [31].

Silk fibroin has also been used for porous scaffold manufacturing mainly because of its excellent mechanical properties. However, its degradation rate is considerably slow and there is some concern about its cytotoxicity [28].

Properties such as biodegradability, biocompatibility, adhesiveness, foldability in different forms, and chemical modification versatility make chitosan (a cationic derivative from chitin) a promising biomaterial for several applications in TE [32]. In addition to these properties, chitosan can form hydrogels in situ and carry growth factors and adhesion proteins.

Alginate crosslinked with Ca^{2+} has been used in TE as a cell carrier in vivo and as fluid scaffolds, in the form of particulate hydrogels. Nevertheless, using $CaSO_4$ to prepare these hydrogels hinders the control of the gelation process, resulting in non-uniform structures that directly affect the cellular response.

The functions and applications of hyaluronic acid in TE are basically associated to its structural characteristics and possible chemical modifications of the polymer, which determine its rheological, solubility, hydration and specific cell recognition properties. Hyaluronic acid is non-immunogenic, biocompatible, and biodegradable; however, its application during scaffold preparation requires prior modification of the polymer, since the native hyaluronic acid has limited mechanical properties and low residence time in vivo. Considering that it can be obtained in different forms, solid or fluid scaffolds, hyaluronic acid has been successfully used for the regeneration of hard and soft tissues.

Over the last few years, in order to have better control of the biodegradability and mainly to improve the mechanical properties of scaffolds, efforts in TE have been directed to obtain composite scaffolds that can mimic in vivo systems.

Beyond crosslinking, chemical modification, addition of additives and reinforcing agents (fibers and particles), several studies have been combining biocompatible polymers, which have limited mechanical properties, with different inorganic materials. The addition of these materials, especially ceramics, can improve the mechanical properties of the new scaffold, provide essential osteoconductivity for the regeneration of tissues and enable the mineralization of bone tissues.

Biodegradable polymers have also been combined with bioactive molecules to improve biological properties of new materials, accelerate cellular processes involved in tissue regeneration, as well as promote specific cellular recognition. Some studies have also associated platelet-rich plasma with natural and synthetic polymers, aimed to improve the properties of the fibrin network and to enable a controlled release of growth factors and cytokines, which accelerate regeneration and healing of tissues [33–35].

Therefore, the material choice for scaffold production must consider the advantages and disadvantages of these materials, as well as the intended application, and it is still a major challenge in TE.

Fabrication Techniques

Several technologies have been used to fabricate different types of scaffolds (Fig. 6), among those which stand out are: solvent casting/particulate leaching, gas foaming, freeze-drying, thermally induced phase separation, electrospinning, rotary jet spinning, rapid prototyping, and bioreactors [36–41].

Despite advances in TE, scaffold manufacturing processes are still limited. Conventional technologies usually depend on time-consuming, inconsistent, inflexible and laborious manual processes, which use toxic organic solvents, porogenic materials with difficult size control and removal of pores and have format limitations [42].

Therefore, the chosen processing technique must generally comply with the following criteria:

- The process or production shall not affect material properties, such as its biocompatibility or physicochemical properties.
- The technique must allow for a control of porosity, size, distribution, and interconnectivity of pores.
- Different groups of matrices must exhibit minimal variations in their properties, when processed under the same conditions.

The main features that distinguish many selected technologies are the desired application and/or the use of solvents, heat, pressure, or additives responsible for pore generation (Table 2) [3].

