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Mini review

Macro-, micro- and mesoporous materials for tissue engineering applications

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Abstract: The design of three-dimensional materials with multiscale pore architecture currently represents a relevant challenge for tissue engineering. In the last three decades, degradable and resorbable biomaterials have been variously manipulated to generate macro/micro/mesoporous templates able to guide and facilitate basic cell activities concurring to the sequence of events triggering *in vitro* and *in vivo* regeneration of tissues. In this context, an accurate control of porosity features (i.e., pore size and distribution, pore interconnectivity) as a function of the peculiar properties of constituent materials is extremely demanded to not compromise scaffold mechanical properties and stability and replying local micro-environmental features from structural and functional point of view. Herein, an extended overview of consolidated and emerging approaches to design macro-, micro-, and mesoporous materials has been reported, underlining among differences mainly due to the peculiar properties of used biomaterials (i.e., polymers, ceramics, composites).

Keywords: porous materials; electrofluidodynamics; silica materials; tissue engineering; biomaterials

1. Introduction

Current approach in tissue engineering involves the use tailor-made 3D porous templates to guide all the main biological mechanisms—cell attachment, proliferation, differentiation and

subsequent extracellular matrix formation, both *in vitro* and *in vivo* [1]. The development of three-dimensional platforms with multiscale pore architecture is still a key point in tissue engineering. In the last three decades, biocompatible, biodegradable and porous materials have been manipulated to generate macro/micro/mesoporous networks able to guide and facilitate basic cell activities involved in the sequence of events that trigger the regeneration *in vitro* of new tissues (Figure 1) [2]. In agreement with the ideal regeneration strategy, porous scaffolds can promote the formation of new tissue by the morphological signals due to adequate spaces, namely porosity, and sufficiently extended surfaces able to direct cellular attachment, migration, proliferation, and desired differentiation of specific cell phenotypes throughout the scaffold. In this context, a proper definition of 3D scaffold architecture is crucial to reproduce all the required signals at the macro-, micro- and nanoscales, corresponding to tissue, cellular, and molecular sizes in a specific tissue, respectively (Figure 2) [3,4]. In particular, the fine control of pore size distribution in combination with the use of selected polymers with controlled degradation kinetics and in context with cells and molecular drugs, allows forming *in vitro* bio-hybrids to be converted in ex-novo tissues directly *in vivo*. The optimization of porosity and pore interconnectivity is also mandatory to improve oxygen and nutrient permeation and transport, and metabolite removal, in order to support the processes of tissue vascularization and/or mineralization, fundamental for late processes of regeneration [5]. Meanwhile, an accurate control of porosity features is also crucial to not compromise scaffold mechanical properties and stability, in order to reproduce micro-environmental features, from structural and functional point of view.

In the last years, the definition of highly controllable processing techniques (Table 1) enabled to design custom made scaffolds with different pore resolution or accuracy as a function of the specific technology used. Herein, we will illustrate different processing techniques for scaffold fabrication, distinguishing between conventional technologies, additive manufacturing and electrofluidodynamics.

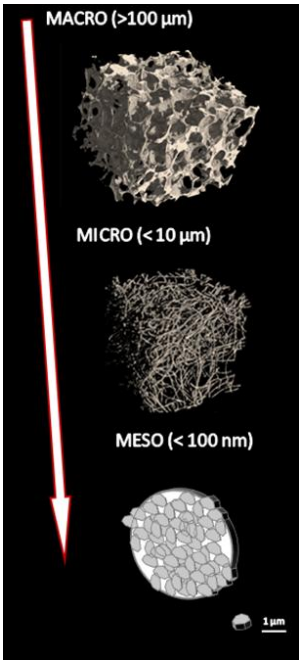

Figure 1. Pore architectures at different size scale: macro- micro- and mesopores.

