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Polymer-Based Microparticles in Tissue Engineering and Regenerative Medicine

Mariana B. Oliveira and João F. Mano

3Bs Research Group-Biomaterials, Biodegradables and Biomimetics, University of Minho, AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, Caldas das Taipas, Guimarães 4806-909, Portugal

Institute for Biotechnology and Bioengineering (IBB), PT Government Associated Laboratory, Guimarães, Portugal

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Different types of biomaterials, processed into different shapes, have been proposed as temporary support for cells in tissue engineering (TE) strategies. The manufacturing methods used in the production of particles in drug delivery strategies have been adapted for the development of microparticles in the fields of TE and regenerative medicine (RM). Microparticles have been applied as building blocks and matrices for the delivery of soluble factors, aiming for the construction of TE scaffolds, either by fusion giving rise to porous scaffolds or as injectable systems for in situ scaffold formation, avoiding complicated surgery procedures. More recently, organ printing strategies have been developed by the fusion of hydrogel particles with encapsulated cells, aiming the production of organs in in vitro conditions. Mesoscale self-assembly of hydrogel microblocks and the use of leachable particles in three-dimensional (3D) layer-by-layer (LbL) techniques have been suggested as well in recent works. Along with innovative applications, new perspectives are open for the use of these versatile structures, and different directions can still be followed to use all the potential that such systems can bring. This review focuses on polymeric microparticle processing techniques and overviews several examples and general concepts related to the use of these systems in TE and RE applications. The use of materials in the development of microparticles from research to clinical applications is also discussed. © 2011 American Institute of Chemical Engineers *Biotechnol. Prog.,* 000: 000–000, 2011
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Introduction

Tissue engineering (TE) is a field that applies the principles of biology and engineering to the development of functional substitutes for damaged tissue.[1] Many of the currently proposed TE strategies are based on the use of hydrogels and porous scaffolds.[2] Advances in the field of TE and regenerative medicine (TE&RM) were possible through the development of alternative systems, which can conjugate the advantages and simultaneously the elimination of drawbacks of both kinds of systems. In this context, particles have been suggested as injectable or moldable systems in which cells can adhere and proliferate in a solid substrate. These systems offer the possibility of injecting the isolated particles into the defect. Moreover, besides the polymeric particles alone as injectable systems, encapsulated bioactive agents or in vitro preseeded cells can be delivered in the defect using particles as vehicles.

The use of particles can be discussed in terms of the dimension of the objects (Figure 1). In a nanoscale perspective, particles for modeling cell behavior by gene delivery have been used in cell therapy with special emphasis in the treatment of cancer and immune system diseases.[3–5] In TE&RM strategies, nanoparticles could be used to deliver bioactive substances either to the cell surrounding medium or directly into the interior of the cells by internalization. The release of proteins [including growth factors (GFs)] or low molecular weight differentiation agents can target and control the behavior and the fate of the cells, as schematically represented in Figure 1A.[6–8] Despite the relevance of nanoparticles in this field, this review will focus mainly on the use of polymeric microparticles.

The use of microparticles in TE&RM may have different purposes, which include (i) the incorporation of microparticles in hydrogels or porous scaffolds (Figure 1B) aiming for the formation of pores,[9] (ii) the achievement of the complex delivery systems for macromolecules (e.g. dual release profile systems),[10] or (iii) the incorporation of osteoconductive materials in the system.[11] The injection of microparticles loaded with bioactive molecules aiming for controlled delivery (Figure 1C) has also been performed, relying on diffusion,[12] polymer degradation or using responsive polymers properties to trigger the release of the molecules.[13] These particle diameters usually range from 1 μm to 10 μm.

Regarding particles with sizes varying from 10 μm to 1,000 μm, scaffolds with interconnected porosity have been obtained by the sintering or chemical agglomeration of microparticles [Figure 1E(b)].[14–16] The use of separate particles offers high surface area for cell expansion [Figure 1E(c)].[17,18] The in situ formation of scaffolds by cell-induced aggregation of injected microparticles has also been

Correspondence concerning this article should be addressed to J. F. Mano at jmano@dep.uminho.pt.

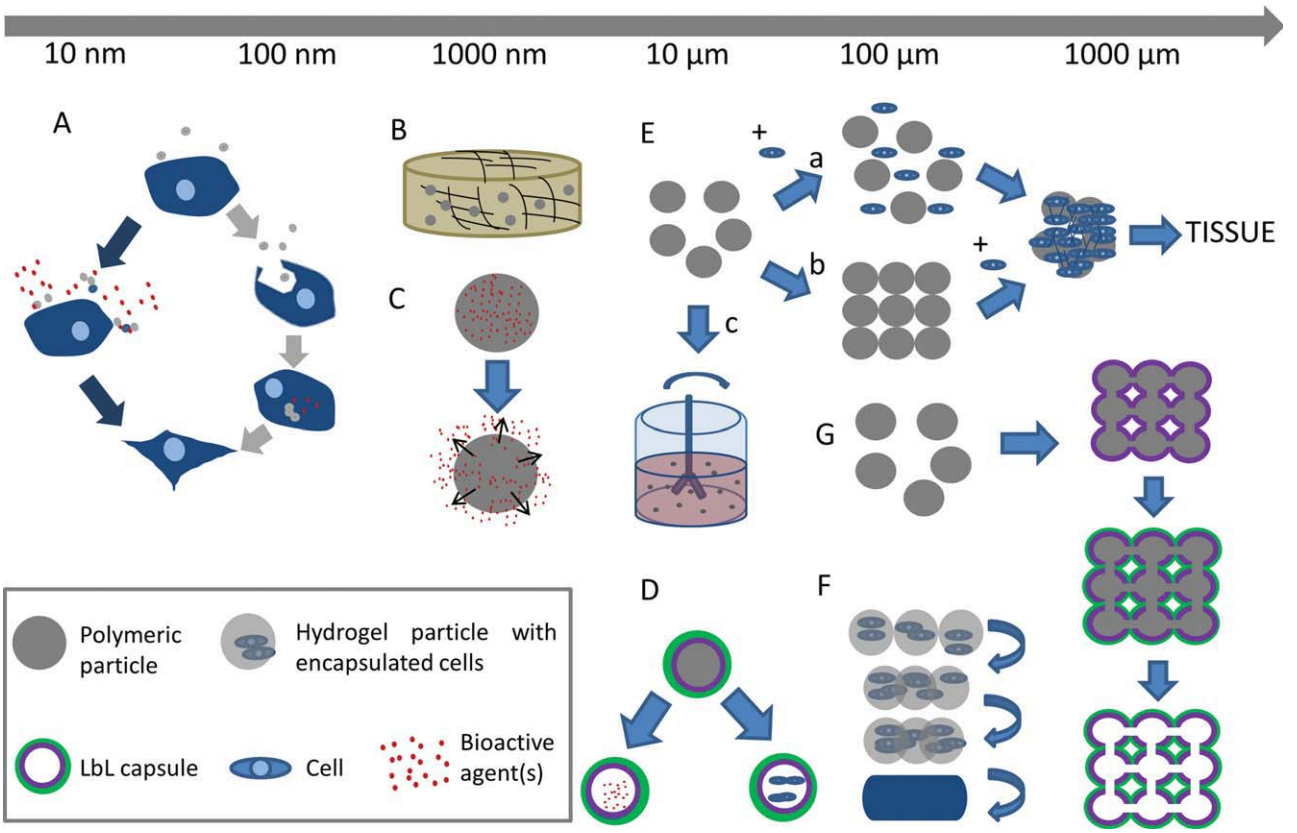

Figure 1. Schematic representation of distinct uses of particles in the TE field according to size.