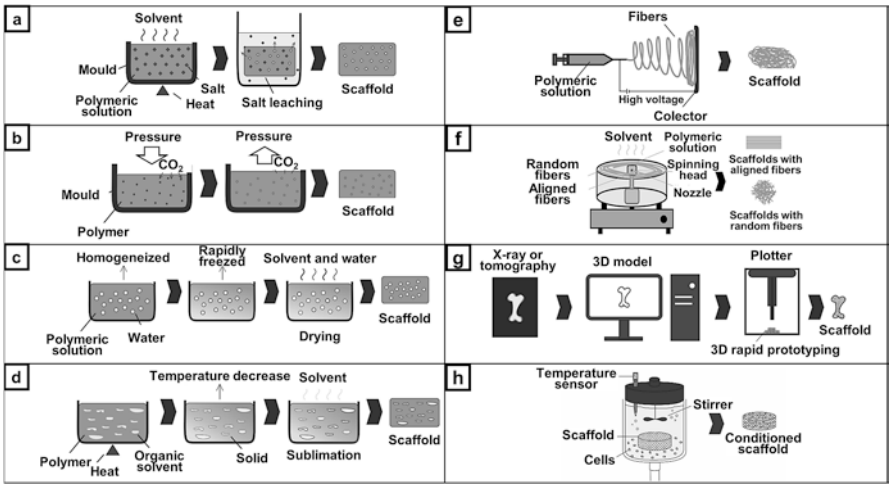

Fig. 6 Schematic illustration of the fabrication process of scaffolds: (**a**) solvent casting/particulate leaching; (**b**) gas foaming; (**c**) emulsion freeze-drying; (**d**) thermally induced phase separation; (**e**) electrospinning; (**f**) rotary spinning; (**g**) 3D printing; and (**h**) bioreactor

Table 2 Parameters of scaffold fabrication processes

Fabrication method	Heat	Solvent	Pressure	Electric field	Productivity	Process control	Porosity	Pore interconnectivity
Solvent casting/particulate leaching	Yes	Yes	No	No	Low	Yes	High	Low
Gas foaming	No	No	Yes	No	Low	Yes	High	Low
Emulsion freeze-drying	No	Yes	Yes	No	Low	Yes	High	Low
Thermally induced phase separation	Yes	Yes	No	No	Low	Yes	High	Low
Electrospinning	No	Yes	No	Yes	Low	Yes	High	High
Rotary jet spinning	No	Yes	No	No	High	Yes	High	High
Additive manufacturing	No	No	No	No	High	Yes	High	High

Solvent Casting/Particulate Leaching

This method involves mixing particles of a water-soluble salt (sodium chloride, sodium citrate) in the solution of a biodegradable polymer. The mixture is placed on a mold with the desired shape and the solvent is then removed by evaporation or freeze-drying (Fig. 6a). To obtain a porous structure, the salt particles are leached. This method, besides simple, allows for adequate control of the pore size and porosity, which can be obtained by the salt/polymer ratio and the particle size of the salt added. However, the geometric shape of the pore is limited to the shape of the cubic crystals of the salt and the removal of soluble particles from the interior of the polymeric matrix becomes difficult for thick scaffolds, limiting their thickness between 0.5 and 2 mm. In addition, the limited interconnectivity of the pores prevents uniform cell inoculation and tissue growth.

In order to overcome these issues, the group of Ma and Choi (2001) has developed scaffolds of biodegradable polymers with spherical pores and controlled interconnectivity, using paraffin beads to generate the pores. The main advantage of this method is that it can provide a porous network completely interconnected. Besides, paraffin is insoluble in water and some water-soluble polymers can be used in the scaffold production by this technique [43].

Gas Foaming

Gas foaming can be used to fabricate highly porous polymeric foams without using organic solvents. In this technique, a gas such as carbon dioxide (CO_2) is applied using high pressure, enabling the formation of a single polymer/gas phase. Subsequently, the pressure is reduced to create a thermodynamic instability of the dissolved CO_2, resulting in nucleation and pore growth and enabling for foam formation (Fig. 6b). The advantages of this method are the absence of organic solvents and the possibility of manufacturing polymeric foams with high porosity [44]. Besides that, processes that do not include heating allow for the incorporation of temperature-sensitive biomolecules. The disadvantage of this method is the production of structures with closed pores without sufficient interconnected pores [19].