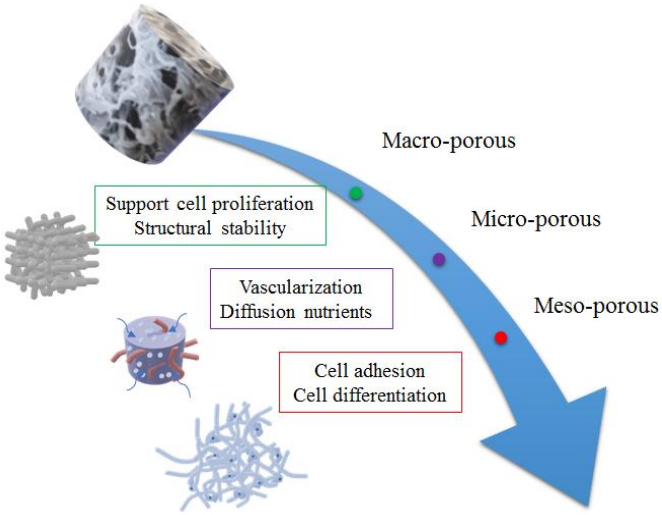

Figure 2. Pore architectures at different size scale: biological targets.

Table 1. Processing techniques to design scaffolds.

Processing technologies		Average pores (μm)	Compressive strength (MPa)	Refs.
Conventional	Thermal induced phase separation (TIPS)	0.10–400	1–100	[6,7]
	Salt leaching	50–300	11–28	[8,9]
	Solvent casting/particulate leaching	50–300	1–10	[10,11]
	Phase separation/salt leaching	150–600	0.1–1.5	[12]
	Gas foaming	20–500	3–50	[13,14]
	Emulsion freeze-drying	40–300	0.3–5	[15,16]
	Melt extrusion	400–500	8	[17]
Additive based	Stereolithography	300–500	14	[18,19]
	Selective laser sintering	30–800	10–20	[20]
	Bioprinting	50–500	0.1–5	[21]
Electrofluidodynamics	Air jet spinning	0.1–20	0.5–5	[22]
	Electrospinning	0.010–45	1–3	[23]

2. Conventional technologies

Conventional techniques are highly flexible methods to design 3D matrices which combine the multiple requirements to obtain optimal bio-instructive scaffolds. Commonly applied techniques for the fabrication of porous scaffolds include thermally induced phase separation [24], salt leaching [25], solvent casting/particulate leaching [26], phase separation/salt leaching [27], particle sintering [28], gas foaming [29], emulsion freeze-drying [30] and melt extrusion [6]. One of the most interesting strategies to design porous scaffolds (exceeding 95%) involves the use of phase separation techniques to generate porous network by binary or ternary polymer/solvent mixtures [29].

Basically, the polymer is dissolved in a solvent and a phase separation is induced by lowering the solution temperature [31] or adding a non-solvent to the solution [32]. Thermal induced phase separation or TIPS is a complex process, depending on the interplay between thermodynamic and kinetic evolution of the polymer solution cooling process. In particular, a liquid–liquid phase separation occurs when the applied temperature is higher than the solvent crystallization temperature or higher than the freezing point, while a solid–liquid phase separation takes place when the solvent crystallization temperature exceeds the coolant temperature [33]. Then, the system is cooled down to the desired quenching temperature using a ramp temperature profile, until to remove the solvent in order to obtain the porous structure. Solvent removal can be performed by either freeze-drying or freeze-extraction [34]. This technique assures the formation of an intrinsically interconnected porous structure through a simple fabrication process. The process can be tuned with manipulating the processing parameters to fabricate scaffolds with the desired characteristics and pore morphologies. Moreover, inorganic materials can be incorporated into the scaffold matrix to enhance the bioactivity and mechanical properties of the scaffold. Meanwhile, biologically active materials (i.e., structural proteins, polysaccharides) may be also used to promote the ex-novo formation of natural tissues. However, some limitation concerns the capability to customize the porous network along the scaffold thickness thus limiting the possibility to generate complex architectures to mimic hierarchically organized tissues like bone. Hence, the potential use of additive or subtracting manufacturing techniques in combination with physical/chemical principia of TIPS, may be successfully addressed to the fabrication of hierarchical structures which more accurately mimic the local microenvironment of hard tissues.