(A) Nanoparticles can be used for release of bioactive agents to the cell culture medium (dark arrows) or for cell internalization (light arrows). (B) Incorporation of microparticles in 3D systems for enhancement of the matrix properties. (C) Use of microparticles for the delivery of bioactive agents to control cell behavior. (D) Hollow capsules obtained by LbL which, after core-leaching, may contain encapsulated bioactive agents for controlled release or liquefied medium with cells. (E) Microparticles in the range of 100–1,000 μm can be used: (a) in combination with cells to obtain cell-induced aggregation; (b) to allow the formation of scaffolds with interconnected porosity after particle agglomeration by sintering or solubilization methods; (c) as cell microcarriers for cell expansion. (F) Use of hydrogel particles with encapsulated cells for organ printing and mesoscale self-assembly. (G) Fabrication of 3D porous constructs obtained by a LbL strategy.

suggested [Figure 1E(a)].[19,20] Cell encapsulation in hydrogel particles has led to cell delivery strategies and new scale-up biofabrication methods, such as organ printing (Figure 1F).[21,22]

The wide versatility of these systems is associated with the variables that can be modeled to obtain an optimal system for a specific application, such as the particle composition, size and shape, existence of porosity, cell culture conditions, or incorporation of bioactive agents. Silva et al. reviewed concepts for the use of materials in particulate form for TE as well as the specific requirements for bone tissue regeneration applications.[23,24]

During the last decades, several techniques have been used to process microparticles for applications in TE&RM. A general overview on such processing techniques will be presented. Several examples and general concepts related to the use of microparticles in TE&RM applications will also be reviewed. A discussion on the most commonly used materials in microparticle TE&RM strategies will be performed, as well as some comments on the bridge between the more fundamental studies and the systems applied in the clinical practice.

Methods for the Preparation of Microparticles for Tissue Engineering

Microparticle manufacturing has been widely developed in the pharmaceutical industry to obtain effective drug release systems.[25] Parameters have been optimized to obtain controlled release profiles and particle size has been adjusted for each particular application. Such lessons can be transposed to the production of microparticles for specific applications in TE&RM. In this case, different specifications should be taken into account, so other important aspects could be considered, such as the control of cell behavior onto the surface of the particles or the degradation profile. Furthermore, parameters such as pore interconnectivity,[20,26,27] surface topography,[28] surface chemistry, or particle size must be considered, which usually have different requirements in pure drug delivery systems.

The preparation of microparticles requires several considerations about their manufacturing, modification, and manipulation. In most applications, one should have control over particle size, shape, surface characteristics, and porosity. The method should also ideally allow the production of large quantities of particles with a narrow size distribution. The most important methods for microparticle production will be reviewed, focusing on methodologies that have been adopted or have potential for TE&RM applications.

Emulsification

Emulsification is the most commonly used approach for the fabrication of microparticles. In this technique, the polymer is dissolved in an organic solvent, and this mixture is in

a larger volume of another liquid with opposite solubility. The method relies on the principle that during the evaporation of the organic solvent, polymeric particles are formed.[29] The double emulsion technique is a modification of the previously referred method, in which a compound (e.g. a bioactive agent) is introduced via emulsion or dispersion in the system, which allows the encapsulation of several agents.[30] Pure polymeric microparticles are usually produced by a single oil-in-water (o/w) emulsion. One advantage of this technique is the ease with which it can be used to generate microgels. Depending on the process conditions, the size distribution of the gels can be minimized. On the other hand, the process typically has a larger size distribution than other synthesis approaches and there is little control of the resulting shapes as the emulsification process typically produces spherical droplets.[31]

Spray drying

In spray-drying methodology—which is widely used in pharmaceutical/drug delivery approaches[32,33]—a polymeric solution is directly injected through a heated nozzle and evaporated by a hot stream of flowing gas. The size of the particles is dependent on several parameters such as polymer/drug solution loading rate, drying temperature, and spraying rate.[34] The main advantage of this technique is its reproducibility and the dispersion uniformity that can be obtained. However, the high temperatures reached during the processing can induce the degradation of encapsulated agents.[29,35]

Hot melt

Hot melt encapsulation is used for the encapsulation of drugs with polymeric coatings. Presieved drug particles are mixed with melted polymer, followed by its suspension in an agitated nonmiscible solvent, heated above the melting point of the polymer. Particles ranging from 1 μm to 1,000 μm can be easily obtained, in large-scale production, after the cooling of the polymer. Narrow distribution of the particle size can be achieved.[36] A major drawback of this technique is the possibility of denaturation or degradation of the molecules due to the high temperature,[29] as well as the impossibility of using it for cell encapsulation.

Gelation

The gelation method uses a polymeric solution, which is extruded and dropped in a hardening bath containing a slowly stirred solution responsible for the crosslinking of the polymer. This technique allows the manipulation of the size of the particles controlling the diameter of the extrusion needle, polymer flow rate, or the polymer concentration. Different combinations of polymers can be presented in the initial liquid formulation, including stimuli-responsive macromolecules.[37] Encapsulation of living cells and other living organisms has been performed using gelation method, which shows its usefulness in biotechnology field.[29,38,39] Such particles can be then surface modified, using for example layer-by-layer (LbL) methodology, permitting to produce liquid-core shells for cell encapsulation[40,41] (Figure 1D).

Particles obtained by gelation have already been obtained using microfluidic systems: Capretto et al.[42] used a microfluidics chip ("Snake mixer slide," Thinxxs, Germany) to test different gelation techniques for sodium alginate. Microparticles with ideal size for cell encapsulation (300–600 μm), with spherical shape and narrow size distribution were obtained by a partial gelation approach: a sodium alginate solution with small amount of $BaCl_2$ (an ionic crosslinker of alginate) was injected through a tube, followed by the injection of an oil phase in another tube positioned in the squeezing channel of the Y-shaped conduct. The particles obtained in the w/o emulsion were then gellified in a $BaCl_2$ solution.

Superhydrophobic surfaces

A new processing method based on the rolling of water drops over superhydrophobic surfaces proposes the fabrication of hydrogel and polymeric spheres depositing drops of liquid precursors containing the polymer and other substances onto the surface.[43] This method presents advantages over conventional gelation and emulsion techniques as the contact of the dispensed drops with an outer liquid environment is avoided. Encapsulation of living cells is also possible strengthening the potential of this technology into TE applications.[43] Moreover, this methodology permits the encapsulation of therapeutic molecules with high efficiency and under mild conditions.[44] This processing method also avoids the exposure of the microparticles to stirring forces and allows to obtain precise shaped and sized structures. In this method, the collection of the particles is also facilitated as the drops can be easily removed as the contact area with the surface is very small.

Coacervation

In coacervation technique, the solubility of the polymeric solution is decreased by the introduction of a contrasting component. Two distinct phases are obtained: one containing the coacervate phase and other containing the supernatant, allowing the encapsulation of both hydrophilic and hydrophobic drugs.

Compared with the emulsion methods, the loss of polymer and drug is significantly lowered. As disadvantages, particles tend to agglomerate, batch-to-batch variability is common and nonapproved solvents are used.[29,45] The parameters that influence the formation of microcapsules by this technique have already been studied: according to Dong et al., for peppermint oil encapsulation in gelatin/gum arabic capsules, the pH value of the preparation solution and stirring speed affected the particle size.[46] Also, polymer concentration affected the shape of the particles. Moreover, the use of different crosslinkers influenced the size distribution of the particles.

