Structural and Biological Characterization of Scaffolds

INTRODUCTION

The most important function of a bone tissue engineering (TE) scaffold is its role as a template that allows cells to attach, proliferate, differentiate and organize into normal, healthy bone as the scaffold degrades. Figure 1 illustrates the most important factors involved in the design of TE scaffolds and their interdependencies. Depending on the final application, scaffold requirements include matching the structural and mechanical properties with those of the recipient tissue and optimization of the micro-environment to support cell integration, adhesion and growth, issues that have become known as structural and surface compatibility of biomaterials.

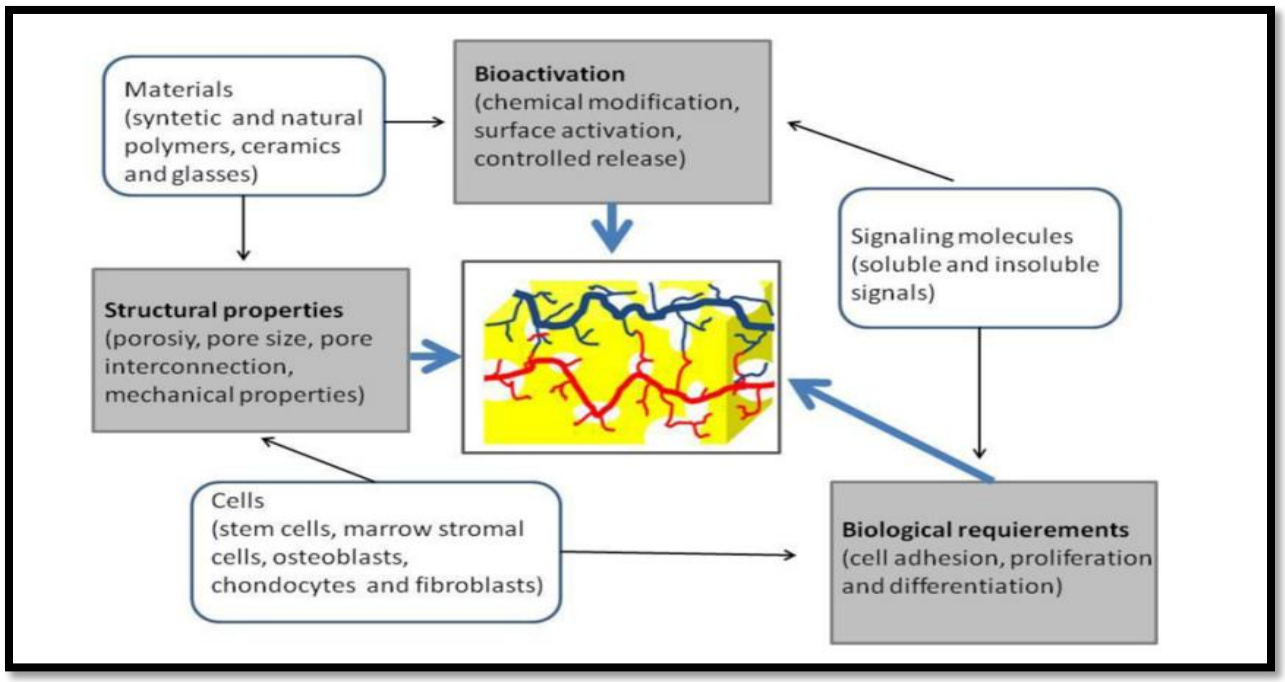

Figure 1 Schematic diagram of key factors involved in the design of optimal scaffolds for bone tissue engineering.

Scaffolds have to fulfil many requirements, such as osteoconductivity, appropriate rate of biodegradation, interconnected porosity and pore diameters in a wide range (e.g. 10–500 mm), microporosity, suitable mechanical strength and structural integrity. Therefore, the complete characterization of scaffold structure and properties is essentially the first step in the process of developing successful bone engineering scaffolds.

Characterization of scaffolds is far from trivial as they are inherently complex structures due to their porous nature. An impressive number of suitable methods are available for scaffold characterization: multi-scale characterization methods have to be used, however, not all methods will be relevant or applicable in every case. One of the problems in choosing adequate characterization techniques is the identification of the key parameters required to adequately characterize scaffolds.

Porosity is one such property, Porosity is a measure of the void space in a material that can be determined from the ratio of the void volume to the bulk material volume. Porosity is known to play a role in determining cell seeding efficiency in addition to the diffusion properties and mechanical strength of a scaffold.

However, it is not a unique parameter (i.e. structurally different materials can have identical porosities) and it cannot be used on its own to sufficiently characterize scaffolds. Rather than basing the scaffold characterization on one parameter, such as porosity, it has been suggested that a multi-parameter approach is needed.