3. Additive technologies

Additive manufacturing technologies are forcefully emerging as feasible technological solutions to develop three-dimensional instructive scaffolds with highly ordered architectures for tissue and organ regeneration. They generally consist in layer-by-layer fabrication strategies suitable to reproduce porous platforms with complex shapes and microstructures since 3-D model data, by high degree of automation, good accuracy and reproducibility. They are currently used to generate different patterns to exert basic structural functionalities required to support viability of cells within the 3-D printed network. In the last 10 years, several studies have widely demonstrated the enormous potential of additive manufacturing (AM) to design tailor-made scaffolds to properly guide cell activities for the regeneration of different kinds of soft and hard tissues [35]. This is possible by an accurate control of the morphological features (i.e., pore size distribution, pore volume, and pore interconnectivity, anisotropy) which can be optimized by the implementation of less invasive processing routes able to easily manipulate either synthetic biomaterials than biological ones (i.e., proteins, polysaccharides) [36]. During the last years, a large variety of 3D printing-based techniques have been implemented to design porous scaffolds for the replacement of tissues and organs. Each technique presents benefits and disadvantages in terms of feasibility, material processability, strut resolution, and productivity [37]. For example, layer-by-layer structures can be fabricated by AM technologies based on ultraviolet (UV) light photopolymerization. They include stereolithography (SLA) [38], and selective laser sintering (SLS) [39] that reproduce complex designs by fast processing and high resolution. These methods allow fabricating 3-D scaffolds by hardening a photopolymer resin under the controlled exposure

to UV light or another similar power source. In these cases, much attention is required for the selection of cytocompatible photo-initiators to minimize damaging effects on cell membrane, protein, and nucleic acids, ascribable to the formation of free radicals that may potentially restrict their use in tissue engineering applications [40]. More recently, AM technologies have been also adapted to design 3-D *in vitro* models able to bridge traditional cell culture and *in vivo* modeling. This innovative approach allows predicting relevant aspects of *in vivo* behavior, only traditionally assessed by animal implants and/or human trials [41]. Respect to traditionally used *in vitro* 2-D models with significant limitations to recapitulate the complex tissue microenvironment [42], novel approaches based on 3-D bioprinting can combine main advantages of consolidated rapid prototyping (RP) techniques with innovative biofunctionalization strategies, thus providing much more physiologically relevant information about organogenesis, disease progression, and molecular release onto specific targets. In perspective, novel biomaterials in the form of powder or bioinks could be used to implement innovative approaches for a rational printing of cells and biomacromolecules derived from native extracellular matrix (ECM) in order to generate *in vitro* and/or *in vivo* tissue analogue structures. By the use of multiple bio-inks and cell types will be possible to guide *in vitro* and *in vivo* generation processes [43], to design and rapidly fabricate mechanically stable, functional, human-scale tissues such as the mandible, calvarial bone, cartilage, and skeletal muscles.

3.1. Electrofluidodynamics

Electrofluidodynamic techniques (EFDs) are emerging as highly flexible and low-cost AM processes able to manipulate biomaterials by utilizing electrostatic forces, giving the unique opportunity to design 3D ECM-like platforms to guide cells activities during *in vitro* regeneration/degeneration processes. By a solid knowledge of EFDTs fundamentals, it is possible to revisit conventional approaches in order to develop new cutting-edge strategies to process/assemble biomaterials in the form of micro-/nano- particles and fibers with intriguing properties for tissue engineering, cancer therapy and nanomedicine [44] (Figure 3). By the application of electric forces generated by high voltage electric fields, they allow generating ultrafine biodegradable fibers from micro down to the nanoscale. By an accurate optimization of process conditions, it is possible to fabricate different fiber-based platforms with multilevel architectures, able to variously interact with cells in order to trigger specific biological activities (i.e., adhesion, proliferation, cell metabolism) as a function of specific micro-, submicro- or nanotexture [45]. The main advantages of these techniques lie in a large customization of the process that allows the production of fibers made of different materials variously assembled by tailored experimental setups, to generate a plethora of different devices with peculiar topological (i.e., surface roughness, fiber anisotropy) or biochemical signals due to physical/chemical entrapment of biopolymers (i.e., proteins, polysaccharides) and/or active molecules (e.g., drugs, growth factors) [46,47]. Hence, EFDs and, in particular, the electrospinning, are the most promising processes to design temporary extracellular matrix (ECM) analogs by multicomponent fibers able to simultaneously confer structural properties and bioactivity to the scaffolds, to more efficiently support the main regeneration processes of the skeletal system (i.e., bone [48], nerve [49] and skeletal muscle [50]. Recent advances in nanotechnologies have allowed the renew of EFDs processes also to engineer more complex platforms able to deliver drugs and/or growth factors sustainably, timely and controllably to a specific target. For instance, Additive

