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1 **Reviewing recently developed technologies to direct cell activity** 2 **through the control of pore size: from the macro- to the nanoscale**

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13 14 **Abstract**

15 Scaffold pore size plays a fundamental role in the regeneration of new tissue since it has been
16 shown to direct cell activity *in situ*. It is well known that cellular response changes in relation with
17 pores diameter. Consequently, researchers developed efficient approaches to realize scaffolds with
18 controllable macro-, micro- and nanoporous architecture. In this context, new strategies aiming at
19 the manufacturing of scaffolds with multiscale pore networks have emerged, in the attempt to
20 mimic the complex hierarchical structures found in living systems. In this review we aim at
21 providing an overview of the fabrication methods currently adopted to realize scaffolds with
22 controlled, multisized pores highlighting their specific influence on cellular activity.

23 24 **Keywords**

25 Scaffold pore size, cell activity, hierarchical structure, multiscale pore architecture, cellular
26 response.

27 1. Introduction

28 In tissue engineering, cell fate can be modulated through several methods aiming at directing cell
29 response to achieve the formation of a healthy new tissue. The composition, morphology and
30 surface topography of scaffolds can furnish the right cues to guide cells in the generation of the
31 newly developed tissue. Among others, pore size plays a fundamental role in defining topological
32 features which contribute to obtain a functional interface between cells and material [1, 2]. Tuning
33 scaffold pore size can serve to mediate cellular response *in situ* (similar to surface
34 functionalization) through the tailoring of cell cytoskeleton arrangement. Cell membrane receptors
35 interact with the multiscale topographical features of the scaffold inducing cytoskeleton
36 deformation and assembly with a direct effect on cell functionalities (adhesion, proliferation, gene
37 expression) and morphologies [3]. Hence, scaffold pores with controllable diameters over multiple
38 length scales were developed to mimic complex living hierarchical structures. For instance, several
39 studies investigated human bone topological features to resemble its unique hierarchical structure
40 at different scales [4, 5]. Pore size affects the response of the hosting cells in a different way [1]:
41 nanopores (< 300 nm in size) [6, 7] promotes cell adhesion increasing the surface area, micropores
42 (0.3-100 μm in size) [6, 8] enhance the permeability of the scaffold and facilitate cell migration
43 while macropores (>100 μm in size) [9, 10, 11] provide space for vascularization and tissue
44 ingrowth, favor gas diffusion, nutrients supply and waste removal (Figure 1). The effect of pore
45 size on cell activity has been extensively investigated (Table 1), as it represents an efficient mean
46 to modify the tissue response *in vivo* by acting on geometrical features instead of compositional
47 cues [12] [11]. However, gaining a full picture of how different cell types react to constructs with
48 certain pore size is still a challenge for tissue engineering, as well as applying available
49 technologies to produce scaffolds with hierarchical porous structure and high pore

50 interconnectivity [13]. Several techniques have been adopted to optimize the manufacturing and
51 modelling of structures with controlled, engineered pore size across a variety of length scales.
52 They include conventional fabrication techniques such as salt leaching, gas foaming, phase
53 separation, freeze-drying, freeze-casting, solid-state porogen thermal decomposition, cell
54 encapsulation, electrospinning [14, 15, 1]. Nonetheless, these traditional methods do not allow to
55 obtain a precise control of scaffold architecture and to achieve reproducible size and shape of pores
56 [16, 17]. Additive manufacturing (AM) techniques using computer-aided design (CAD) modeling
57 introduced remarkable improvements in terms of repeatability and accuracy on scaffold micro-
58 and macrotopography. Despite that, a strict control of nanopores is difficult to achieve with AM
59 technologies. So far, stereolithography, selective laser sintering, selective laser melting, electron
60 beam melting, 3D-bioprinting, direct laser writing, fused deposition modeling are the more
61 common AM technologies applied in tissue engineering [18, 19]. Furthermore, the use of AM
62 technologies allows to control not only the pore size but even the pore geometry which has been
63 recently reported to be an effect on cell response [10, 20].

64 In this review we analyze recent papers published in the last five years, in which the specific effect
65 of pore size on cell activity has been investigated. The influence of nano- micro- and macropores
66 size on tissue response are illustrated in the following sections, together with the manufacturing
67 techniques used (Table 1). Finally, we also report current examples of novel approaches applied
68 to achieve hierarchical structures with multiscale pore architecture.