Grinding

The grinding method was proposed by Elkharraz et al.[47] In this time-saving approach, microparticles were prepared by the grinding of thin drug-containing ethylcellulose films in cryogenic conditions. Because of the low temperatures and mechanical disruption of the films, this technique is not compatible with cell encapsulation. Particle size and shape of the microparticles could be controlled by both films' thickness and milling time. The encapsulation efficiency as well as the in vitro drug release depended on the physical state of the drug in the ethylcellulose matrix (dispersed or dissolved).

Electrospraying

Electrospraying (ES) is a slightly modified form of the electrospinning process in which an electric field is applied to a polymeric solution extruded from a syringe. The applied high voltage potential forces the polymer to form a jet that, using specific parameters, enables the formation of micro/nanoparticles. The most important variable distinguishing ES and electrospinning is the polymer concentration used in the process; ES requires relatively smaller concentrations to generate particles.[48] The great advantage of ES over other commonly used methods is the fact that it is a one-step process that does not make use of organic solvents or crosslinking agents. The effects of low atmospheric conditions in the encapsulation efficiency have been studied: bigger particles were obtained with decreasing pressure. Moreover, with specific controlled temperature and pressure, uniform and spherical particles were obtained.[49]

Cell encapsulation was successfully achieved by Xie and Wang using alginate beads electrosprayed into a gellifying $CaCl_2$ solution.[50] Particles ranging from 200 μm to 2 mm could be obtained with narrow size distribution operating under low flow rates with high voltages applied to the nozzle.

Using this method, poly(lactic-*co*-glycolic acid) (PLGA) microparticles could be patterned, with or without adhesive proteins (such as collagen), into substrates through a mask into a nonadhesive substrate. After cell seeding, cell patterns could be seen, as cell growth only occurred in the PLGA particles.[51]

A variation of this method is dual-capillary ES system, consisting on two separate flow channels formed by two coaxially aligned capillaries. It allowed a single-step production of monodisperse, 100% drug encapsulation of PLGA-coated drug particles, with diameters ranging from 165 nm to 1.2 μm.[52] Different from a single-capillary ES system, the dual-capillary ES system can separate the drug and the coating material in a core (drug)–shell (polymer) structure.

Supercritical fluid mixing

In supercritical fluid mixing, a polymer/bioactive agent solution is sprayed in a supercritical (sc) fluid (usually $scCO_2$), which results in the dissolution of the solvent in the supercritical phase and precipitation of the polymer.[29] When a supercritical fluid such as carbon dioxide is used as a nonsolvent, the simple tuning of the processing conditions (pressure and temperature) can tailor the final structure of the microparticles. Also, any subsequent drying step is avoided, as the obtained porous structure is a dry product free of any residual solvent. The selective solvating power of supercritical fluids allows the separation of a particular component from nontoxics and nonflammables. Furthermore, the structure and functionality of the incorporated molecules are maintained.[11]

The polymers must be suitable for the precipitation step, satisfying requirements in molecular weight, crystal structure, glass transition temperature, and solubility. Several works allowed the production of particles in the range of 1–2 μm, whereas in others, particles in the range of 30–120 μm were produced,[11,53–56] showing the versatility of this technique regarding particle size.

Microfabrication

Recently, microfabrication methods have been used to make nano/microparticles with monodisperse size distribution, in which solid templates are used.

Soft lithography is a group of techniques using an elastomeric mold with topological features to generate micro or even nanostructures. Guan et al. combined two different soft lithography strategies to develop a simple approach to fabricate highly uniform polymer microparticles with controllable sizes, platelike structure, and well-defined lateral shapes using common polymers.[57] However, the collection of individual particles after preparation by soft lithography has not been easy because of the insolubility of the most commonly used elastomers in mild solvents as water, or to their nonbiodegradability. Hydrogel templates were considered in the methodology of collecting the formed particles by simply dissolving the templates in aqueous solutions, or even to implant the particles in the body inside biodegradable templates. For example, gelatin was proposed in this context as it exhibits a sol–gel transition with temperature that permitted to mechanically produce efficient templates.[58] Using such material, the size of the particles could be adjusted from 200 nm to >50 μm, the drug loading capacity was 50% or higher, and the initial burst release was minimal.[58]

Considering microfluidic-based processes, stop-flow lithography, a continuous microfluidic process, was applied to produce a large amount of cell-laden hydrogel particles.[59] A prepolymer solution-containing cells was flowed through a microfluidic device, and arrays of individual particles were repeatedly defined using pulses of UV light through a transparency mask.

Applications of Microparticles in Tissue Engineering and Regenerative Medicine

Microcarriers for cell expansion

Cell expansion is an important issue in most of the TE&RM strategies as the number of cells harvested and isolated from the patient may not be sufficient. The most common way of expanding cells is to culture them as a monolayer on the bottom of a culture dish.[2] However, these substrates are not amenable to scale up because of the larger growth surface areas required. Microcarrier cultures can provide sufficient cell numbers of the appropriate phenotype to assist in the repair or regeneration of damaged or degenerated tissue and allow the inclusion of specific GFs or extracellular matrix (ECM) proteins to further aid proliferation and differentiation, increasing production capacity with improved control, reducing requirements for culture medium as well as labor effort, and lowering the risk of contamination.[60]

Microcarrier culture was introduced by van Wezel in 1967[18] to mass-produce viral vaccines and biological cell products using mammalian cells. Since then, a wide range of commercially available microcarriers have been successfully used for the production of a variety of biological products at the analytical and industrial scales.

The number of cells that attach on the surface of microcarriers depends on its diameter (usually ranging from 100 μm to 400 μm), on the size distribution—that must be as narrow as possible—and in the porosity of the carriers. The specific density of the microcarriers might also be slightly higher than that of the culture medium to enable suspension with agitation. The retrieving of the cells is dependent on the microparticle composition and in the degree of porosity.[17] Some drawbacks are associated with the use of microcarriers. The generation of shear stress in the surface of the microcarriers during stirring in the bioreactors may impair

cell attachment, growth, and differentiation processes. The risk of collision between the microcarriers can damage cell integrity.

Microcarriers have been widely used for cell proliferation of several tissues such as bone,[61,62] cartilage,[63–65] blood vessels,[66] and skin[67] as well as in the differentiation studies.[68,69] Malda et al. reviewed the application of microcarriers for bone and cartilage cell growth.[17]

There are still issues to be optimized related to the detachment and isolation of expanded cells from these particles. The use of toxic enzymes—usually trypsin—frequently used for cell retrieval both in monolayer culture and in microcarrier culture has been overcome using poly(*N*-isopropylacrylamide) (PNIPAAm)-containing particles.[70] The use of this synthetic temperature-responsive polymer, exhibiting a low critical solution temperature (LCST) close to the body temperature, allowed cell detachment with the lowering of temperature, in an analog strategy to cell-sheet engineering,[71] in which the structure of the ECM could be maintained. Another possibility for cell expansion avoiding exposure to toxic enzymes for cell retrieving is the simultaneous implant of the cells with their expansion supports, i.e. the microcarriers, in the defect. Considering cases of application of this concept in the field of skin regeneration, this approach also allowed the circumvention of the use of GFs and layers of feeder cells—used in traditional two-dimensional (2D) in vitro techniques of autologous skin expansion—which may lead to tissue rejection.[72,73]