Electro Spraying (AES) [51]—in other words, integration of electrosprayed nanoparticles into electrospun fiber network—has been proposed as an interesting route to control "separately" and "independently" release and functional properties of the scaffolds in order to address cell activities during the "ex-novo" formation of novel extracellular matrix. More recently, similar approach has been successfully proposed for the fabrication of bioactive coatings with interesting antimicrobial activity to fight resistant antibacterial populations in oral cavity [52], paving the way towards a new functional coating with no relevant increase of process complexity and resource costs.

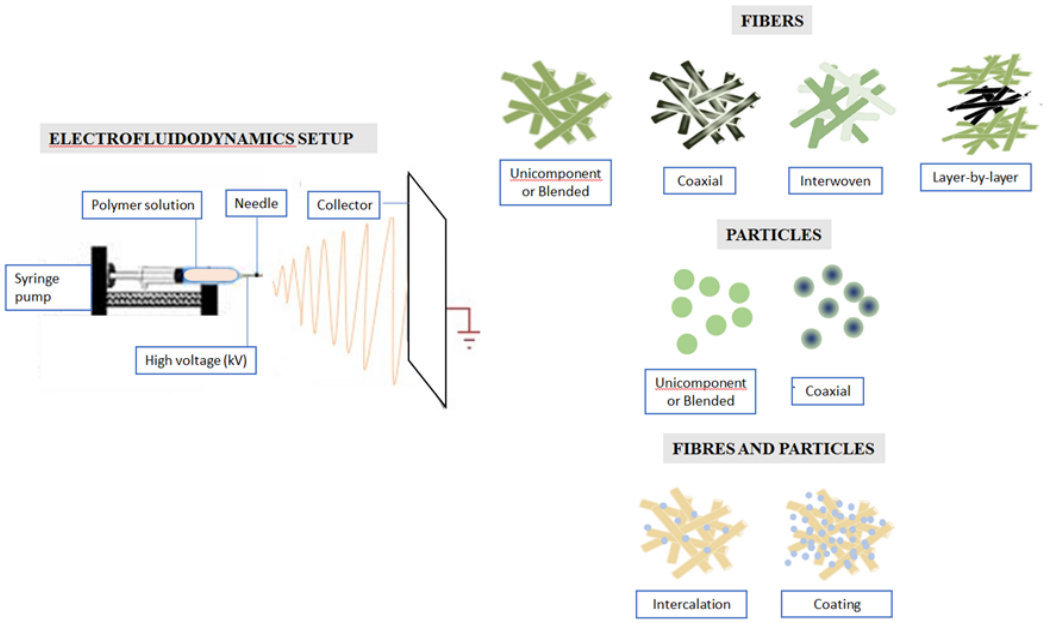

Figure 3. Electrofluidodynamic setups to design fibres and/or particles at micro- and submicro-scale.

4. Design of mesoporous materials

Among the nanoscale materials, a class of mesoporous materials—i.e., pores with a range between 2 to 50 nm—have become the subject of intense research worldwide because of their unique physical and chemical properties like tunable sizes, shapes (spheres to rods), uniform cylindrical mesopores and high surface areas easily for functionalization. Owing the mentioned structural features, the diverse range of biomedical applications as effective delivery vehicles for pharmaceuticals and bioactive molecules or as host materials for bioimaging, biocatalytic, and biosensing agents, have been widely recognized [53,54]. The vast majority of recent publications have centered around biological applications with a majority dealing with drug delivery systems. Several other bio-based articles on mesoporous systems concern biomass conversion and biofuels, magnetic resonance imaging (MRI) studies, ultrasound therapy, enzyme immobilization, antigen targeting, biodegradation of inorganic materials, applications for improved digestion, and antitumor activity [55–57]. The types of mesoporous materials range from carbon materials, metal oxides, metal sulfides, metal nitrides, carbonitriles, metal organic frameworks, and silica materials. Investigations conducted to elucidate the biocompatibility of these materials on biological systems, are still a subject of intense debate.