Freeze-Drying

This method consists of creating an emulsion, by homogenizing a solution of polymer (in an organic solvent) and water, rapidly cooling the emulsion to trap the liquid inside the structure and removing the solvent and water by freeze-drying (Fig. 6c). Scaffolds with high porosity and large pore sizes can be manufactured by this method [45, 46]. Nevertheless, the pores obtained do not show high interconnectivity. Besides, porosity and pore size are affected by parameters such as freezing temperature and cooling rate [47]. This technique has been widely applied to fabricate scaffolds mainly used in soft TE.

Thermally Induced Phase Separation

In this technique, the polymer is primarily dissolved in an organic solvent at high temperature, and the phase separation (liquid–liquid or solid–liquid) is induced by a temperature decrease of the solution. Subsequently, the removal of the solidified solvent is accomplished by sublimation, generating a porous polymeric scaffold (Fig. 6d). The pore morphology of the polymer depends on the solvent, the concentration of the polymer solution, and phase separation temperature.

An advantage of this method is that the scaffolds obtained generally have good mechanical properties. However, this method normally results in scaffolds with pore sizes between 20 and 500 μm, but mainly smaller than 100 μm, which are not ideal for the regeneration of many tissues [48].

Electrospinning

Electrospinning is a process of fibrous scaffold preparation that employs an electric field to control the formation and deposition of polymeric fibers on a given substrate (Fig. 6e). The geometry of these fibers is mainly influenced by parameters such as viscosity, electrical conductivity, and surface tension of the polymeric solution.

This technique can fabricate scaffolds with fiber diameters ranging from micrometers to several hundred nanometers [49, 50]. A wide variety of polymeric blends has been electrospun for the formation of scaffolds with high surface area, high porosity, and low density, used mostly for fibrous TE [51]. However, the productivity of this process is low and high electrical fields are necessary for fiber formation.

Rotary Jet Spinning

The rotary jet spinning technique consists on inducing the formation of fibers from a polymeric solution in an organic solvent, through the action of a centrifugal force, which ejects the solution, generally through nozzles at the head spinning, also evaporating the solvent (Fig. 6f).

This technique presents many advantages such as high efficiency and productivity, good process control, and fabrication of highly aligned and porous scaffolds [52, 53]. However, it is necessary to evaluate if the organic solvent was totally evaporated throughout the process, since it can cause scaffold toxicity.

Additive Manufacturing

Additive manufacturing (AM) is a technology based on the advances of computer science that has emerged for the production of custom models, such as layer by layer (Fig. 6g). Specifically, in TE, this technique combines knowledge of computed

tomography, magnetic resonance imaging, and computational models (CAD) to construct 3D scaffolds.

The main advantages of this technology are the possibility of manufacturing scaffolds with customized geometries, the fabrication of scaffolds with anisotropic structures, and total control of the manufacturing process by computers.

The most commonly used AM methods in TE are the powder-based technologies of selective laser sintering (SLS) and electron beam melting (EBM). From these technologies, custom models of hard body parts can be produced with precise control of morphology. However, the porous structures produced by these techniques affect mechanical properties of the scaffolds, and reduce the scaffold integrity [54].

Both processes fuse powder in a specific geometry of the model to be printed, SLS fuses or sintered the powder through a carbon dioxide laser, and EBM melts the powder with an electron laser beam.

Li et al. (2018) and Salmoria et al. (2018) produced scaffolds by an SLS process from a commercial magnesium alloy and a composite of poly(L-*co*-D,L) lactic acid (PLDLA) and bioglass, respectively, and the reported results showed biocompatibility and mechanical properties appropriate to bone repair [55, 56]. Yan et al. (2018) produced titanium mandibular scaffolds by EBM and reported that the mandibular defect was completely recovered after 2 months of in vivo implantation using 12 animal models [57].