69

70 **2. Nanopores**

71 The scaffold nanofeatures are largely studied since cell-cell and cell-substrate interactions
72 occurred at the nanoscale. Briefly, when cells interact with a substrate, they explore the

73 environment by expanding lamellipodia and filopodia [21]. When the substrate is suitable for the
74 attachment, cells develop focal adhesions (FAs) that successively elongate and generate mature
75 adhesions known as fibrillar adhesions (FBs) [22]. The evaluation and characterization of FAs and
76 FBs allow analyzing the influence of nanotopography on cellular activity. Many approaches have
77 been used to fabricate nanotextured surfaces from a large variety of materials with the aim to
78 investigate the effects on cell behavior. Among others, the presence of nanopores can affect cell
79 response and scaffold architecture can be optimized to achieve the wanted biological effect. For
80 example, Zhang et al. [23] developed a porous hexagonal molybdenum sulfide (MoS_2)
81 nanostructure, composed by many interconnected nanoflakes with a size of 5–8 nm. The authors
82 fabricated the nanoporous architecture by a bottom-up hydrothermal method using the fluorine-
83 doped tin oxide (FTO) coated glass as substrate [24]. This fabrication technique involves the use
84 of high temperature (400 °C) and pressure for growing single crystals from an aqueous solution
85 [25]. The high-resolution transmission electron microscopy (TEM) images showed the MoS_2
86 nanoflakes created a lattice spacing with a pore size of 0.63 nm. The effect of the nanostructured
87 MoS_2 biointerface on mesenchymal stem cells (MSCs) attachment, spreading, and formation of
88 focal adhesion was studied, by considering a flat substrate as control. Scanning electron
89 microscopy (SEM) revealed the presence of more protrusions on cells grown on the nanoporous
90 MoS_2 compared with that on control. Moreover, vinculin expression of cells on the flat substrate
91 was low, whereas the vinculin intensity increased significantly for that on the nanostructured
92 MoS_2 , demonstrating a higher concentration of FAs. Besides, the enhanced cell adhesion on the
93 nanoporous surface, authors proved the nanotopography capability to induce the osteogenic
94 differentiation of MSCs (Figure 2). Another recent study conducted by Greiner et al. [26]
95 demonstrated for the first time that 31.93 ± 0.97 nm pores present within endogenous collagen type

96 I fibers are sufficient to induce the osteogenic differentiation of human stem cells. A collagen-like
97 scaffold which mimics the collagen pore structures was developed by self-assembly of silicon
98 dioxide (SiO₂) nanoparticles linked together by a thermally induced crosslinking reaction of oleic
99 acid molecules [27]. The obtained substrates showed pore size of 34 ± 14 nm that directly lead to
100 the successful osteogenic differentiation of adult neural crest-derived inferior turbinate stem cells
101 (ITSCs). In contrast, nanocomposites with 18 ± 4 nm pores and flat glass substrates did not induce
102 the differentiation of ITSCs. This SiO₂ porous nanocomposite could be employed as coating for
103 micro- or macroporous scaffold to mimic the physiological bone architecture and guide the
104 endogenous stem cells towards the osteogenic phenotype. The influence of a nanoporous structure
105 on cell behavior was also analyzed by Merhie et al. [7] who used the electrochemical anodization
106 process to produce the anodic porous alumina (APA) substrate. Anodizing is an electrolytic
107 process which allows to obtain oxide coatings of 5 to 25 μ m in thickness on a metallic component
108 placed in acid solutions normally under DC voltages. Oxidation occurs at the surface, resulting in
109 the formation of a porous oxide film that is adherent to the underlying metal substrate [28]. The
110 porous architectures obtained presented various pore sizes (approximately 60, 80, 100 and 120
111 nm) depending on different exposition times of etching solution (0, 10, 20 and 30 min). The Neuro-
112 2A (N2a) mouse neuroblastoma cell line seeded on each nanoporous substrate adhered and
113 differentiated mainly on the substrate with small pores. Indeed, SEM and confocal fluorescence
114 images showed neuron-like cell shape, with several neuritic extensions, whereas for substrates
115 with larger pores, the cell cytosol appears with no preferred direction. Many studies reported the
116 fabrication of nanopores on the titanium (Ti) surface applied in bone implants [29, 30, 31, 32, 33,
117 34, 35]. For instance, oxidative nanopatterning [36, 37] and electron-beam lithography (EBL) [3]
118 can be applied to form nanopores on Ti alloys. The oxidative patterning/etching is a chemical

119 surface treatment that produces nanoporous networks by exposing the substrate to oxide solutions
120 [38]. In EBL, on the other hand, a resist layer is directly nanopatterned by directly writing on the
121 surface with focused electron beams [39]. These nanofabrication techniques allow to enhance the
122 anchorage of the implants by increasing adhesion, migration, proliferation and mineralization of
123 osteo-like cells and MSCs.