Delivery of bioactive agents

Some studies have addressed different strategies that may enhance tissue regeneration, most of them involving the use of GFs. Polymeric microparticles may be used to deliver relevant therapeutic and bioactive factors on implantation in TE applications. The easiest way to add proteins and peptides to polymeric systems is their direct loading into the polymeric matrix. However, if proteins are incorporated into hydrogels without any further modification, typical release profiles show a rapid burst release during the initial swelling phase. If the retardation of protein release from the presented approaches is not sufficient to provide enough protein for long-term applications, carriers may be added to hydrogels to retain the bioactive factors for an extended time. Such systems may be made of different biodegradable and nonbiodegradable materials offering a tunable control over the release rate. Using nondegradable systems, protein transport out of the device is driven by diffusion and specific interactions between the protein and the matrix. Mass transport occurring through polymer chains or pores is the only rate-limiting step of the process. In degradable systems, the particles inside the hydrogel degrade and the encapsulated protein is released.[74]

Tabata and coworkers proposed the use of polyanhydride microspheres for controlled release of proteins[75,76] for TE&RM strategies. The same group has developed gelatin particles for the treatment of arteriosclerosis obliterans by the release of erythropoietin,[12] regeneration of intravertebral disc by the delivery of platelet-rich plama,[77] and osteoarthritis using basic fibroblastic growth factor (bFGF) testing their effectiveness in animal models.[78] More works using microparticles in this ambit have been developed by these authors regarding osteochondral regeneration as well as the induction of angiogenesis.[79–82]

Distinct biomaterials have been proposed for the delivery of bioactive agents from particles. For example, Santo et al. proposed carrageenan as a material to develop hydrogel beads with the ability to incorporate GFs, with the purpose of stimulating angiogenesis, obtaining a controlled release profile.[83] Bessa et al. have used silk fibroin for the loading of human recombinant bone morphogenic proteins (BMPs), and tested its release in vitro and in vivo.[84]

A different approach for the delivery of bioactive agents relies in the application of external stimuli to trigger the release, instead of strategies purely relying on the degradation or diffusion of the drug from the microparticles. Soontornworajit et al. developed a hybrid particle–hydrogel for oligonucleotide-mediated pulsatile protein release at-will.[85] Affinity particles (composed of streptavidin-coated polystyrene) were functionalized with aptamers (biotinylated aptamers) and embedded in agarose hydrogels. Aptamers bind strongly to proteins, which in this case was platelet-derived growth factor (PDGF). The release of the GF from the aptamer was triggered by a CO-mediated aptamer–protein dissociation. The release experiments showed that the protein release rate in the aptamer-incorporated hybrid dramatically decreased in normal conditions as compared with the control composite.

Considering specific applications of microparticles in tissue repair, several examples will be explored focusing on applications for bone, cartilage, and neuronal tissues.

Regarding bone repair, single-GF delivery strategies have been proposed. For example, Meinel and coworkers suggested a strategy for bone regeneration by releasing insulin-like factor I loaded in PLGA incorporated in an alginate–tricalcium phosphate composite particles.[86] Also, Ichinohe et al. developed a system tested in rabbit skull defects using titanium nonwoven fabrics combined with fibroblast growth factor-2 (FGF-2) release from gelatin hydrogel microspheres.[87] However, and especially in bone TE, the presence of different bioactive agents may be needed at different stages of the healing process and tissue regeneration, in which both the formation of mineralized tissue and the penetration of the structure by a highly vasculatized structure are important. Therefore, it is reasonable to consider concepts in which dual or multiple release of different molecules takes place with different release profiles. Mooney and coworkers[10] developed a platform in which the first approach involved the mixing of lyophilized vascular endothelial growth factor (VEGF)—a well-established initiator of angiogenesis—with PLGA particles before processing the polymer into a porous scaffold; this resulted in a rapid release profile of VEGF. The second approach involved pre-encapsulating some GF (in this case, PDGF) in PLGA microspheres and then fabricating scaffolds from these particles. The combination of both approaches was utilized to incorporate VEGF and PDGF, which promotes the maturation of blood vessels by the recruitment of smooth muscle cells to the endothelial lining of nascent vasculature into scaffolds. Also, a sequential VEGF and BMP-2 delivery, using PLGA microspheres loaded with BMP-2, embedded in a polypropylene scaffold, surrounded by a gelatin hydrogel loaded with the endothelial GF could be obtained. In combination with local sustained BMP-2 release, VEGF significantly enhanced ectopic bone formation compared with BMP-2 alone; however, in the orthotopic defects, no effect of VEGF on vascularization was found, nor was bone formation higher by the combination of GFs, compared with BMP-2 alone.[88]

Regarding cartilage tissue regeneration, several approaches have been developed. Considering pure microparticulate systems, PLGA microspheres were loaded with transforming growth factor-β3 (TGF-β3), successfully leading to the chondrogenic differentiation of mesenchymal stem cells (MSCs).[89] Injectable biodegradable hydrogels (composed of oligo(poly(ethylene glycol)fumarate) with laden-MSCs, combined with gelatin microspheres loaded with TGF-β1 GF was also used proposed for cartilage regeneration.[90]

Aiming neuronal tissue repair, injectable degradable particles composed of PLGA, poly(L-lactic acid) (PLLA), and poly(ethylene glycol) (PEG) were proposed for the delivery of brain-derived neurotrophic factor (BDNF). A prolonged release was obtained for periods of time greater than 60 days, and the delivered BDNF was bioactive.[91] Also, magnetic alginate microspheres were used for the positioning and controlled delivery of nerve growth factor (NGF), allowing the precise control of the delivery place.[92] PLGA microsphere composition has also been modulated to control the burst effect. However, the formulations with more appealing controlled delivery response showed increased NGF denaturation.[93]

Incorporation of microparticles in traditional tissue engineering matrices

The incorporation of particle systems in conventional hydrogels or scaffolds might have different objectives in TE strategies, such as the insertion of GFs and differentiation agents in the system by encapsulation in microparticles, which has been discussed in the previous section. This section will focus on the effect of introducing microparticles in matrices for the enhancement of some property of the matrix, such as mechanical properties, degradation, cell migration, or production of porosity.

The generation of porosity in compact structures has been used to allow cell penetration and migration in a biomaterial structure. Pectin degradable polymeric microparticles in calcium phosphate cement were suggested for porosity induction. This also allowed an adequate drug release profile from the same structure.[94] After 4 months, the cement structures were macroporous and interconnectivity between pores could be seen. A similar approach has been developed by Ruhé et al. suggesting the incorporation of porous PLGA particles in a cement.[9] Relying on the in vivo hydrolysis of the particles (the tests were performed in rats for 12 weeks), a macroporous structure was obtained. An approach based on the enzymatic degradation of poly(trimethylene carbonate)—a material with rubberlike properties—microparticles in a ceramic cement also allowed to obtain macroporosity in the presence of enzymes; in this case, although the compressive strength of the composite decreased with the loading of the particles, the toughness was improved, preventing the fragmentation of the cement.[95]

The incorporation of polymeric particles in hydrogels or sponges has also been used to improve the mechanical properties, namely the stiffness of these structures. Different approaches have been performed, either having the microparticles of a similar material of the matrix or incorporating particles of a different material. Injectable scaffolds composed of PLGA microparticles in a chitosan hydrogel have been prepared.[96] The final composite hydrogel showed lower swelling ratio than the chitosan hydrogel, and higher elastic stiffness. Also, Kaplan and coworkers have developed a structure consisting of incorporated silk microparticles in a silk sponge, to increase the elastic stiffness of the construct. They observed that having increasing values of elastic moduli with the increasing load of microparticles per sponge, that also shown to affect the differentiation of MSCs in osteogenic lineage and calcium desorption in the presence of BMP-2 and other osteogenic factors.[97]

Cell encapsulation, organ printing, and mesoscale self-assembly

Cell encapsulation is a strategy in which a pool of living cells is entrapped within a semipermeable polymeric matrix, which can have the form of a microparticle.[98–100] Also, it aims to protect the transplanted cells from attack by the host immune system without immunosuppressive agents.