Another promising AM technique that has emerged recently is 3D cell printing (or 3D bioprinting), which enables the fabrication of cell-embedded scaffolds using a one-step fabrication process in order to mimic complex structures. The challenge of this technology is to develop appropriate bioinks containing living cells in conjunction with microfluid systems capable of supporting cells and to present properties adequate to printability [58].

Choi et al. (2016) developed a bioink using decellularized skeletal muscle and applied the cell-printed technology to produce functional muscle embedded with myoblast cells mimicking the structure and function of skeletal muscle [59]. Ahn et al. (2017) noted that cell viability and printability are closely related [60].

This AM technology still presents many challenges, including the development of bioinks, long fabrication time, and limited thickness of the scaffolds.

Bioreactors

Scaffold conditioning using bioreactors has become a new and interesting approach for TE applications (Fig. 6h). Bioreactors are systems with adjustable parameters capable of stimulating biotransformation or cell expansion using whole cells or its components [61]. These systems can be used for three main applications on TE: cell expansion in vitro, cell viability maintenance during cultivation on scaffolds and validation of the scaffold function and cell differentiation [62].

Although TE seeks to create 3D scaffolds capable to regenerate or even replace tissues and organs, most research is limited to thin layered structures because of the poor diffusion of nutrients and oxygen through thicker scaffolds to the cells on static

cultures. Besides that, cells are not able to proliferate and uniformly distribute on the scaffolds, when cultured in vitro on static setups [63, 64].

Santoro et al. (2015) studied the influence of perfusion flow on tumor cells, comparing dynamic culture using bioreactors with static cultures. The application of a bioreactor with perfusion flow improved cell distribution and proliferation on electrospun PCL scaffolds [65].

Scaffolds must withstand the same conditions of the tissue where it will be applied. Moreover, the cells seeded on the scaffold must be able to differentiate into the desired cells. Therefore, bioreactors must simulate in vivo conditions for scaffolds, controlling mechanical, electrical and physicochemical parameters, such as temperature, pH, flow, oxygen, nutrient, and shear stress [66].

Lee et al. (2008) produced composite scaffolds based on PCL and type I collagen and evaluated its response at conditions of high pressure and flow, similar to physiological vascular conditions. The scaffolds presented good stability and biomechanical properties and were able to support cell adhesion and the proliferation of endothelial and smooth muscle cells [67]. Shepherd et al. (2018) produced scaffolds of type I collagen using freeze-drying and evaluated the effect of combining a flow bioreactor with megakaryocytes to the production of platelets. The system was capable of retaining the cells and effectively releasing platelets [68].

In addition to the culture and conditioning applications of bioreactors, they have been widely used in the new scaffold production technique of decellularization and recellularization. Decellularization consists of using chemical, enzymatic, and/or physical agents to remove cellular components of tissues and organs, leaving just the structure of a biological scaffold. Through this process, the decellularized extracellular matrix (dECM) has its structural integrity preserved and presents similar properties of the native tissues or organs, without being immunogenic [69]. After the decellularization, the material obtained (dECM) can be used for whole organ recellularization [70–72] or to create scaffolds using other fabrication techniques [73–76], such as AM [77].

Nichols et al. (2018) produced porcine decellularized lung scaffolds and used a bioreactor for its recellularization with different cells able to promote lung regeneration. The bioengineered (recellularized) lungs were then transplanted to pigs and did not indicate any rejection [78].

Jang et al. (2017) used 3D cell printing of heart tissue-derived decellularized extracellular matrix to create scaffolds for cardiac repair. A pre-vascularized patch was developed showing therapeutic efficiency [79].

Applications

Scaffolds have been studied for numerous applications on TE, considering all types of tissues, such as bone, cartilage, and skin, among others. In the last few years, different types of scaffolds, materials, and fabrication techniques have been combined, seeking an ideal scaffold for clinical applications. Some of these recent combinations for various scaffold applications are presented in Table 3.