124 Recent literature highlighted the role of nanopore size in mediating cell attachment, proliferation
125 as well as differentiation and maturation. So far, nanopores are mainly applied for hard tissue
126 devices [40] but the effect on other cell phenotypes has been recently investigated [7] thanks to
127 novel nanofabrication technologies which offer the possibility to tailor nanometrical pore size on
128 both metals and polymers in order to engineer the most promising conditions to each tissue
129 engineering application.

130

131 **3. Micropores**

132 The microporous structure has a leading role in the interaction among small molecules and proteins
133 as well as in the mechanical properties of the substrate at cellular level. Indeed, interconnected
134 micropores directly influence the scaffold porosity, as porosity is related to the volume of empty
135 pore space present in the construct. Therefore, micropores have a key role in scaffold permeability,
136 protein adsorption and biodegradation rate. Furthermore, micropores induce a capillary force that
137 anchors cells to the surface and drives them to migrate within the 3D structure [41]. Stachewicz et
138 al., [18], compared the pore size produced by electrospinning polylactide-*co*-glycolide acid
139 (PLGA) scaffolds in two configurations: aligned and randomly oriented nanofibers. Indeed, the
140 electrospinning technique allows to fabricate nanofibrous mats by extruding a polymer solution
141 contained into a syringe through a high potential difference between the metallic needle and a

142 collector [42]. The average pore sizes for the aligned and random fibers were $0.92 \pm 0.57 \mu\text{m}$ and
143 $2.30 \pm 1.33 \mu\text{m}$, respectively. *In vitro* tests showed that the proliferation of MC3T3-E1 cells was
144 much limited for aligned fibers as the size and circularity of pores were larger for the random
145 fibers' construct. The electrospinning technique was also used by Abeyayehu's group [43] to
146 fabricate fibrous scaffolds of various morphologies made from polydioxanone (PDO). Different
147 pores and fibers diameters were obtained by varying the initial solution concentration: 60 mg/mL
148 scaffolds featured fibers with a diameter of 400 nm and pores with an approximate diameter of 1.5
149 μm , while 140 mg/mL scaffolds contained fibers with a diameter of 2.4 μm and pores with a
150 diameter of 18 μm . The authors examined how scaffold architecture affected both mast cell
151 inflammatory response and angiogenesis. More specifically, they analyzed the only effect of pore
152 size by altering pore diameters without changing fiber size. With this aim they used an air-flow
153 mandrel approach, which increases the average pore size throughout the scaffold (from
154 approximately 1.5 μm to 4.5 μm). The bone marrow-derived mast cells (BMMC) were then seeded
155 and the immune signals IL-33 and LPS were evaluated through the ELISA test. The results
156 highlighted how the presence of micropores can modulate inflammatory cytokine secretion and
157 the angiogenic response, thus demonstrating that large micropores reduce the inflammation and
158 promote angiogenesis.

159 Finally, a scaffold with precise architecture and microporous structure was produced by using melt
160 electrospinning technology [44] obtaining a 3D mesh with a pore size of 50 μm from a top view
161 perspective. Melt electrospinning technique forms well defined filaments with small diameters that
162 can be deposited into 3D architectures using additive manufacturing principles (Figure 3) [45, 46].
163 The effects of microfibrillar architectures on human skeletal stem cell (hSSC) behavior were
164 investigated in terms of cell geometry and yes-associated protein (YAP) expression. An increase

165 in nuclear YAP expression, collagen formation and mineral deposition was observed at 24h post
166 seeding. Moreover, cells appeared spread and elongated on the surface, demonstrating the
167 influence of 3D fibrous extracellular matrix (ECM)-like architecture on hSSC behavior.

168 The possibility to modulate micropore size is a key strategy to improve the biological outcomes of
169 tissue engineering device in terms of both cell regrowth and inflammatory process. To date, only
170 few advanced processing technologies having a strict control of 3D pore size are available (e.g.
171 electrospinning and melt-electrospinning) with a restricted number of processable materials.
172 Therefore, the development of 3D architectures with optimized micropore size still remains a
173 challenge for many applications and new technologies are required to process a wide variety of
174 materials controlling their pore size at the microscale.