4.1. Mesoporous silica materials

The mesoporous silica materials (MSM) could be synthesized through hydrogels or sol–gel method and even its physicochemical properties can be designed according to the conditions used [58]. In the synthesis of these highly ordered porous materials, structure controlling agents of amphiphilic nature are used, and those agents are associated in supramolecular arrangements. The agents could be ionic, neutral or surfactants. Hydrothermal sol–gel synthesis is from large chains of cationic surfactants that act as templates or pore-forming agents. Depending on the materials used and the synthesis conditions, different pore sizes, types of ordered structure and particle morphology of silicon oxide can be obtained. The three phases generated by these materials are: hexagonal (honeycomb type), typical of MCM-41, SBA-15 and HMS; cubic, characteristic of MCM-48; and the laminar, is presented in the MCM-50 and is unstable. However, the MSM are group of materials for example the M41S family consists of the materials MCM-41, MCM-48 and MCM-50, with structures characterized by their respective X-ray diffraction diagrams [59]. The most stable and common phase at low concentrations of surfactant is the hexagonal form (MCM-41). The cubic phase (MCM-48) whose structure is more complex, consists of two systems of three-dimensional channels that accommodate one another forming a cubic symmetry. Finally, high concentrations of surfactant favor the formation of the lamellar phase (MCM-50) in which there is a stacking of mesopores that collapse after removing the surfactant by calcination. M41S mesoporous siliceous materials exhibit characteristics such as a narrow pore range, high surface areas and pore volumes, as well as high thermal stability [60]. Another group is SBA-type mesoporous materials have large pores, thick pore walls and show very high stability. Among this materials Santa Barbara Amorphous (SBA-15 and SBA-16) are amorphous mesoporous silica material, for example SBA-15 has a two-dimensional hexagonal hole structure cubic and cubic symmetry with large specific surface area and pore volume that could provide target sites for cell adhesion (Figure 4). Moreover, SBA-16 is considered as an interesting microarchitecture as it has a 3D cubic cage structure (body-centered cubic ordering of cages with 8 connecting entrances, Im3m symmetry) with multidirectional and large pore systems allowing good accessibility for functionalization [61,62].

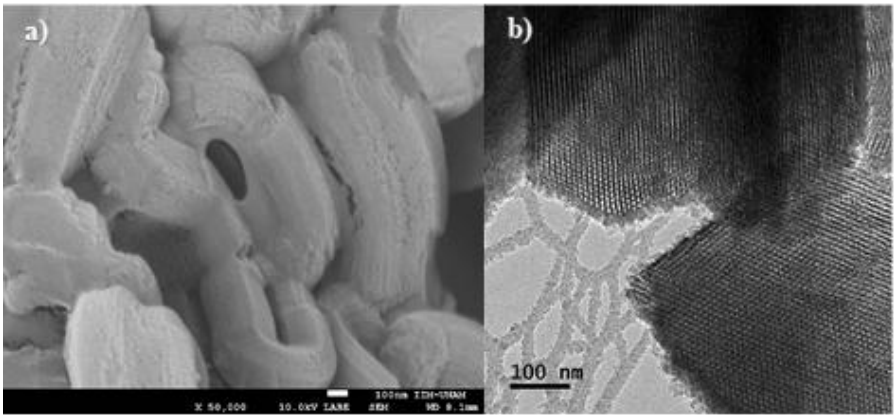

Figure 4. a) SEM of ceramic mesoporous SBA-15; b) TEM of ceramic mesoporous SBA-15.

Mesoporous materials are generally modified by attaching suitable functional groups on their surfaces, to serve as effective host materials for drug or bioactive molecules, biosensors, biocatalysts,

or site-specific bioimaging agents. The functionalized MSM can be different depending on how the surfactant templates are removed, because repeated exposure to organic solvents during solvent extraction accumulate free radicals, with unexpected negative consequences toward cells. However, calcination results in free of possible residual surfactants, generally preferable for biological applications [63,64]. The biological applications of MSM are ideal for adsorbing and holding up pharmaceuticals or bioactive substances into their mesopores and biologically active molecules, due to mesopore sizes, large pore volumes, high surface areas, highly ordered mesoporous structures and their easy surface functionalization. The dimensions of MSM pores are suitable size for passive delivery of drugs into cells, giving a biologically friendly material. The biocompatibility issues are mainly associated with how the MSM interfere with a variety of biological processes depending on the cell type. The nature and structural features of the MSM composition, make a more stable system for different biological applications. In addition, many types of MSM show nontoxic effects in biological systems if they are prepared and applied at the right dosages. For these reasons, MSM have long emerged as candidate materials for biomedical applications.