Table 3 Application of scaffolds in different tissues

Tissue application	Material	Scaffold type	Fabrication technique	Reference
Bone	Hydroxyapatite	Microparticles	3D printing	[80]
	PDLLA-*co*-TMC	Fibrous	Electrospinning	[81]
	Chitosan/alginate	Solid	Freeze-drying	[82]
	PU/hydroxyapatite	Fibrous	Electrospinning	[83]
	Chitosan/gelatin/alginate/hydroxyapatite	Beads	Foaming	[84]
	Strontium hydroxyapatite/chitosan	Membrane	Freeze-drying	[85]
	PLGA	Membrane	Solvent casting/particulate leaching	[86]
	Bone (ECM)	Solid	Decellularization	[87]
Cartilage	Keratin	Fibrous	Electrospinning	[88]
	Cartilage (ECM)	Solid	Decellularization	[89]
	Cellulose/alginate	Solid	3D printing	[90]
	Chitosan/PVA/$CaCO_3$	Fibrous	Electrospinning	[91]
	PBLF	Microspheres	Emulsion	[92]
	PU/hydroxyapatite and PU/PEO	Solid	3D printing	[93]
	Cellulose	Fibrous	Freeze-drying	[94]
	PEG/heparin	Fibrous	3D printing/electrospinning	[95]
	PLGA/dECM	Solid	3D printing	[96]
	Hyaluronic acid	Hydrogel	–	[97]
Cardiac	Cardiac tissue (ECM)	Solid	Decellularization	[98]
	PANI-PGS	Membrane	Solvent casting/particulate leaching	[99]
	Gelatin/hyaluronic acid	Solid	3D printing	[100]
	PU-siloxane	Membrane	Thermally induced phase separation	[101]
	PLCL	Fibrous	Electrospinning	[102]
	PLA/chitosan	Fibrous	Electrospinning	[103]
	Collagen-alginate	Membrane	Freeze-drying	[104]
	Protein/polysaccharide	Sponge	Freeze-drying	[105]
	PGS/PBS-DLA	Membrane	Solvent casting/particulate leaching	[106]
Neural	PU	Solid	3D printing	[30]
	Nerve (ECM)	Solid	Decellularization	[107]
	PCL/gelatin	Fibrous	Electrospinning	[108]
	Gelatin methacrylate/PEGDA	Solid	3D printing	[109]

(continued)

Table 3 (continued)

Tissue application	Material	Scaffold type	Fabrication technique	Reference
Epidermal	Chitosan–agarose	Hydrogel	–	[110]
	PCL/gelatin/collagen type I	Fibrous	Electrospinning	[111]
	Silk fibroin-keratin	Membrane	Freeze-drying	[112]
	Fibrin	Membrane	3D printing	[113]
	Skin (ECM)	Solid	Decellularization	[114]
Other tissues	WSC/galactose and WSC/collagen	Hydrogel	–	[115]
	Silk fibroin	Solid	Freeze-drying	[116]
	PLGA/ECM	Membrane	Freeze-drying	[117]
	PPY/PDLLA	Membrane	Emulsion	[118]