Cell encapsulation in biodegradable hydrogels has been widely studied[99] and offers numerous attractive features for TE, including ease of handling and a highly hydrated tissue-like environment for cells.[101] Cell microencapsulation and implantation provides a promising platform for cell therapy to treat a vast array of clinical disorders.[102] Immobilized cells may be used to produce new tissues or bioactive agents with therapeutic effect. The main advantage of the microencapsulated cell-based delivery system is the continuous production and secretion of the bioactive agents from the microencapsulated cells, thereby eliminating the requirement for purification and encapsulation of unstable therapeutic factors and the need for multiple dosages.

Alginate is the most commonly used polymer for encapsulating cells as it can be easily gellified in mild conditions, is noncytotoxic, and its quality can be constantly ensured. The mechanical strength and elasticity of these encapsulating structures should be high enough to guarantee consistent therapy over prolonged periods, and their manufacturing process must not only be reproducible but also meet the demands for medical approval.[103] Other polysaccharides such as carrageenan have been proposed to encapsulate cells in particles.[104]

The deep understanding, biomimicking, and using of developmental mechanisms of embryonic histogenesis and organogenesis can represent a promising step for developing new TE solutions.[22] Regarding this concept, self-assembled tissue spheroids—obtained by cell encapsulation techniques—can be used as a possible alternative to classic solid biodegradable scaffold in a bottom-up LbL approach for the processing of artificial organs. In this application, microparticles—composed of a material and encapsulated cells—represent a "bioink" that takes part in a scale-up technology. Two approaches can be explored in this concept: the placement of drops that harden in place, which can be considered analogous to an "inkjet" system, or the assembly of prefabricated "voxels," i.e., the spheroids is used as building blocks, as represented in Figure 1F.

These systems represent the arising of new demands in TE&RM: (i) high cell density for fast tissue assembly and (ii) the development of tissue maturation methods. Moreover, new approaches on materials design are required; although conventional TE&RM systems play a role of cell growth substrate, they are also expected to serve as mechanical support to the site of the defect while new tissue in in vivo conditions is formed; however, fast degradation rates are required in organ printing, so the microspheres can serve just as a support for cell growth and assembly in in vitro

conditions, and once mature cells are obtained, the remaining system is the new tissue. Organ printing advantages rely on the fact that it is an automated technique that offers a pathway for scalable, reproducible, mass production of tissue engineered products. The positioning of different cell types can be fully controlled in three-dimensional (3D) arrangements; high cell density tissue can be created and the problem of vascularization in thick tissue constructs can be solved even in in situ conditions.[22]

Jakab et al.[105] demonstrated that closely placed cell aggregates in 3D gels can self-organize into metastable tissue constructs of desired shape. Moon et al.[106] developed a bioprinter that can be used to print 3D patches of smooth muscle cells encapsulated in collagen enabling multilayered structures with controlled spatial resolution. In this work, a gradient of concentration of collagen could be obtained along the increasing height of the system, which shows the potential of this technique to create patterned 3D constructs. Rivron et al.[107] reviewed several aspects on tissue assembly and organization, such as the design of the initial geometry of the patterns, which showed influence in the arrangement of the structure, type of produced ECM and in the type of load mechanical forces, which can influence expression of genes and proteins, cell proliferation, and migration, among others. Other analyzed factors were the integration of signals, the manipulation of cell adhesion and ECM properties and cell as the integration of native features of the system, such as vascular or neural networks.

A close attempt to obtain shape-controlled systems is the application of mesoscale self-assembly, also known as the micromasonry approach—which relies in the self-regulated aggregation of smaller units to obtain an object with a desired shape. This concept can be observed in nature, for example, in the formation of coral reefs. Self-assembly is a natural attempt of lowering the energy of the system, and usually the most common interactions between the units are of hydrophilic/hydrophobic character.[108] Khademhosseini and co-workers[109] demonstrated a method for creating centimeter-scale cell-laden hydrogels through the assembly of shape-controlled PEG microgels randomly placed on the surface of a high density hydrophobic solution. The self-assembly process was guided by the surface-tension forces at the liquid–air interface. The same group also surveyed the current techniques for controlling cell aggregation, proliferation, and extracellular matrix deposition, as well as approaches to generating shape-controlled tissue modules.[110]

Microparticle aggregates

Regarding microparticle aggregation, two distinct approaches have been proposed, namely the production of scaffolds by microparticle agglomeration or the formation of hybrid systems mediated by cell aggregation.

Microparticle Sintering/Agglomeration. The microparticle sintering/agglomeration approach relies in the random packing of microspheres with further aggregation by physical or chemical means to create a 3D porous structure. Several works have been proposed aiming the production of 3D scaffolds, and both natural and synthetic materials have been used.

Although this approach is interesting because of the control of pore size of the scaffold by controlling the size of the particles,[111] avoiding typical procedures such as salt leaching[112] or gas foaming[26] and other porogen use, these structures lose their injectability potential. The porosity of the obtained scaffolds is generally low but the interconnectivity between the pores is kept.

Laurencin and coworkers have applied this technique for the development of synthetic-PLGA-based microsphere matrices for bone repair. The researchers have tried different approaches by developing sintered microsphere-based matrices[14,15,113] or gel microsphere matrices.[113] In sintered microsphere matrices, the microspheres were first obtained by a solvent evaporation technique. The 3D structures were then processed by heating the prefabricated PLGA microspheres above the glass transition temperature. The polymer chains were fused with neighboring polymer chains and thus form contacts between neighboring microspheres. In this case, no hydroxyapatite was incorporated in the particles. In the gel microsphere matrix methodology, the PLGA gel microspheres are obtained by emulsion with poly(vinyl alcohol) (PVA), and hydroxyapatite was incorporated. After agglomeration more production steps were followed including air drying, freeze drying, rehydration with salt leaching, and freeze drying again. Sintered particles showed to be more versatile than particles obtained by gel microsphere matrices and to have more adequate pore interconnectivity for bone TE purposes. Although the gel microsphere matrices showed higher elastic modulus due the incorporation of hydroxyapatite, the final pore interconnectivity was not appealing for cell proliferation.[113]

The in vitro behavior of sintered hybrid chitosan/PLGA microparticles has also been tested. Pore sizes, pore volume, and mechanical properties of the scaffolds could be manipulated by controlling fabrication parameters such as sintering temperature and sintering time. The presence of chitosan on microsphere surfaces increased the alkaline phosphatase activity of the preosteoblastic cells and upregulated gene expression of osteogenic markers.[114] Considering the sintering technique, a drawback resulting from the use of heat as a particle binder is the impairment of cell encapsulation in the particles or the possible denaturation of loaded bioactive agents.