175

176 **4. Macropores**

177 The diffusion of nutrients and oxygen is an important feature in the design of a bioengineered
178 implant and can be modulated by tailoring macropores shape and size. Macroporous structures
179 provide space for angiogenesis by allowing cellular infiltration and the development of vascular
180 system within the scaffold. Indeed, a rapid vascular infiltration is needed to sustain tissue ingrowth
181 *in vivo*, in addition to efficient gas diffusion and nutrients supply [47]. Recently, various studies
182 were conducted to define the ideal macroporous patterns to direct cellular activity. For instance,
183 Torstrick and coworkers [48] proposed the use of the salt leaching technique to realize a
184 macroporous structure with pores size determined by salt particle diameters. In salt leaching
185 method, salt crystals are blended with a polymer solution or placed into a mold and a polymer is
186 then added to fill in the remaining spaces. The polymer is subsequently hardened, and the salt is
187 removed via dissolution in a solvent such as water or alcohol [49]. This technique has been adopted

188 for many years as it allows to achieve a precise control of the pore size and pore morphology [50,
189 51, 52]. In this study, the authors fabricated a porous polyetheretherketone scaffold (PEEK-SP)
190 using sodium chloride with different sizes (200 to 312 μm , 312 to 425 μm , and 425 to 508 μm).
191 The influence of pore size on cellular response was evaluated seeding human femoral osteoblasts
192 and human MSCs (hMSCs) on PEEK-SP and comparing osteogenic differentiation of cells to
193 smooth PEEK. The *in vitro* analysis proved the superior ability of PEEK-SP to induce bone cell
194 proliferation and differentiation. The particulate leaching method was also adopted by Zhao et al.
195 [53] who fabricated 3D porous PCL scaffolds with different macropore size to evaluate the hMSCs
196 response to the macrotopography. Porous scaffolds were produced using paraffin microspheres
197 (100–200 μm , 200–300 μm and 300–450 μm) as porogen. After porogen removal, the surface was
198 functionalized through hydrolysis or aminolysis. The analysis indicated that the hydrolytically
199 treated scaffolds, with a pore size of 200–300 μm , better supported cell growth, while the
200 aminolytic scaffolds performed best with a biggest pore size of 300–450 μm . Regarding both the
201 osteogenic and chondrogenic differentiation of hMSCs in these scaffolds, the deposition of
202 minerals and glycosaminoglycans (GAG) suggested the successful differentiation mainly occurred
203 in constructs with the largest pore size of 300–450 μm despite the variation in surface chemistry.
204 Walthers et al. [54] investigated the critical amount of angiogenesis necessary to sustain a
205 population of implanted intestinal smooth muscle cells (SMCs) within multi-layered scaffolds.
206 Macropores was fabricated by laser-cutting of PCL electrospun mats obtaining an interconnected
207 network with 250 μm pores. After 2 weeks of seeding, cell infiltration, vascular ingrowth, and
208 survival of green fluorescent protein (GFP)-expressing SMCs were measured. The histologic
209 sections of retrieved implants revealed a significant difference between porous and uncut scaffolds
210 which showed little cellular penetration through the outermost layer, and the lack of nutrients

211 supply affected the vitality of the inner layer. In addition, blood vessels were more numerous into
212 the porous rather than in smooth scaffolds.

213 In the last years, the rising of AM techniques has opened the ways to the development of macropore
214 size-controlled scaffolds using a wide variety of materials and permitting a greater control of pore
215 geometry [10, 55]. However, macropore can strongly affect the mechanical performance of the
216 scaffold and the optimal pore size for cell response should be defined avoiding any structural
217 damaging.

218

219 **5. Multiscale Pore Architecture**

220 In order to develop biomimetic scaffolds that resemble the complex living hierarchical structures,
221 constructs having pore size on multiple length scales can be obtained. To achieve this ambitious
222 goal Chen et al. [56], prepared porous gelatin scaffolds using the freeze-drying technique, which
223 involves the sublimation of frozen water directly into the gas phase, resulting in pore formation.
224 The pore sizes of the scaffolds fabricated are largely dependent on the ratio of water to polymer
225 solution and on the emulsion viscosity [57, 58]. To analyze cellular contraction, proliferation and
226 synthesis of ECM, bovine articular chondrocytes were seeded on gelatin substrates with round
227 macropores and interconnected micropores on their walls. Chondrocytes resulted more infiltrated
228 into scaffolds prepared using high concentrations of ice particulates while they deposited on the
229 surface of the control with less interconnected pores. Hierarchical structures for tissue engineering
230 applications were also recently realized by several groups [15, 59, 60, 61, 62, 8, 63, 64]. Jakus and
231 coworkers [15], for example, used the 3D-painting process, a new form of 3D-printing combined
232 with salt leaching. Like fused deposition modeling, this new method extrudes fused thermoplastic
233 polymers through a nozzle to build complex structures through a layer by layer approach directly