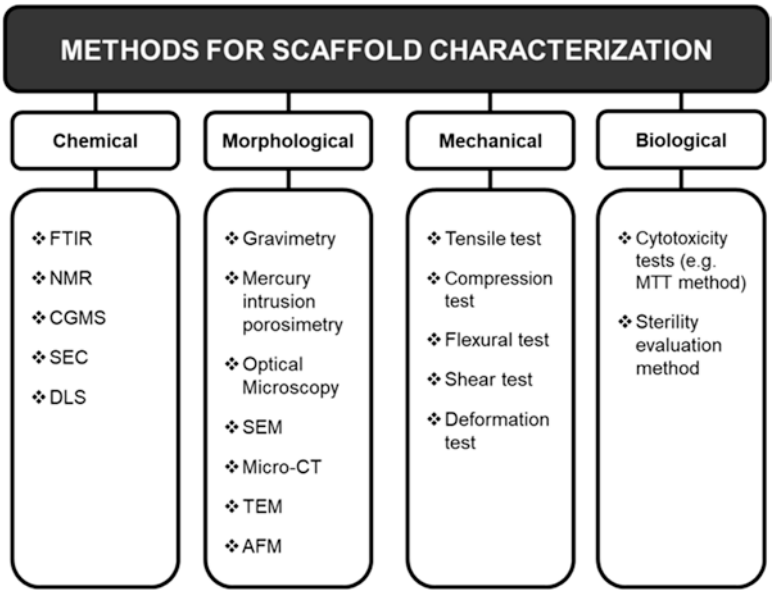

Fig. 7 Methods for scaffold characterization

Methods for Scaffold Characterization

Several analytical methods have been used to characterize the physical-chemical, mechanical, and biological properties of the scaffolds in their different formats (Fig. 7). These methods are usually based on international standards (American Society for Testing and Materials—ASTM) and include known analytical techniques [119].

Scaffolds are primarily characterized by their chemical properties, which include the chemical composition, impurities contained, and chemical nature of the surface groups.

These parameters can be identified and determined quantitatively by techniques such as Fourier Transform Infrared (FTIR) or Nuclear Magnetic Resonance (NMR ^{1}H, ^{13}C or ^{31}P) combined or not with Gas Chromatography (CG) coupled with Mass Spectrometry (MS) or other appropriate analytical methods.

Furthermore, the size of the molecules, or their molar mass, is usually evaluated. The techniques used in this case include size exclusion chromatography (SEC) and dynamic light scattering (DLS). In all cases, complete solubilization of the material in an appropriate solvent is required. SEC is indicated for the determination of the molar mass of linear polymers, while DLS can be used for both linear and branched polymers. These methods are usually comparative; thus, the results must include the solvent used, the temperature in which the measurements were taken, the standard used as reference, and the concentration of the solutions analyzed.

In order to determine the scaffold morphology (porosity, pore size, interconnectivity, tortuosity, roughness, and topography), a variety of equipment and software have been used. The most common methods include gravimetry, mercury intrusion porosimetry, optical microscopy (OM), scanning electron microscopy (SEM), and micro-computed tomography (micro-CT) [120].

The gravimetric method determines, in a fast and simple way, the total porosity of the material; however, the measurement accuracy is limited.

Mercury intrusion porosimetry determines total volume, average diameter, and the size distribution of pores, but has the disadvantages of high toxicity, high cost of mercury, and the possibility of scaffold collapse with the high pressures required by this method.

Optical microscopy is mainly employed in preliminary observations of scaffolds. Although it has a number of advantages including simple preparation of samples and low cost of analysis, the OM resolution is limited mainly to sizes around 200 nm, preventing a detailed characterization of the structures.

Through the association of micrographs obtained by SEM and computer software, it is possible to determine the average pore diameter and porosity, in addition to obtain an estimated interconnectivity and pore wall thickness. However, to ensure accurate measurements, samples must be carefully sectioned to avoid changes in the porous structure. In addition, the sample sensitive to the high vacuum required by this technique must be properly fixed to prevent its collapse.

Micro-CT accurately provides all information about the 3D morphology of the scaffolds and has the advantage of being non-destructive and not requiring pretreatment of the sample with toxic chemical compounds. However, this technique still presents a high cost and is not suitable for scaffolds containing metals.

Besides these methods, the average nanoparticle diameter and surface (topography and roughness) of the scaffolds can be observed by transmission electron microscopy (TEM) and atomic force microscopy (AFM), respectively.

The mechanical properties evaluated in the scaffolds usually involve tests related to stress and strain or that show the response of these scaffolds to the application of a physical force.

The scaffolds must be evaluated under conditions that mimic the intended application. Besides, a special assembly of the evaluated specimens may be required depending on the size and format of the scaffold and the equipment used.