4.2. Mesoporous calcium phosphate materials

Calcium phosphate (CaP) materials are widely used in orthopedics and dentistry bone regeneration field, due to their structure and properties. For medical applications they are used in the form of bone cements, paste, scaffolds, or coatings. CaP family is composed of calcium cations and ortho, meta, or pyrophosphate anions, and sometimes hydrogen or hydroxide ions are present. CaP in form of calcium is commonly found in milk, blood, also the major inorganic constituents of bone, teeth and other calcified tissues. A wide variety of CaPs, from the individual phases to nano-CaP, biphasic and triphasic CaP formulations, composite CaP coatings and cements, functionally graded materials (FGMs), and antibacterial CaPs are studied [65]. CaPs can be manufactured in both porous and dense forms, as well as powders, granulates, coating or in the form of injectable systems. Their combination with hydrogels (i.e., PVA) may be also efficaciously used to modulate cement properties (i.e., setting or hardening properties) thus influencing micro- and mesopore architecture [66,67].

Among all calcium phosphates, those with designed and interconnected porosity present very interesting features to be employed in biomedicine, since their interconnected porosity allows transport of body fluids within the bioceramics, enhances their degradation, and increases the possibility of proteins and cells colonizing them [68]. Porosity of CaP is fundamental important for ingrowth of bone. Traditionally, CaP was macroporous (~100 μm). Biological and medical significance of calcium phosphates but studies have shown that increasing the pore volume may accelerate the kinetic process of biological apatite deposition. Mesoporous CaP bioceramics can permit the ingrowth of bone tissue and cells and therefore, enhance the bone formation [69]. Resorption serves to replace CaPs coating or cement with bone, either by cells or dissolution processes, dependent on the phase content of the CaP, particle size, crystallinity and porosity. Increasing porosity enhances the dissolution rate, which can explain the large differences in solubility of different HAp scaffolds [70]. For CaP mesoporous materials the rate of bone substitution may take 3 to 36 months, while the normal resorbability rate is between a few months and years [71]. This trait is important respect to short and long term biologically desired properties where mesoporous CaPs bioceramics, resemble the best prospect in biomaterials.

5. Biomedical applications of meso- and microporous composite scaffolds

Although porous and mesoporous materials (PMM's) were initially developed for catalysis, their potential was quickly discovered for many other applications such as sensors, optical materials, photocatalysis, electrical systems and above all in the field of medical research. It is precisely in this field in which scientists have shown a greater interest in PMM's as biotechnological materials due to its high potential to host different host molecules thanks to its high specific surface area and particular geometry. These characteristics and their low toxicity make promising PMM's vehicles useful for the release of drugs and as a biomaterial for tissue engineering regeneration strategies [72]. It was in 2001 when these materials were proposed, for the first time, as controlled drug release systems [73]. Its ordered porous structure and homogeneous pore size favor the reproducibility of the processes of adsorption and release of biomolecules, its high specific surface area gives them great adsorption capacity and its high pore volume allows to house the amount of drug required, also on its surface. There are silanol groups capable of reacting chemically with organic molecules in a process called functionalization that allows controlling the biomolecule-matrix interaction and thus modulating the adsorption and release processes. Subsequently, in 2006 it was demonstrated that these systems can act as implantable bioceramics with capacity for bone regeneration. Thus, the appropriate combination of these two characteristics has led to the development of bioceramics able to locally release drugs for the treatment of diseases related to bone tissue, such as bisphosphonates, very potent drugs that have low intestinal absorption and of which only small doses are necessary. These characteristics make these systems excellent candidates for use in bone regeneration technologies (Table 2) [74].

