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Review

Role of pore size and morphology in musculo-skeletal tissue regeneration

Roman A. Perez [a,b,*,1], Gemma Mestres [c,1]

[a] Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 330-714, Republic of Korea
[b] Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, Republic of Korea
[c] Department of Engineering Sciences, Uppsala University, Box 534, 751 21 Uppsala, Sweden

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ABSTRACT

Biomaterials in the form of scaffolds hold great promise in the regeneration of diseased tissues. The scaffolds stimulate cellular adhesion, proliferation and differentiation. While the scaffold composition will dictate their biocompatibility, their porosity plays a key role in allowing proper cell penetration, nutrient diffusion as well as bone ingrowth. Porous scaffolds are processed with the help of a wide variety of techniques. Designing scaffolds with the appropriate porosity is a complex issue since this may jeopardize other physico-chemical properties. From a macroscopic point of view, parameters such as the overall architecture, pore morphology, interconnectivity and pore size distribution, have unique roles in allowing bone ingrowth to take place. From a microscopic perspective, the adsorption and retention of proteins in the microporosities of the material will dictate the subsequent cell adhesion. Therefore, the microstructure of the substrate can determine cell proliferation as well as the expression of specific osteogenic genes. This review aims at discussing the effect of micro- and macroporosity on the physico-chemical and biological properties of scaffolds for musculo-skeletal tissue regeneration.

Contents

Abbreviations: ALP, alkaline phosphatase; BCP, biphasic calcium phosphate; BMP, bone morphogenetic protein; BMSC, bone marrow stromal cell; BSA, bovine serum albumin; CDHA, calcium deficient hydroxyapatite; CPC, calcium phosphate cement; μCT, X-ray micro-computed tomography; GAG, glycosaminoglycan; HA, hydroxyapatite; hBDC, human bone-derived cell; hBMSC, human bone marrow stromal cell; MSC, mesenchymal stem cell; PCL, poly □-caprolactone; PDLLA, poly-DL-lactic acid; PLLA, poly-(L-lactic acid); PLGA, poly-(lactic-co-glycolic acid); PVA, polyvinyl alcohol; SBF, simulated body fluid; SEM, scanning electron microscopy.

* Corresponding author at: Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 330-714, Republic of Korea.
E-mail address: romanp@dankook.ac.kr (R.A. Perez).

[1] Both authors have contributed equally in the manuscript.

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1. Introduction

Recent advances in tissue engineering have emphasized the need to properly design scaffolds to allow cells to attach, migrate, proliferate and differentiate [1,2]. While the composition and surface chemistry of scaffolds dictate the ability of cells to initially attach, the morphology of the scaffold plays a key role in controlling their ability to migrate [3,4]. Besides allowing cell penetration, a proper architecture of the scaffold will allow nutrients and oxygen to flow into it as well as to remove waste produced by the cells to increase cell survival and hence to regenerate tissue [2,3]. Thus, scaffolds need to be designed to present enough porosity, which not only has to have pores big enough to allocate cells, but also needs to present interconnections to allow cell migration between the different pores.

Nevertheless, the pores not only play a significant role in allowing cell penetration and migration, but also significantly influence the physical properties of the scaffold [5–7]. For instance, the increase in the porosity is known to exponentially decrease the mechanical properties, whereas on the other hand, the permeability can be largely increased with increased porosity [2,8]. In order to optimize scaffolds for tissue engineering, all these parameters need to be balanced to guide proper tissue regeneration. The total porosity, the pore size and pore size distribution as well as the pore morphology are some key parameters that play a critical role in balancing the physical and biological properties of the scaffolds. Furthermore, these properties need to be balanced with the degradation of the scaffold. Ideally the scaffold should degrade at the same time as new natural tissue is being formed, which growth may be stimulated by the biomaterial [2,9]. The porosity is known to increase the ability of fluids to penetrate the structure and therefore enhances the degradation [4].

While pore sizes and pore interconnections in the range of hundreds of microns are relevant for cells to migrate and proliferate, pore sizes in a smaller range also play pivotal roles in tissue engineering [10,11]. These pores are usually few microns in size and are involved mainly on the initial adsorption of proteins on the surface of the materials. Cells interact with biomaterials through cell–protein interactions through the transmembrane proteins. Therefore, it is believed that the increase in protein concentration may significantly affect cell fate [12–15]. Besides the ability to adsorb proteins, these small sized pores are also known to allow the regulation of cell behavior, playing key roles in directing stem cell fate.

The scope of this review is to provide a detailed description on how the different pore sizes and morphologies may affect the overall in vitro and in vivo tissue regeneration. At the same time, the review will discuss how the change in porosity or pore size affects other physical parameters that may also play important aspects in the overall bone regeneration such as the mechanical properties. While there are many types of scaffolds made of different materials and compositions, we do not aim to describe the different sources and the differences among them, but rather describe studies that have been able to analyze specific parameters of the scaffolds while maintaining other parameters constant. For this purpose, polymeric based materials are of great ease to work since their mouldability allow to fully control their morphology and porosity as desired. While the porosity of ceramics can be controlled to a lower extent due to the high sintering temperatures, metallic materials will be overlooked as scaffolds in this review due to their inherent low biological properties as well as the possible release of ions that may cause severe adverse reactions [16,17]. For this purpose, the review will describe works mainly on polymeric materials as well as some ceramic materials that have allowed performing comparative studies.

2. Types of porosity

Porosity is the quantification of void spaces within a material. The most common methods to measure the porosity are mercury intrusion porosimeter, capillarity and permeability methods. The main advantage of the mercury porosimeter is that it allows quantifying the pore size distribution of the pores' neck (detection limit of 0.06 μm) by incorporating mercury into the scaffolds through the use of pressure. The pore size distribution can also be obtained below this size by means of nitrogen adsorption. Other measurement techniques are based on imaging methods, such as micro computed tomography (μCT), scanning electron microscopy (SEM) as well as atomic force microscopy (AFM). These imaging techniques can reach high resolutions. For example, AFM can resolve pores as small as 500 nm, although the area of sample that can be studied is too small to obtain pore size distributions.

Besides the porosity of the samples, the pore size distribution is of great importance as well. The porosities of dense materials are classified in three different types according to the IUPAC: micropores (<2 nm), mesopores (2–50 nm) and macropores (>50 nm). Nevertheless, for tissue engineering, it is commonly used a slightly different description of the pore sizes. In this sense, we will adopt in this review the nomenclature used by biomaterial scientists to describe the pore sizes of scaffolds, which classifies pore sizes as macropores (>50 μm) and micropores (<50 μm). Therefore, we will only distinguish two different types of pores throughout the review and will not consider the pore ranges established by IUPAC.

3. Macroporosity

Scaffold macroporosity plays a critical role in the regeneration of damaged tissues. Macroporosity is aimed to allow cell penetration, which may then trigger the integration with the host tissue and increase the chances for key processes to take place (e.g. blood vessel ingrowth). The optimum pore size for scaffolds lies in the range between 100 and 400 μm [2,18,19]. Nevertheless, it is still unclear whether scaffolds with, for instance, homogenous pore size distribution perform more efficiently than scaffolds with heterogeneous pores.

These macroporosities can be obtained by a wide variety of techniques that include freeze drying, solvent casting, rapid prototyping or laser sintering, among others [2,20,21]. The fabrication methods will determine the pore morphologies and pore size, and hence need to be carefully chosen. Scaffold fabrication techniques are indicated and shortly described in Table 1. For further details about each of these