Mechanical tests include compression, tensile, flexural, shear, and deformation performed on servo-hydraulic equipment and can be performed on dry or swollen scaffolds to mimic conditions in vivo. The parameters generally measured are the modulus of elasticity (Young's modulus) and the shear modulus that indicates the scaffold stiffness.

The swelling and degradation profiles of scaffolds are also important parameters and are usually determined by a gravimetric method in medium, which mimics conditions in vivo.

The swelling profile of the scaffolds is generally obtained at 37 °C in phosphate buffered saline (PBS) at pH between 7.2 and 7.4 or in Dulbecco's Modified Eagle's Medium (DMEM). Degradation tests may also be carried out under these conditions or in the presence of suitable enzymes.

From the biological point of view, scaffolds are characterized primarily by the in vitro biocompatibility of the materials used in their fabrication.

This evaluation is performed through cytotoxicity tests, especially by cell viability, which consists of placing the scaffold directly or indirectly in contact with a culture of animal cells and verifying the cellular changes that resulted by different mechanisms, including the incorporation of vital dyes or the inhibition of the formation of cellular colonies. The most used cell viability methods are the neutral red incorporation method and the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide).

Furthermore, the sterility of scaffolds is also evaluated by counting the total bacteria and fungi (molds and yeasts), using the pour-plate method, surface-spread method, and serial dilution method.

Conclusions and Future Perspectives

During the last few years, important advances have been made in regenerative medicine, especially in the TE field. According to the bibliometric analysis of this work, the last two decades presented increasing scientific production and new techniques related to scaffolds. However, existing therapies still have several limitations. In fact, no combination between cells, scaffolds, and bioactive molecules have fulfilled all the necessary criteria to mimic the conditions in vivo and effectively promote the regeneration of different tissues.

A specific combination between cell type, culture regime, and scaffold must be carefully selected, since it has been demonstrated that the physicochemical characteristics of the scaffolds directly affect the cellular behavior and, consequently, the

process of tissue regeneration. In addition, the incorporation of bioactive molecules in this system has been shown to contribute to accelerate these processes.

Therefore, an ideal combination of these parameters for the effective regeneration of different tissues is still a challenge, and researchers have been increasingly directing their work to biodegradable and biocompatible materials, undifferentiated cells, and autologous bioactive molecules.

The studies developed so far indicate that future advances in TE depend on new systems that can modulate cellular behavior and result in functional and effective tissues.

Many challenges are still limited to the multidisciplinarity of this area and the complexity of the biological systems involved in the regeneration of different tissues. Its success depends on combined efforts of researchers to understand, modulate, and optimize the results of basic sciences and clinical applications.

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Appendix

Keywords	No. of occurrences (1990–1999)	No. of occurrences (2000–2009)	No. of occurrences (2010–2019)
3D printers	–	–	369
Additive manufacturing (or rapid prototyping)	–	107	112
Bioactivity	–	65	207
Biocompatibility	11	429	1562
Biodegradability (or biodegradation)	36	244	287
Bioreactors	6	225	428
Bone	13	427	1444
Cardiac tissue engineering	–	13	67
Cartilage	27	350	633
Ceramics	–	128	181
Composite scaffolds	–	32	183
Electrospinning	–	161	1221
Epidermis	2	27	47
Fluidic devices (or fluids)	–	11	34
Freeze-drying	2	106	284
Gas foaming (or foaming or foam)	3	20	34
Mechanical properties	8	177	765
Metals	–	18	81
Morphology	9	180	360

Keywords	No. of occurrences (1990–1999)	No. of occurrences (2000–2009)	No. of occurrences (2010–2019)
Natural polymers	–	17	56
Nerve regeneration	6	81	251
Particulate leaching	–	10	31
Polymer	33	601	941
Scaffolds	10	944	2174
Solid	–	54	20
Spinning (fibers)	–	26	231
Surface property	4	364	769
Synthetic polymers	–	13	38
Thermally induced phase separation	–	9	54
Tissue engineering	33	2564	5748

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