Singh et al.[115] have prepared PLGA particles by precision particle fabrication (based on a spraying technique) and the integration between particles was achieved by ethanol soaking. Bilayered, multilayered as well as GF-containing-gradient-scaffolds were obtained. In a recent work, the same author has prepared microparticle-based scaffolds without the use of sintering agents such as heat and solvents, which are not cytocompatible. The cell viability was maintained using subcritical CO_2 for the sintering of the particles in the presence of cells at near-ambient temperatures. Moreover, the foaming properties of CO_2 allowed the structure to have interconnected porosity.[116]

Regarding the use of natural-origin polymers, Malafaya et al.[16] have developed chitosan scaffolds also prepared by agglomeration of microparticles. The particles were prefabricated by precipitation and agglomerated in a heat-induced process. Scaffolds obtained by this technique were proposed for osteochondral regeneration by the effective differentiation of adipose tissue-derived MSCs in osteogenic and chondrogenic media.[16] A bilayered scaffold was further produced, consisting of a composite part (composed of chitosan and hydroxyapatite composite) and a purely chitosan composed part, linked by a chemical crosslinking process, responsible for an integrative bone and cartilage interface. The scaffolds were cultured in a bioreactor, which allowed the immersion

of the different parts in different media, in which the bone part was able to mineralize and mineralization of the cartilage part was impaired.[117]

Chitosan particles were also preloaded with insulin to assess its effect in chondrogenic differentiation. High encapsulation efficiency and adequate release profiles were obtained, as well as chondrogenic differentiation of a prechondrogenic murine mesenchymal cell line.[118] The assessment of the mechanical properties was further performed, concluding that particle agglomerated structures show high mechanical properties, as well as the in vivo performance of the structures in rat muscle-pockets models, which showed that the in vivo functionalities can be obtained, including neovascularization of the tissue in early stages of implantation.[119]

In a different concept for the use of microparticles, the assembly of paraffin particles was recently proposed so they are used as sacrificial templates to construct scaffolds for TE. Free packet paraffin particles were precharged and further coated with natural-origin polyelectrolytes of opposite charges (positively charged chitosan and negatively charged alginate) using a 3D LbL methodology based on a perfusion technique; the particles were then leached out to produce scaffolds with walls just composed of nanostructured multilayers (Figure 1G), presenting mechanical integrity and high in vitro compatibility.[120]

Cell-Induced Aggregation. Isolated particles and cells previously incubated on their surface may be used to be injected to a defect using minimal invasive procedure and avoid aggressive strategies for cell detachment, such as the use of enzymes. Particle agglomeration after implantation is expectable as a consequence of cell proliferation and ECM, as represented in Figure 1E(a). Playing the role of a 3D scaffold, those systems emerged as an alternative for traditional TE systems, that is, porous scaffolds and hydrogels.

A traditional porous scaffold is not an injectable system, thus requiring invasive surgery procedures to place it in the defect spot. Obtaining a scaffold with the adequate shape of the defect may be achieved using computer tomography techniques and relying on the surgeon technique to adapt the scaffold to an ideal shape during the surgery.

Injectable hydrogels have been considered a convenient TE strategy[121,122] due to the possibility of cell encapsulation in a highly hydrated ECM-similar viscoelastic milieu where the cells proliferate,[2] allowing cell delivery after degradation of the hydrogel and avoiding cell seeding posterior to hydrogel manufacture. However, some disadvantages have been pointed to hydrogel systems: the ability to homogeneously encapsulate cells has been questioned because of the deposition of the cells on extremes of the hydrogel where the access to oxygen is easier,[21,123] and the cell spreading with a correct morphology due to the high content of water has not been observed. Microparticle systems for implantation share the advantage with traditional scaffolds of allowing cells to proliferate in a solid substrate, which is essential for the attachment of adherent cells. Such systems lead usually to construct with better mechanical properties when compared with hydrogels, which are not considered appealing for some tissue regeneration applications such as bone.

Microparticles for cell-induced aggregation have been produced from both synthetic materials and natural polymers. The mostly used synthetic material in the processing of microparticles in the context of cell-induced aggregation has been PLGA.[45,124–126] To obtain cell-induced aggregation of microparticles, PNIPAAm has been grafted with acrylic acid to control microparticle syneresis—a time-dependent shrinkage effect on thermoresponsive polymer hydrogels—which was proven to affect cell viability and proliferation, compromising the binding of the microparticles by cells.[127] Also, cell-induced aggregates were obtained using particles from a recombinant elastinlike polymer (ELP) in a study about the influence of particle crosslinking in the formation of aggregates.[128] The studied osteoblastlike cell line was sensitive to the variation of crosslinking degree of the particles, showing ability to form aggregates only in the highest crosslinking condition. Among natural polymers, gelatin microcarriers are the most commonly used for TE. In the majority of the cases, commercially available gelatin microbeads are chosen in this context as their cytotoxicity is already studied, production protocols are already optimized, and there is a wide availability of different sizes and porosities.[28,129–131] Chitosan particles have also been prepared by several processing techniques such as emulsion, spray drying, and ES.[27,132,133] The use of collagen has also been proposed as well as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV).[134,135] The production of blended constructs has also been proposed. PLLA microparticles have been grafted with chitosan-graft-lactose, which is nontoxic to chondrocytes and can stimulate the biosynthesis of chondro-specific ECM in a 2D culture.[19] The formed hybrid structures of cells/microcarriers have PLLA bulk properties, which maintain the cell phenotype, and are expected to deliver the chondrocytes to the cartilage defects in an injectable manner.

To stimulate osteoconductivity, PLGA microspheres have been immersed in simulated body fluid (SBF) to induce the production of apatite, obtaining a polymer/ceramic osteoconductive composite-coated system adequate for bone TE.[136]

The degree of aggregation can be regulated controlling different culture conditions, including mixing intensity in dynamic cultures, particle size, particles porosity, surface morphology and charge, oxygen tension, duration of culture, initial cell number, and degree of crosslinking, among others. The following paragraphs will focus on the discussion of the effect of these parameters in cell-induced aggregation of microparticle strategies and in supporting examples.

Stirring speed, which leads to shear stresses in the surface of the microparticles, has been shown to affect the differentiation of human pluripotent stem cells (HES-3), downregulating the expression of pluripotent makers, compared with the results obtained in static cell culture conditions.[137] On the other hand, the performance of dynamic cell culture in microcarriers loaded with osteoblastlike cells enhanced the expression of osteogenic markers and mineralization,[138] showing that this parameter requires specific study to different cell types. A problem related to the speed of agitation of the microparticles is the increasing probability of collision between particles with increasing speed, leading to cell structure damage, as well as to the decrease of cell/microcarrier contact time.[139]

Porosity of the particles is also an issue affecting cell proliferation ability. Macroporous systems allow increased surface area per bead, thus allowing the attachment of a larger number of cells.[139] Moreover, porosity permits an efficient diffusion of nutrients and oxygen and makes neovascularization of new formed tissue easier.[9,26,131]

Oxygen tension is an influencing variable in the differentiation of chondrocytes when cultured in microcarriers. Low oxygen tension did not affect the proliferation of these cells; however, it allowed for the maintenance of chondrogenic phenotype, avoiding undifferentiation.[140]

A high initially seeded cell number in the microparticles can allow a faster covering of the particles.[141] Considering an example of ceramic particles cultured with osteoblastic cells, when seeded at high density, the cells adhered well to the microsphere surface, bridging the surface gaps during the first 5 days and proliferating actively with prolonged culturing, covering the surface almost completely after 15 days. Although cells seeded at low density adhered well and multiplied with increasing culturing time, they could not effectively cover the entire surface of the microspheres even after 15 days of cell culture.[142]

