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Recent advances and future perspectives on porous materials for biomedical applications

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"The applications of porous materials in biomedicine range from their use as bioreactors, diagnostic devices and drug-delivery platforms to cell culture substrates for tissue engineering scaffolds"

Porous materials are emerging as functional materials for biomedical applications since they offer a high surface area, as well as tunable pore features. The pore size, shape, chemistry, and softness can be customized by the organic or inorganic nature of the material and the synthesis method [1]. According to their pore size, the International Union of Pure and Applied Chemistry classifies them into microporous (<2 nm), mesoporous (2− 50 nm), and macroporous (>50 nm) [2]. Nowadays, the applications of porous materials in biomedicine range from their use as bioreactors, diagnostic devices and drug delivery platforms to cell culture substrates for tissue

engineering scaffolds. For each case, the pore structure requirements are different, and their performance is strongly related to the pore properties [1,3].

In general, mesoporous materials are synthesized using soft or hard templates. Soft-templating involves the use of block copolymers or surfactants which self-assemble to define the pore structure, whereas hard-templating (nanocasting) uses a rigid mold with the desired pore morphology and size. Further, the water droplet templating method was developed to fabricate mesoporous honeycomb-like films. The procedure is performed under a humid airflow, in which water droplets in the air act as templates. A novel and powerful technique, 3D-printing, consists of the layer-by-layer deposition of material (natural or synthetic) from a predesigned computer model that enables creating structures with different pore sizes and shapes with high accuracy. At lower scale, polymeric porous materials can be easily prepared by the crosslinking reaction of polymer chains, giving rise to polymeric networks constituting hydrogels with pores in their inner structure [4].

Reliable characterization of the complex pore structures is still a major challenge [5]. There are experimental techniques for the characterization of porosity such as gas adsorption, X-ray diffraction, small angle X-ray, X-ray computed tomography, mercury porosimetry, scanning and transmission electron microscopy, thermoporometry, nuclear magnetic resonance methods, positronium annihilation lifetime spectroscopy, and electron tomography. However, each method offers a limited length scale of applicability [6]. Surface area and pore structure analysis by gas adsorption is still the most popular technique because it allows assessment of the most complete range of pore sizes, from micropore to mesopore, and to some extent, even macropores (pore width > 50 nm) [7]. Nitrogen gas at liquid nitrogen temperature (77 K / -196.15 °C) is the preferred choice for gas adsorption studies due to the ease of accessibility of liquid nitrogen in most laboratories and a long history of use as adsorptive. Adsorbed amounts of N_2 are measured (volumetrically or gravimetrically) as a function of relative pressure (absolute/saturation pressure). The resulting isotherms reflect mechanisms of pore filling, the physics of which can be used to infer pore volume and pore size distributions [8]. In addition to gas adsorption, mercury porosimetry is used for the characterization of larger macropores up to 400 μm. Controlled pressure is applied for mercury penetration into the pores of a material. The amount of pressure required is inversely proportional to the pores size: the smaller the pore the higher the pressure needed to penetrate into the pore. Therefore, the combination of nitrogen adsorption and mercury

porosimetry is important for the characterization of porous materials; together, these techniques allow the elucidation of the pore structure over a wide range from pore widths <4 nm up to at least ≈400 μm [5].

For drug delivery applications, porosity plays a key role in the drug encapsulation capacity and in the controlled release. Engineering the pore size and its response to environmental changes enables one to (a) encapsulate molecules with different properties, (b) decrease their undesired release by partial diffusion before reaching the target site and (c) program a sustained or burst drug release, thereby decreasing secondary effects. Dendritic polymers, for instance, have an inherent porous structure that has been used to encapsulate small molecules as cargo. As they have well-defined structural components, i.e., core, interior and surface, the functionalization of each of these sections allows one to control the type of molecules to be hosted in each part of the dendritic polymer. A recent example emulated the dendritic structure in the highly popular mesoporous silica nanoparticles, achieving radial pore morphology, with large pores (>3–50 nm). Such nanocarriers showed to have 100% encapsulation capacity of the anti-inflammatory interleukin (IL)-22 and an excellent stabilization and transport through intestinal membranes [9]. An alternative approach that has gained interest in the last few years, is the use of crosslinked polymeric materials with nanometer dimensions, so-called nanogels. The pore properties of nanogels can be easily controlled by the monomer composition and by modulating the crosslinking density. Thermoresponsive polymers are commonly used to build nanogels that respond to temperature variations caused either by gradients in the tissue or by external sources. N-isopropyl acrylamide (NIPAM)-based nanogels have been used to deliver proteins into the skin, to treat genetic skin diseases [10]. The inherent skin thermal gradient was shown to be sufficient to trigger the collapse of the nanogels' pores, resulting in the release of the encapsulated protein. Controlling crosslinking parameters like density and crosslinker size, was shown to have an effect on the protein encapsulation and release performance [11].