234 from a computer aided design (CAD) model [65]. The difference with 3D-printing technique is
235 the material processed, made almost entirely out of water-soluble salt. The resulting polymeric
236 structures are highly porous and contain a low percentage of solid material [15]. More specifically,
237 authors synthesized a 3D-printable ink using PLGA and a water-soluble salt as porogen; then the
238 salt was dissolved and removed from the printed structure, obtaining a multiscale pore architecture
239 formed by controlled macropores and interconnected micropores on the filaments surface (F-
240 PLGA). F-PLGA scaffold increased hMSCs attachment, viability, proliferation and matrix
241 synthesis capabilities when compared to 3D printed PLGA construct. Kim et al. applied a novel
242 modified electrohydrodynamic direct-jet printing (EHDP) to fabricate a hierarchical 3D structure
243 composed by collagen nanofibers assembled into 3D macroporous structures [59]. In this
244 processing technique designed by authors, the machine moved automatically according to the path
245 designed by a CAD model. As a target, EtOH was used as media with a grounded copper plate
246 immersed in the bath. After dispensing the 3D fibrous structures, the EtOH was removed with
247 water [66]. The *in vitro* analysis, performed by culturing MSCs, proved that this hierarchical
248 collagen structure provided a suitable biomimetic environment to efficiently induce the cell-cell
249 and cell-substrate interactions. The group of Novotna and coworkers, on the other hand, developed
250 hierarchical 3D porous calcium phosphate scaffolds with high pore interconnectivity by using *in*
251 *situ* polyurethane foaming technique [60]. With this method the foam is formed by carbon dioxide
252 bubbles generated *in situ* via reaction of water with isocyanate groups [67, 68]. The pore size can
253 be controlled by optimizing the reactant composition, namely by modifying the water,
254 diisocyanate, polyol, and hydroxyapatite ratio. The study showed these *in situ* foamed scaffolds
255 were well supportive to proper attachment and viability of normal human cells and can potentially
256 be used in bone tissue engineering applications. Furthermore, Hu et al. designed bioactive

257 nanoparticle/PCL (BNPCL) hierarchical porous scaffolds with tunable performance and well-
258 defined pore size [61]. With this aim, authors employed the solvent evaporation of 3D printed
259 water-in-oil high internal phase emulsion (HIPE) templates, containing hydrophobically modified
260 hydroxyapatite and silica nanoparticles in the oil phase. This innovative approach allowed to
261 achieve a multiscale pore architecture with macropores formed by 3D-printing and micropores
262 from HIPE templates (Figure 4). The in vitro biomineralization study suggested that the BNPCL
263 scaffolds possessed excellent apatite formation ability (bioactivity). Another additive
264 manufacturing technique, stereolithography, was employed by Sherborne et al. [8]. This method
265 is based on the spatially controlled solidification of a liquid resin by photo-polymerization, in a
266 layer-by-layer manner. A pre-defined pattern is illuminated on the surface of a resin using a
267 computer-controlled laser beam or a digital light projector with a computer-driven building stage.
268 As a result, the illuminated resin solidified to a defined depth, causing it to adhere to a support
269 platform [69]. Authors photo-polymerized a 3D scaffold from a polymeric emulsion known as
270 High Internal Phase Emulsions (PolyHIPEs) and produced hierarchical and repeatable pore
271 structures [8]. Indeed, micropores with diameters of 1-50 μm governed by emulsion templating
272 and macropores (100 μm size) dictated by additive manufacturing, were obtained. PolyHIPE
273 scaffolds were compared, in terms of cell viability, to a commercial product that had a similar
274 macroscopic architecture but lacked the internal micropores of the PolyHIPE construct. MLO-A5
275 cells, a murine osteoblast cell line, improved their proliferation capability and deposited
276 significantly greater amounts of mineralized ECM when seeded on PolyHIPE, demonstrating the
277 beneficial effects of the hierarchical structure onto cell activity. Hierarchical architectures of
278 primary (macroscale) and secondary (microscale) pores was also developed [63, 64]. For instance,
279 Morgan et al. [63] designed a multicompartamental scaffold with a precise 3D microporous