Surface properties of the microparticles also influence cell behavior. For example, surface roughness and general topography influence the ability for cells to attach and proliferate.[28] For example, gelatin particles with diameter ranging from 280 μm to 350 μm were fabricated by cryogenic freeze drying and modified by incorporation of bFGF.[143] Microparticles with gyrus-patterned surface demonstrated the highest cell attachment rate and higher cell growth, in particular on bFGF combined particles. Also, the charge of the particle surface can determine the adsorption of proteins, which are the intermediates for efficient cell adhesion.[144]

The degree of crosslinking of the particles as well as the nature of the crosslinker can affect properties of the particles such as stiffness, which has been proved to affect stem cell differentiation[145] and osteoblastlike cell proliferation and mineralization ability.[146] The crosslinking parameters of microparticles also affect their degradation rate and the form of the degradation debris of the particles.[133]

Considering the time needed for aggregation of particles by cells, analyzing examples from Table 1, for particles ranging approximately from 50 μm to 350 μm, and considering different cell types, after 7 days of cell culture, particle aggregation has not usually occurred. However, in general, after 14 days, particle aggregation can be observed. Although these are the most common results, this does not mean that some cell types characterized by rapid proliferation rates cultured in optimal conditions could not induce a faster particle aggregation. On a bioreactor specially prepared for particle agglomeration, after 10 days of cell culture in porous particles, a skinlike cell construct was obtained in vitro, forming agglomerates of apparently 1 mm.[130] Considering in vivo studies, for example in rabbit knees, PLGA particles were injected in a 6×3 mm^2 $\times$ 2 mm defect, and after 6 weeks, a tissuelike structure was formed.[125] However, cell aggregation, as the phenomenon studied in in vitro conditions, started much earlier. In conclusion, the time frame in which cells are able to agglomerate particles may vary according to the tissue type and several cell culture conditions, which makes this topic a difficult, yet interesting, discussion.

Microcarriers seeded for short periods of time can provide constructs suitable for injection, which can be delivered into defects. However, in large defects, tissuelike materials obtained from larger cell culture times might be useful.

Several cell culture conditions showed to be effective in the formation of cell/microparticle aggregates. Static cell culture in tissue culture plates has been performed by Gan et al.[127] and Cruz et al.[132] However, to guarantee an even adherence of the cells in the particles, agitation of the particles—manually,[19] with an oscillatory stirrer[28,124] or using spinner flasks[126]—has been performed for a few hours before the culturing in static conditions. Dynamic cell culture has been proven to enhance mass transfer, improving the access of cells to oxygen and nutrients and an effective waste elimination. For cell expansion purposes, dynamic cell culture conditions using a spinner-flask bioreactor is the commonly used strategy, which has already been used for the formation of aggregates as well.[27,147]

In Table 1, the state of the art on cell-induced particle aggregation systems is presented, considering the used materials, processing methods, studied parameters, cell culture conditions, and intended tissue application.

An Overview of the Materials Used for Microparticle Production: From the Lab to the Market

Regarding the particular case of microparticles, one of the most widely used polymeric materials is PLGA, a slowly biodegradable, noncytotoxic, and Food and Drug Administration (FDA)-approved synthetic polymer. The use of synthetic polymers in TE&RM is known to show some hampers; although they can be easily modified to change their chemical composition and molecular weight, polyesters such as PLGA and PLLA release acidic degradation products, and in opposition to natural polymers, their chemical structure is not similar to the natural ECM.[149] Although natural-origin polymers contain domains similar to biological macromolecules, which are metabolically recognized, and generally avoid the stimulation of chronic inflammation and immunological responses, their mechanical properties are usually lower than the ones of synthetic polymers. However, an appropriate mechanical strength of materials is required in several TE applications, as well as efficient degradation profiles. The two opposite properties have been balanced by the combination of natural and synthetic polymers.[150,151]

The most commonly used natural polymer in particulate-shaped systems aiming for the production of scaffolds composed of agglomerates of particles—either by sintering or by cell-induced aggregation—is chitosan, a marine-derived polysaccharide. Besides possessing interesting chemical properties, such as groups that allow chemical crosslinking for the improvement of mechanical properties, biologically, it is a polymer with low cytotoxicity and antimicrobial activity.[152] Regarding cell encapsulation, alginates—an algae-derived class of polymers—are often used for their biocompatibility and mild ionic crosslinking conditions.[149] Gelatin has also been widely used to produce particles, either for cell encapsulation or for cell-induced aggregation. This animal-origin polymer, despite its considered cytocompatibility, has led to some problems such as contaminations and allergies, which are frequently seen as a limitation.[149] For cell expansion purposes, commercially available dextran (Cytodex 1 and Cytodex 2), collagen-coated (Cytodex 3), and gelatin (CultiSpher) microcarriers are available.[17] The most widely used polymer for this purpose is dextran, a natural polymer produced by some bacteria,[153] which has shown to facilitate cell attachment and proliferation of a wide range of cells.[17]

Stimuli-responsive materials have risen as an interesting way of controlling the behavior of biomaterials, for example, for delivery of bioactive agents in a controlled manner ("at-

Table 1. State of the Art of Microparticles Used for Cell Induced Aggregation. Comparison of Different Systems are Made Considering Material, Processing, Diameter, Pore Size, Cell Type and Culture Conditions and Applications

Material	Processing	Diameter (μm)	Pore Size	Studied Variables	Cell Culture Conditions	Cell Type	Application	References (Author, Year)
Synthetic polymers								
PLGA	Porogen leaching phase separation in a w/o single emulsion	~50	4.7 ± 1.0 μm	Study of the effect of the porosity on cell viability, proliferation and differentiation	Suspension cultivation in spinner flask followed by injection in mice	3T3L1 (preadipocyte cell line)	Adipose tissue	Chung et al., 2009[20]
PLGA	o/w emulsion and solvent extraction	30–80	–	–	In vivo study in 6 weeks New Zealand white rabbit knees	Articular cartilage chondrocytes from 6 weeks New Zealand white rabbits	Hyaline cartilage	Kang et al., 2006[125]
PLG	ProLease®	52–68	–	Effect of the encapsulation of $Mg(OH)_2$	Microsphere-chondrocyte system incubated for 4 h at 37°C in shaker incubator. In vitro culture in agarose-covered 12-well plates	Chondrocytes isolated from articular cartilage of 2–10-day-old calf gleno-humeral joints	Cartilage	Mercier et al., 2005[124]
PLGA	o/w emulsion solvent evaporation method	80–90	–	Different microcarrier surface charges	Initial seeding in spinner flask followed by constant agitation	Chondrocytes obtained from bovine joint	Articular cartilage	Chun et al., 2004[126]
PLLA (with chitosan-graft-lactose)	Emulsion solvent evaporation and posterior immobilization of chitosan-graft-lactose by aminolysis and subsequent grafting	80–120	–	–	Seeding and culture in a tube with agitation every 15 min of the first hour	Chondrocytes isolated from the cartilage of rabbit ears	Cartilage	Tan et al., 2010[19]
P(NIPAM-HEMA)	Free radical precipitation polymerization	–	–	Concentration of acrylic acid on syneresis of the microgels; consequences of the syneresis in cell adhesion and proliferation	Culture flask (static)	HepG2 cell line	Liver	Gan et al., 2010[127]
ELP	Superhydrophobic surface deposition	~800	–	Influence of two different crosslinking conditions in cell response and ability of cells to aggregate particles in vitro	Static cell culture	SaOs-2 (osteoblast-like cell line)	Bone	Oliveira et al., submitted
Natural polymers								
Gelatin	CultiSphere-GL	166–370	~30 μm	Influence of chondrogenic and osteogenic media in the differentiation; influence of shear stress in	Agitation for 5 min in the first 5 h after cell seeding and subsequent culture in spinner flask	Human dermal fibroblasts isolated from patients submitted to regular plastic surgery	Cartilage and bone	Sommar et al., 2009[129]