The attractiveness of porous polymeric materials goes beyond their use as mere transporters of small cargos, as they are currently being applied as shielding scaffolds of functional proteins. The combination of proteins with artificial materials able to alleviate the damage caused by the environment, e.g., proteolytic activity of proteases, on their natural conformation is of high importance for the therapeutic applications of those. Specifically, the use of polymer nanogels is meaningful for biocatalytic proteins, i.e., enzymes, due to their intrinsic properties, i.e., high water

content, their flexibility, and their high porosity. Additionally, the multipoint interactions between the polymeric chains and the amino acids exposed on the surface of the proteins usually enhance the performance of the biomacromolecule. Enzyme-nanogel hybrids are thereby emerging as stable, highly active therapeutic bioreactors for severe diseases, e.g., ischemia or organ injury [12]. Such bioreactors consist in the embedment of antioxidant enzymes into flexible and porous nanogels that allow the entrance of reactive oxygen species through the polymeric network and its transformation into harmless compounds, which are released from the bioreactor [13]. The porosity and the thickness of the nanogel must be here controlled to shelter the protein from environmental insults and, at the same time, to reduce the diffusional issues that would exclude the substrates to reach the active site of the protein. Importantly, the porosity of these protein-nanogel systems is closely related to the flexibility of the scaffold. Highly entangled networks, with smaller pores, will increase the stiffness of the envelop and, consequently, the dynamism of the inner enzyme required to perform the catalysis can be jeopardized [14]. Therefore, a thorough control on the porosity of the shelter material is key for the operability of the bioreactors.

Tissue engineering scaffolds represent not only a mechanical support that replaces the damaged tissue they are implanted on, but also a niche where cells can grow, proliferate and eventually deposit a healthy and functional tissue. From the earliest definition of tissue engineering, it seemed clear that scaffolds would require the use of materials that permit certain fluid flow, to transfer nutrients to cells and allow for waste removal [15]. Nowadays, scaffolds can be developed on the shape of hydrogels, sponges, fibrous materials, 3D printed mesh structures and more. One of the main characteristics that define these different types of scaffolds are the architecture and water entrapment capacity that, at the same time, are defined by the macroporosity of the structure. In fact, the porosity of the fabricated scaffolds (size, shape and degree of porosity) has been shown to affect the transport of nutrients, the local mechanical properties and even the oxygen tension sensed by cells within. In turn, these changes on the cell microenvironment result on the regulation of important parameters such as the metabolic activity and the cytoskeletal tension that downstream affect the regulation of signal transduction and ultimately cell survival and fate [16]. Lately, it has also been demonstrated that the porosity of the scaffold can also determine the likeliness of vascularization to occur when implanted *in vivo* and somehow modulate the immune response of the host [17]. Thus, porosity has become a key parameter in the design of scaffolds for tissue engineering applications [18].

All examples discussed above highlight the versatility of porous materials and the extensive applications that they can offer in the biomedical field. Despite the huge knowledge gained about their applicability in drug delivery, few clinical studies have been reported for polymeric materials. Future studies should be focused on exploring their biodegradability and clearance, as well as their pharmacodynamics and pharmacokinetics before performing clinical trials [19]. Regarding enzyme-nanogel hybrids as bioreactors, the next step forward to a scalable application should be focused on improving the reusability and storage of the bioreactors and on reducing the mass transport issues of the substrates through the porous envelop, while preserving the integrity of the enzymes [20]. For porous materials applied in tissue engineering, the challenge remains in providing pore sizes relevant to the cell microenvironment (≈ 100 μm) while allowing for controlled spatial distribution and interconnectivity of these in a reproducible manner and further preserving the integrity of the scaffold until the maturity of the growing tissue [21]. In general, future efforts should be focused on overcoming the synthetic challenges to reach their real applications in clinics, for instance, the development of more scalable synthesis procedures, which still allow control of the pore features.

Author contributions

All authors contributed to the manuscript writing. All authors have read, corrected and approved the final version of the manuscript.

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