The design of advanced porous ceramic materials for the regeneration of bone tissue is one of the great current challenges in the investigation of biomaterials. The possibility of obtaining bioceramics as macroporous pieces would allow to apply them both in the in situ regeneration of bone tissue and in the manufacture of cellular solids for application in tissue engineering [75,76]. Given that the fundamental role in regeneration will be played by the cells, it is essential to obtain supports in the form of pieces with a designed porosity similar to that of natural bone and thus allow the tissue formed to fulfill its different physiological functions. Therefore, it is interesting to develop forming methods that provide interconnected macroporosity in the range of 20–1000 μm while preserving the intrinsic mesoporosity of the starting materials [37,77]. In this way, mesoporosity allows to harbor biomolecules of clinical interest and macroporosity allows the growth of bone tissue, its oxygenation and finally its vascularization. On the other hand, by functionalizing the external surface of the material with peptides or growth factors that act as osteoinductive signals, it is possible to attract bone-forming cells and induce them to fulfill their function [78,79].

Implantable materials may improve their osteointegration properties due to the presence of mesoporous CaP bioceramics. The bioactivity mechanism is the dissolution and release of calcium and phosphate ions to elevate the local concentrations and favor the precipitation of apatites on the ceramics surface [68]. Mesoporous CaP bioceramics are widely used in the field of bone regeneration, due to their good biocompatibility, osseointegration and osteoconduction that involves recruitment of progenitor cells, adhesion and proliferation of cells in the CaPs to differentiate towards the osteoblastic linage [69]. Even though, the compressive strength is fairly good, being higher than that of normal bone. CaPs mesoporous wider limitation for clinical application is the moderate mechanical properties. CaPs have low impact resistance related to their primary ionic bonds and

relatively low tensile stress (6 to 10 MPa) because of their porosity, which attend as initiation sites for crack propagation [71]. CaPs coatings, cements and scaffold have been integrated with both organic and inorganic materials, and polymers to control the biodegradability and bioactivity, improvement of the mechanical properties, corrosion resistance [80]. The use of mesoporous CaPs for healing bone defects, is limited. Many properties as setting time, cohesion/washout resistance, injectability, macroporosity, mechanical properties, long-term degradation, drug eluting properties, and biological response can be improved by adding a synthetic or natural polymeric phase [81].

Table 2. Main applications of mesoporous materials for tissue engineering.

Materials	Pore size	Structure	Key Results	Refs.
MCM-41	3–5 nm	Hexagonal	The principal advantages of mesoporous are the following: (1) a large surface area and pore volume provide great potential for drug adsorption and loading within the pore channels and (2) excellent mesoporous structure and an adjustable pore size enable better control of drug loading and release kinetics. *In vitro* bioactivity studies by soaking three different mesoporous materials, SBA-15, MCM-48 and MCM-41, in simulated body fluid showed an apatite-like layer is formed on the surface of SBA-15 and MCM-48 materials after 30 and 60 days, respectively, allowing their use in biomedical engineering for tissue regeneration. MCM-41 also exhibits a bioactive behavior when its walls are doped with phosphorus or when small amounts of bioactive glasses are added.	[59,60,64]
MCM-48	2–3 nm	3D cubic		
SBA-15	5–10 nm	Hexagonal		
CaP	20–500 nm	Porous and dense forms, powders, granulates	The osteoconductivity and osteoinductivity that CaP possess, make of them a suitable candidate for the regeneration of bone. Its surface topography guide protein adsorption and consequently cell adhesion. In addition to solubility and topography, CaP properties such as surface charge and crystallinity affect several key precursor events such as protein adsorption and cell adhesion, which ultimately modulate osteoblastic differentiation.	[61,62,69]

Examples for natural polymers are: alginate, chitin, chitosan, silk, hyluronate, cellulose, gelatin, soybean, albumen, collagen, and chondroitin sulfate. Synthetic polymers include polyethylene glycol (PEG), poly(ethyl) acrylate, polyesters and polyethers, polyacrylic acid (PAA), fibrin, PLGA, poly(glycolic acid) (PGA), polycaprolactone (PCL), poly-L-lactide acid (PLLA), amide fibres, polyamide fibres [81]. In tissue engineering, both synthetic and natural polymers are considered for scaffold development. Such scaffolds are similar to soft tissues that are present in the human body and the advantages of polymer scaffold are that they provide interconnected porosity, varying surface chemistry, and distinctive geometries for regeneration of tissues. The important properties of the scaffold can be enhanced by carefully selecting the polymers, with the appropriate constituent material and process of fabrication [40,82]. Moreover, synthetic polymers are preferred in the field of biomedical science because they show higher degradation and controlled porosity which can be tailored based on the application. The polymer/ceramic composites are a class of materials scaffolds

