Materials Science and Engineering C 49 (2015) 807–815

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Mimicking the quasi-random assembly of protein fibers in the dermis by freeze-drying method

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ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form 22 December 2014
Accepted 23 January 2015
Available online 24 January 2015

Keywords:
Gelatin
Freeze-drying
Microstructure
Recrystallization
Skin tissue engineering

ABSTRACT

Freeze-drying is extensively used for fabrication of porous materials in tissue engineering and biomedical applications, due to its versatility and use of no toxic solvent. However, it has some significant drawbacks. Conventional freeze-drying technique leads to the production of heterogeneous porous structures with side orientated columnar pores. As the top and bottom surfaces of the sample are not in contact with similar environments, different rates of heat transfer in the surfaces and the temperature gradient across the sample establish the preferential direction of heat transfer. To achieve a scaffold with a desirable microstructure for skin tissue engineering, freeze-drying method was modified by controlling the rate of cooling and regulation of heat transfer across the sample during the freezing step. It could create a homogeneous porous structure with more equiaxed non-oriented pores. Freezing the polymeric solution in the aluminum mold enhanced pore interconnectivity relative to the polystyrene mold. Recrystallization process was discussed how to influence the mean pore size of the scaffold when the final freezing temperature varied. Higher final freezing temperature can easily provide the energy required for the recrystallization process, which lead to enlarged ice crystals and resulting pores.

1. Introduction

Tissue engineering is a field of research, which is aimed at regenerating tissues and organs. Cells, scaffolds, and growth factors are the three main components for creating a tissue-engineered construct [1]. Tissue engineering scaffold provides an extracellular matrix (ECM) analog, which functions as a necessary template for host infiltration, a physical support to guide the proliferation and differentiation of cells into the functional tissues or organs and insoluble regulator of cell activity [2]. The efficiency of tissue engineering scaffolds rests upon a few essential features. They include biocompatibility, controlled biodegradability, low or non-antigenicity, adequate mechanical strength, and a suitable microstructure [3]. Design variables for producing optimum scaffold microstructure include the provision of permeability, adequate space for growth and the development of sufficient transport pathways within the porous material [4]. Scaffold permeability is defined by a combination of five important parameters: (1) porosity, (2) pore size and size distribution, (3) pore interconnectivity, (4) pore interconnection size and distribution, and (5) pore orientation [5]. These parameters must be manipulated in order to yield a suitable homogeneous three-dimensional porous structure. A sufficiently high porosity is required to allow for cell seeding and diffusion of nutrients throughout the matrix. The mean pore size must be bounded within the lower and upper limits; the pores need to be large enough to allow cells to migrate into the structure, but small enough to establish a sufficiently high specific surface for a minimal ligand density required for efficient binding of a critical number of cells to the scaffold [6]. Three-dimensional materials, in addition, must provide interconnected pores. Interconnectivity of pores facilitates mass transfer of nutrients and waste products, thereby enabling cells to survive in these regions and homogenous cell seeding in vitro and blood capillary ingrowth in vivo [4].

The scaffold material has also a significant effect on cellular activity. The development of biodegradable polymers to perform the role of a temporary matrix is an important factor in the success of cell transplantation [1]. Collagen is the most abundant protein present in the human body, and it is the major component of the skin. Because of the high cost of pure collagen, variable physicochemical and degradation properties and the risk of infectious diseases transmission, gelatin, the denatured type of collagen has been applied for the fabrication of skin tissue engineering scaffolds. Chitosan has been introduced to the scaffolds as it can enhance blood coagulation, accelerate the wound healing, and improve the stability of gelatin scaffolds [7].

Different techniques have been used to fabricate porous materials in tissue engineering. Commonly used techniques are: particulate leaching [8], emulsion templating [9,10], electro spinning [11], rapid prototyping [12], phase separation [13–15], and mostly freeze-drying [1,16–20].

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http://dx.doi.org/10.1016/j.msec.2015.01.071
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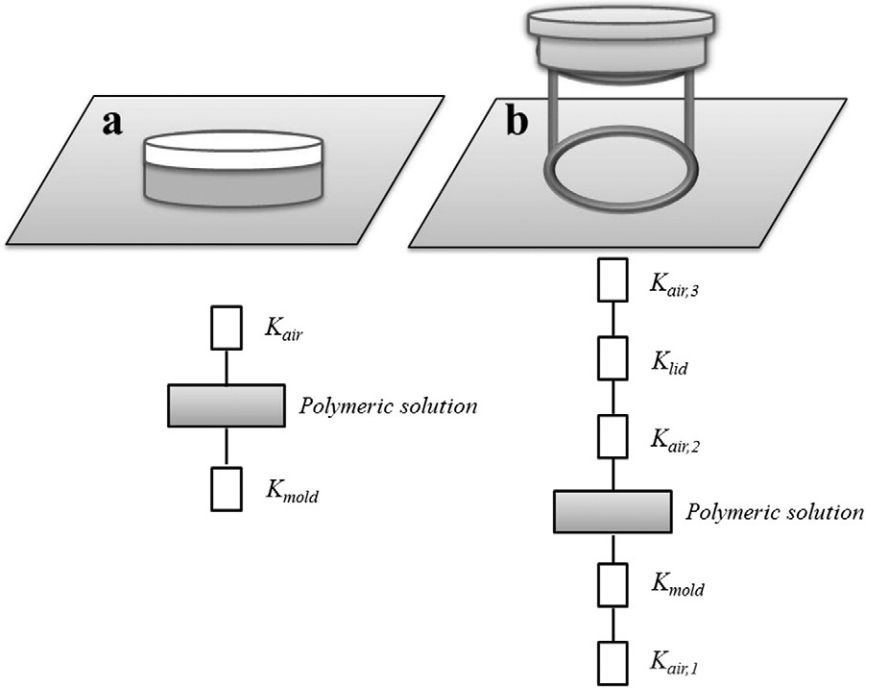

