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Biomaterials
Biomaterials 27 (2006) 1362–1376
www.elsevier.com/locate/biomaterials

Review

A comparison of micro CT with other techniques used in the characterization of scaffolds

Saey Tuan Ho[a], Dietmar W. Hutmacher[a,b,*]

[a]Faculty of Engineering, Division of Bioengineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119280, Singapore
[b]Faculty of Medicine, Department of Orthopaedic Surgery, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119280, Singapore

Received 13 July 2005; accepted 18 August 2005
Available online 19 September 2005

Abstract

The structure and architecture of scaffolds are crucial factors in scaffold-based tissue engineering as they affect the functionality of the tissue engineered constructs and the eventual application in health care. Therefore, effective scaffold assessment techniques are required right at the initial stages of research and development so as to select or design scaffolds with suitable properties. Various techniques have been developed in evaluating these important features and the outcome of the assessment is the eventual improvement on the subsequent design of the scaffold. An effective evaluation approach should be fast, accurate and non-destructive, while providing a comprehensive overview of the various morphological and architectural characteristics. Current assessment techniques would include theoretical calculation, scanning electron microscopy (SEM), mercury and flow porosimetry, gas pycnometry, gas adsorption and micro computed tomography (CT). Micro CT is a more recent method of examining the characteristics of scaffolds and this review aims to highlight this current approach while comparing it with other techniques.
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Keywords: Scaffold characterization; Micro CT

Contents

*Corresponding author. Faculty of Engineering, Division of Bioengineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119280, Singapore. Tel.: +65 94309696.
E-mail addresses: biedwh@nus.edu.sg, g0201956@nus.edu.sg (D.W. Hutmacher).

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doi:10.1016/j.biomaterials.2005.08.035

1. Introduction

In scaffold-based tissue engineering, scaffold architectural features are studied after the design and fabrication process; hence, allowing an assessment of the feasibility and the precision level of the process. These architectural characteristics would include porosity, pore size, surface area to volume ratio, interconnectivity, anisotrophy, strut thickness, cross sectional area and permeability [1]. The mechanical strength and biological functionality of the scaffold are influenced by these characteristics. Various techniques can be used to evaluate them and they would include theoretical assessment, scanning electron microscopy (SEM) analysis, flow and mercury porosimetry, gas pycnometry and adsorption. Researchers have only recently employed micro computed tomography (CT) in the study of scaffolds. The author's research group has performed theoretical calculations, SEM, mercury porosimetry and micro CT evaluation of scaffolds fabricated via various techniques [2–5]. Gas adsorption and flow porosimetry have potential applications in the assessment of scaffold architecture; however, there is yet to be any report of their usage in the current literature.

As one goes about selecting a suitable technique in characterizing scaffolds, the associated virtues and pitfalls of each technique should be scrutinized. Sometimes a combination of techniques is required so as to achieve an in depth study of the scaffold properties. However, the most attractive option is a single technique which is non-destructive, yet capable of providing a comprehensive set of data. It appears that micro CT can potentially fulfill this role. When it was first developed, micro CT was used extensively in the study of the trabecular structure [6] and important structural data can be derived after scanning the specimens, but it is not without its limitations. This review compares micro CT and other scaffold evaluation techniques and the final section dwells on a micro CT study that was conducted on novel scaffolds.

2. Architectural and structural parameters

Molecular transport in tissue engineered constructs is dependent on vasculature growth and diffusion. At the implantation sites, vasculature provides the main mode of transport, while for in vitro culturing, diffusion would be the sole approach. In order to ensure the survivability of the cells, the pore network has to be optimized so as to facilitate molecular transport [7]. Molecular transport would include the exchange of oxygen, nutrient, metabolic wastes and molecular signaling. These biochemical exchanges are essential for cell migration and proliferation. When molecular transport is hampered due to poor diffusion, cell—scaffold constructs exhibit peripheral cellular growth while the interior of the construct undergoes necrosis [8]. In the light of this, scaffolds are evaluated against a series of properties (Table 1), so as to assess the ease of diffusion within the scaffold. Moreover, researchers are able to gauge the mechanical properties and cellular response of the scaffold from these characteristics.

One of the key properties that is widely discussed in the literature is porosity [3,9,10]. Porosity would determine cell seeding efficiency, diffusion and the mechanical strength of the scaffold. High porosity and high surface area to volume ratio are required for uniform cell delivery, cellular attachment and neo-tissue in growth [11,12]. In a landmark study by Langer et al., a porosity of 90% was recommended for optimum diffusive transport within a cell–scaffold construct under in vitro conditions [13]. However, scaffolds with such a high porosity would possess very low mechanical strength. In creating scaffolds for load bearing tissues such as bone and cartilage, mechanical integrity is a concern, hence there must be a compromise between the porosity and mechanical properties of the scaffold [14].

Studies on scaffold design have revealed that besides porosity, other factors such as pore interconnectivity and permeability affect molecular transport. A highly porous scaffold may have non-interconnected pores, thus lowering the diffusion efficiency. Suh et al. cultured chondrocytes on scaffolds of equal porosity but of varying degrees of interconnectivity. He observed superior cell attachment and proliferation in scaffolds with highly interconnected pores [15]. However, one should be mindful that deficient molecular transport may occur in the interconnected pores due to poor permeability. The scaffold permeability describes the size of the pore interconnections or fenestrations and this influences the ease at which a fluid flows through the pore network. Besides fenestrations, permeability is also dependent on porosity, interconnectivity, pore orientation, pore size and distribution [16]. A permeation experiment can be conducted so as to measure the permeability of the scaffold and permeability is calculated using Darcy's law [17,18]. In this investigation, the rate of fluid flow is measured at a known hydrostatic pressure. When viscous fluid flow is assumed, Eq. (1) is used:

$$P_C = \mu FL/hA, \quad (1)$$

where F is the flow rate, L the linear dimension of the scaffold in the flow direction, h the pressure drop across scaffold, A the cross-sectional area of scaffold and μ the fluid viscosity.

Besides permeation measurements, Scheidegger noted that permeability could be derived from known porosity, tortuosity and average specific surface area (Eq. (2)). In his calculations, he simplified the pore network to a series of parallel channels. Tortuosity is defined as the ratio of the length of the twist flow path connecting 2 points to the straight-line distance between the points. Average specific surface area is defined as the ratio of the pore area to the scaffold volume [19]:

$$k = P^3/T^2S^2, \quad (2)$$

where P is the porosity, T the tortuosity and S the average specific surface area.

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