which possess properties that could biologically mimic the properties of bone and the dual benefit of both ceramic and polymer could be huge for tissue engineering (Table 3). Such composites exhibit better biocompatibility, enhanced mechanical property, controlled biodegradation and also increase osteogenesis ability [83,84].

Table 3. Comparison of composite porous scaffolds with different size scale of pores.

Nanopores	Macropores	Materials	Structure	Key Results	Refs.
Nanometric scale (5–10 nm of porogen, columnar (φ20 × 10 mm))	100 μm fully interconnectivity	PLLA/SBA-15	Highly ordered structure via layer-by-layer selective laser sintering (SLS). Porosity degree close to 50/60%	The number of cells on the composite scaffolds with SBA15 was much greater than that of scaffolds without SBA15 at the same time of culture. Compressive strength, compressive modulus and Vickers hardness were improved.	[69]
Sub-micrometric scale (~390 nm)	Micropores formed via Gel Casting Not interconnected pores	nHA/BioGlass /Alumina Composite	Interconnected porous structures with low porosity degree (20–25%)	The composite powder material showed no toxic effects on the MG-63 cell lines; the presence of nHAp and bioactive glass enhanced the biocompatibility and osteoconductivity of the scaffold material. The developed composite scaffold exhibited enhanced mechanical properties, with compressive strength of ~157 ± 2 MPa, tensile strength of ~83 ± 2 MPa.	[70]
Micrometric 0.7–1 μm	200–400 μm Partially interconnected pores	Mg/CHA particles dispersion in porous PCL scaffolds	Random pore architecture. Porosity degree exceeding 80%	Mg,CO_3-doped HA in PCL scaffolds support the *in vivo* cellular response by inducing neo-bone formation as early as 2 months post-implantation, and abundant mature bone tissue at the sixth month, with a lamellar structure and completely formed bone marrow.	[71,72]

Currently several studies have reported that polymer/ceramic composite scaffolds have improved in mechanical properties and cellular response. Kang et al. reported that the good attachment of minerals with polymer scaffolds enhanced the mechanical properties and improved the cell attachment of the polymer scaffolds [85], also Xu et al. reported a mesoporous silica composite scaffold which show that stimulates cell behaviors, had good bioactivity and improved the mechanical properties of the scaffold [86], Mondal et al. reported a nano-hydroxyapatite bioactive glass composite scaffold which exhibited 20–25% porosity, a high compressive strength of 157 ± 2 MPa, also facilitated new cell attachment, growth, and proliferation on its surface, all of which correlates with good osteoconductive properties [87]. Guarino et al. demonstrated that PCL

composite influenced the surface wettability with implications upon cell-material interaction and new bone formation mechanisms. In particular, ion substitution in apatite crystals positively influences the early *in vitro* cellular response of human mesenchymal stem cells (hMSCs), i.e., adhesion and proliferation, and promoted an extensive mineralization of the scaffold in osteogenic medium, thus conforming to a more faithful reproduction of the native bone environment than undoped HA particles and also at *in vivo* cellular response showed the neo-bone formation as early as 2 months post-implantation, and abundant mature bone tissue at the sixth month, with a lamellar structure and completely formed bone marrow [88,89].

6. Conclusions

The applications of macro-, micro- and mesoporous materials is shown to be a promising biomaterial for bone tissue engineering due to its biocompatibility with beneficial influence on structural characteristics of the scaffolds that could represent a new therapeutic strategy to repair bone defects. Moreover, macro-, micro- and mesoporous materials could be an excellent material for bone tissue engineering because its properties and it can ease to mold in various geometries, could give advantages to enhance the biocompatibility and the functionalization for delivery of growth factors and genetic materials to the bone contributing to prepare new kind of scaffolds for bone tissue regeneration.

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Conflict of interest

The authors declare no conflict of interest.

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