Fig. 1. Freezing condition in (a) the conventional freeze-drying method; the mold is not covered and is in direct contact with the freezer tray (K_{mold}: mold conductive resistances, and K_{air}: air convective resistance), (b) the modified freeze-drying method; the mold mounted on the stand and placed into the freezer at the modified freeze-drying method ($K_{air,1}$: air convective resistance, K_{mold}: mold conductive resistances, $K_{air,2}$: conductive resistances made by the stuck air, K_{lid}: lid conductive resistances made, and $K_{air,3}$: air convective resistance).

Freeze-drying has been extensively used to prepare porous polymeric structures for tissue engineering and biological applications. In this method, aqueous solutions are often used to fabricate scaffolds. When compared to the other methods [8–10,13–15], the freeze-drying process does not bring any impurities into the samples and a further purifying process is therefore not necessary. The low temperature of the freeze-drying process helps maintain the activity of biomacromolecules and pharmaceuticals [21]. Due to its advantageous properties, freeze-drying method has been extensively used in biomedical applications. However, this method has some significant drawbacks. In conventional freeze-drying, porous matrices have been produced by quench freezing which results in heterogeneous microstructure with side oriented and columnar pores. Such a structure is undesirable for scaffolds applied in skin tissue engineering, because oriented pores cannot be favorable in a dermal regeneration template as the dermis is a quasi-random assembly of collagen fibers [22]. Despite some deficiencies of the conventional freeze-drying technique, this method is commonly applied for fabrication of tissue-engineered scaffolds, even in current researches. Furthermore, there is a lack of sufficient study on the microstructural properties of scaffolds produced by this method. Therefore, in the previous work, we reassessed freeze-drying method, comprehensively, in terms of the created microstructure [7]. It was concluded that the production of the heterogeneous porous structure with oriented columnar pores is inherent of the conventional freeze-drying and even the combination of the porogen leaching with this method did not improve the microstructure mainly. The suggested solution, in the literature, is the use of a slow constant rate of cooling in the freezing step [5,6,16]. The formation of oriented pores is a result of the temperature gradient developed within the sample. Non-uniform heat transfer across the polymeric solution is the main cause of the temperature gradient and application of the constant cooling rate cannot improve it essentially. Therefore, in this work, we focused on the regulation of heat transfer to reduce the temperature gradient, as far as possible to achieve a uniform structure with equiaxed pores.

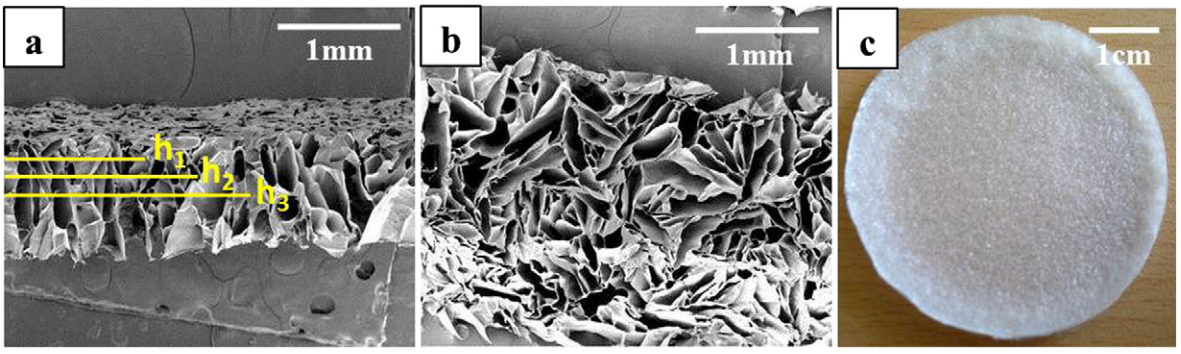

Fig. 2. SEM micrographs of (a) vertical, (b) horizontal cross sections and (c) macroscopic view of the scaffold prepared by conventional freeze-drying. h_1, h_2, and h_3 represent three different depths of the scaffold in which the horizontal cuts were made for the pore size evaluation.