(*Continued*)

Table 1 *Continued*

Material	Processing	Diameter (μm)	Pore Size	Studied Variables	Cell Culture Conditions	Cell Type	Application	References (Author, Year)
				osteogenic differentiation				
Gelatin	CultiSphere-S	70–170	10–20 μm	In vivo regeneration of healthy dermis in humans in the presence of gelatin microspheres	–	–	Skin (dermis)	Huss et al., 2010[131]
Gelatin	CultiSpher-G	130–380	10–20 μm	–	Spinner flask bioreactor; study of aggregates assembly in a maturation chamber	Bovine dermal fibroblasts	Skin (dermis)	Palmiero et al., 2010[130]
Gelatin	W/o emulsion	280–350	–	Influence of different microparticles' surface topographies obtained by different drying processes	Cell culture under continuous agitation	Human keratinocytes (HaCaT) and fibroblasts (HFF)	General tissue engineering	Huang et al., 2010[28]
Chitosan	Spray drying and crosslinking with genipin	1–10	–	Cell adhesion and proliferation chitosan spheres crosslinked by genipin or gluteraldehyde and respective degradation rates	In vivo implant in Wistar rats by intramuscular injection (study after 3 days to 20 weeks)	–	General tissue engineering	Mi et al., 2002[133]
Chitosan	Emulsion crosslinking technique	70 $\pm$ 20 μm and 60 $\pm$ 10 μm for crosslinked particles with 20 and 40 mM genipin, respectively	–	Effect of the crosslinker concentration on cell adhesion and proliferation	Culture flask (static)	Goat bone marrow stromal cells	Bone	Cruz et al., 2008[132]
Chitosan	Electrospraying and thaw-refreeze method	Four ranges: -350 ± 10, -516 ± 13, -626 ± 20, and -890 ± 15	Respective to the four diameter ranges: -95 ± 3.1, -91 ± 2.6, -79 ± 2.7, and -48 ± 3.2 μm	Study of the effect of chitosan concentration in the size and morphology of the microparticles	Spinner flask	Human marrow stem cells	–	Maeng et al., 2008[27]
Collagen type I	Cellagen®	~150	–	–	Spinner flask	Trabecular osteoblasts acquired from patients undergoing total knee arthroplasty	Bone	Overstreet et al., 2003[147]
PHBV	w/o/w emulsion with solvent evaporation	Three ranges: -153.2, -242.5, and -361.8	–	Effect of the particle size	Seeding in a tube followed by cultivation in 24-well tissue culture plates	HepG2, Hep3B	Liver	Zhu et al., 2006[148]
Composites								
Apatite coated PLGA	PLGA microspheres were obtained by an o/w emulsion and solvent extraction t echnique followed by immersion in SBF for 5 days	~52	–	Comparison between the in vitro performance of PLGA and apatite coated PLGA microcarriers	In vivo implantation of microcarriers and rat osteoblasts in subcutaneous sites of athymic mice	Osteoblasts isolated from the calvaria of neonatal Sprague–Dawley rats	Bone	Kang et al., 2008[136]

Table 2. Examples of Well-Accepted Biodegradable Polymeric Particles Used for TE&RM Clinical Applications and Clinical Trials

Application		Material	Commercial Name	Loaded Agent/Cells	References
Dermal subcutaneous augmentation	Clinical use	PLLA	Sculptra®	–	157–159
Cardiovascular disease	Clinical trial	Alginate	–	FGF-2	160,161
Cartilage	Clinical trial	Alginate	–	Human mature allogenic chondrocytes	162
Pancreas	Clinical trial	Gelatin	Gelfoam®	Human pancreatic islets	163
		Alginate	–	Porcine pancreatic islets	164

will") or for cell retrieval from microcarriers.[154] The most common responsive polymer is PNIPAAm, which has a LCST around 32°C, which allows a change in the polymer conformation by simply varying the temperature. However, this polymer shows important drawbacks for TE&RM applications, as it is nonbiodegradable, limiting its implantation.

The relevance of new advanced polymers has recently increased in the microparticle processing field. Polymers such as silk fibroin, which shows enhanced mechanical properties and biocompatibility, have been used for the delivery of BMP-2.[84] Moreover, tailored polymers obtained by genetic engineering such as ELPs, which show reversible responsive behavior to several external stimuli such as temperature and pH, allow the total control of the structure of the polymer. This control allows the insertion of bioactive domains in the polypeptidic chain aiming, for example, for mineralization or improved cell adhesion, as well as for the control of the degradation of the structure.[155] Such polymers may be also used to produce thin smart coatings.[156]

Some examples of the use of microparticles in clinical field and already published clinical trials can be seen in Table 2. Despite the broad range of materials that have been proposed for TE&RM applications, the amount of biomaterials used in human clinical practice is very restrict, and still focusing in synthetic biodegradable polyesters such as PLLA. The range of applications is still restrained to aesthetic subcutaneous filling. Considering the performance of clinical trials, other materials such as PLGA and hyaluronic acid have already been authorized for clinical use in applications such as drug delivery and aesthetic filling, respectively.[165,166] However, published clinical trials with microparticles are still stuck in the use of widely studied and classical materials, such as alginate and gelatin (see Table 2). The use of advanced materials is still very limited in the context of microparticles for TE&RM, although promising data have been reported concerning response to external stimuli.

Conclusions and Future Perspectives

The use of microparticles in the medical field, which has started with drug delivery applications, has been adapted to TE strategies in several approaches. From the first explored concept of cell expansion, by van Wezel, in 1967, particles have taken part in complex cell therapy strategies (in nanoscale conditions)[3] as well as in the delivery of specific fragile macromolecules for induction of tissue regeneration. They have also been applied as tools for innovative TE scaffold construction, by their fusion or even as injectable systems for in situ scaffold formation, avoiding complicated surgery procedures.

However, new possibilities are open for the use of these versatile structures. Polymers that respond to external stimuli have been proposed for different biomedical applications;[154] however, there are not many studies reporting the use of particles made from such smart biomaterials in TE applications. Specific applications of such smart particles are in cell expansion, where cell detachment could be facilitated by the action of temperature or other variable, and in the controlled release of therapeutic soluble factors.

Surface micropatterning has shown to influence cell behavior.[167] Different approaches were already developed[168] to control the topography of surfaces or cell patterning in 2D substrates.[169] Interesting approaches could be achieved if one could transpose such surface modifications to 3D particles, which is a nonstraightforward task. Dipping techniques, such as LbL, are possible methodologies that permit to modify the surface in nonflat structures up to some extent.

Particles with material gradients along the radius could also bring new possibilities in tailoring the degradation profile or controlling the release profile of bioactive agents. Hydrogel particles could also exhibit a controlled spatial distribution of cells, or combinations of different cells, that could bring new insights in microparticles for TE&RM.

Hierarchical systems combining particles on other objects at different length scales could also be envisaged, to develop more sophisticated multiple release devices and to combine multifunctional features in microparticulate biomaterials. In resume, there are still different directions to be followed to design, produce, and use microparticles specifically in TE&RM applications. Such developments should be accompanied by efforts to translate the resulting knowledge into real clinical applications, where regulatory issues should be considered as well as the commercial viability of the products.

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