280 framework. In particular, muscle and vascular templates were constructed from a novel slowly
281 degrading elastomer, poly(limonene thioether) (PLT32i), and were connected via an oxygen
282 permeable vascular-parenchymal interface constructed from rapidly biodegrading poly(glycerol
283 sebacate) (PGS). The macroporous structure constituted by microchannels and grids was
284 fabricated by casting the PLT32i prepolymer onto sintered spheres of poly(methyl methacrylate)
285 (PMMA) within precisely patterned molds followed by photocuring, de-molding, and leaching out
286 the PMMA. The behavior of human umbilical vein endothelial cell (HUVEC) and heart cell seeded
287 on this scaffold was evaluated, by demonstrating the improvements in perfusion and heart cell
288 alignment given by the grids and the enhanced heart cell retention conferred by microscale pores.
289 Finally, we report the study of Chen et al. where controllable and reproducible extrusion deposition
290 and porogen foaming processes were applied to generate highly porous hierarchical scaffolds [62].
291 Particularly, the authors produced three kinds of hydroxyapatite scaffolds varying the particles size
292 of graphite used as porogen (HA-G, HA-nG, HA- μ G). The hierarchical structures were
293 advantageous in terms of biological performance, including biodegradation, proliferation,
294 adhesion, and differentiation. Indeed, SEM analysis indicated that myoblasts adhered much more
295 freely on HA-G, in contrast to the restricted adhesion on normal scaffolds. Moreover, cell
296 interactions and cellular functionalities were further improved with the HA-nG and HA- μ G
297 constructs.

298

299 **6. Conclusions**

300 Porous 3D scaffolds are typically used in tissue engineering applications since each pore size
301 directly affects the cellular response in a different way. Depending on the pore diameters needed,
302 and the type of material used, several conventional and AM techniques can be employed. However,

303 the current main goal among research groups worldwide is to develop a hierarchical scaffold with
304 pore size over multiple length scales, which can introduce significant improvements in terms of
305 biomimetic structure, interconnectivity of pores and final mechanical properties of the scaffold.
306 The overview described in this review clearly indicates that the most performing techniques to
307 obtain a controlled and hierarchical pore architecture are additive manufacturing methods in
308 combination with traditional technologies. Indeed, AM techniques allow to achieve highly
309 interconnected and controlled macro- and micropores, while the conventional methods provide
310 pore size at the nanoscale. In this scenario, the use of melt electrospinning technology is very
311 promising technique as it combines conventional (electrospinning) and AM techniques in one
312 single system providing a nanofibrous matrix with a complex geometry and controlled micro- or
313 macroporous architecture. Furthermore, recent findings highlighted as not only the pore size is
314 pivotal to modulate cell fate but even the pore geometry have a role in controlling cell/structure
315 interactions. So far, few recent studies [70, 71] reported the effect of pore geometry on cell
316 behavior and the implementation of melt-electrospinning devices having a strict control on both
317 pore size and geometry could be a promising strategy to combine pore size and geometry to gain
318 further insights in the knowledge of cell response on different architectures to improve the design
319 of bioinductive scaffolds.

320

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Figure 1 – Schematic representation of different pore size surfaces and their influence on physical properties and cell behavior. Nanopores ($< 0.3 \text{ nm}$) promotes cellular attachment by inducing cells to develop FAs; micropores ($0.3 - 100 \text{ }\mu\text{m}$) improves the permeability of the scaffold and facilitate cell migration; macropores ($> 100 \text{ }\mu\text{m}$) provide space for vascularization, nutrients supply, waste removal and gas diffusion.

Figure 2 – Scanning electron microscopy (SEM) image of MoS₂ nanostructured surface (a) and immunofluorescent staining of the osteogenic markers osteocalcin (OPN) and osteopontin (OCN) expressed by rat bone marrow mesenchymal stem cells (MSCs) after 14 days in vitro. Reprinted with permission from [23].

Figure 3 – Melt electrospinning setup (a) and scanning electron microscopy (SEM) images of PCL scaffolds obtained by melt electrospinning with fibrous layers oriented at 90° (b) and 60° (c). Reprinted with permission from [46].

Figure 4 – Example of hierarchical porous structures basing on 3D-printing of Pickering HIPE templates. Reprinted with permission from [61].