1. CHARACTERIZATION OF SCAFFOLDS MORPHOLOGY AND POROSITY

Methods to visualize and subsequently quantify scaffold structures, e.g.
1-scanning electron microscopy (SEM),

2-micro-computed tomography (m-CT), figure 2 and

3-confocal laser scanning microscopy

are acquiring increasing significance. m-CT, in particular, has been widely used as an essential scaffold characterization tool with the potential to significantly enhance the understanding of pore structures in porous material in the millimetre to micrometre range. This information can be applied to gain knowledge on the correlation between pore structure and mechanical properties and to improve the understanding of the relationship between pore-size distribution and permeability.

As compared to porosimetry methods, m-CT can access both connected and isolated pores enabling the total void volume to be determined. m-CT is also capable of non-destructive assessment of mineralization processes within 3D scaffolds in vitro.

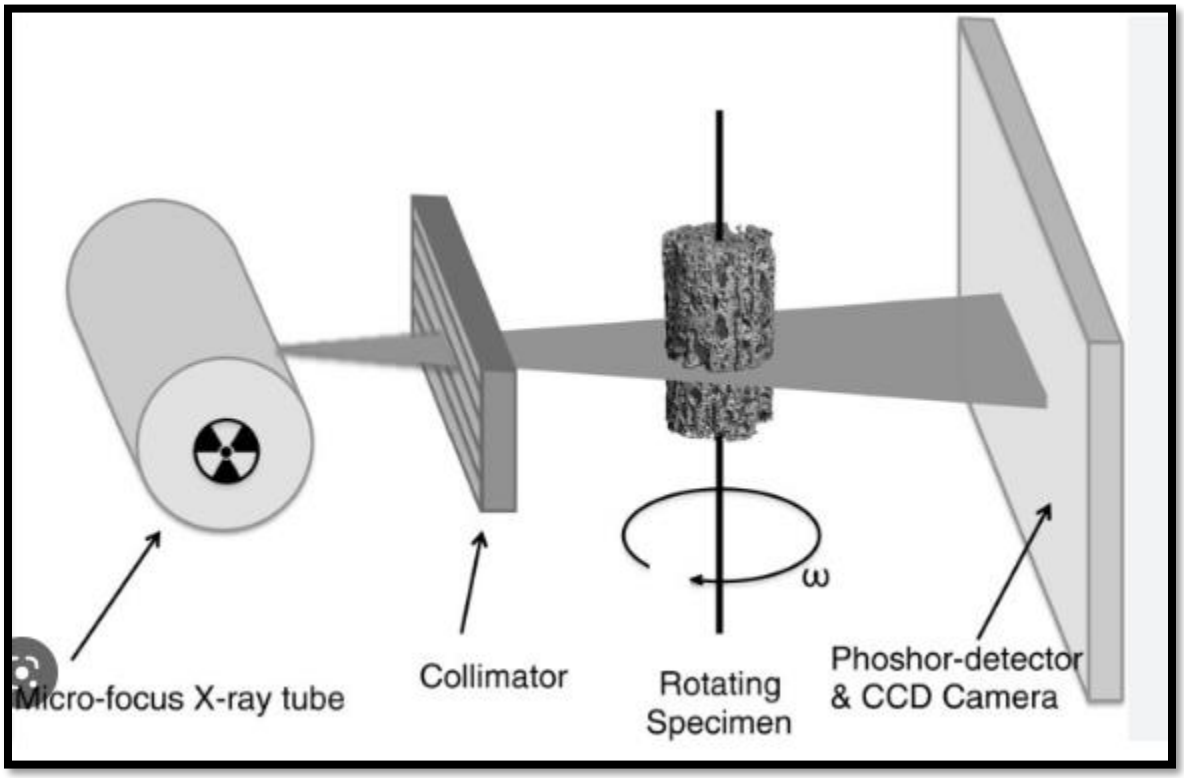

Figure 2 micro-computed tomography (m-CT)

MicroCT equipment is composed of several major components: x-ray tube, radiation filter and collimator (which focuses the beam geometry to either a fan- or cone-beam projection), specimen stand, and phosphor-detector/charge-coupled device camera.

The principle of microCT is based on the attenuation of x-rays passing through the object or sample being imaged.

As an x-ray passes through tissue, the intensity of the incident x-ray beam is diminished according to the equation, $I_x = I_0 e^{-\mu x}$, where I_0 is the intensity of the incident beam, x is the distance from the source, I_x is the intensity of the beam at distance x from the source, and μ is the linear attenuation coefficient. The attenuation therefore depends on both the sample material and source energy and can be used to quantify the density of the tissues being imaged when the reduced intensity beams are collected by a detector array.

MicroCT is established as an essential tool for evaluating bone structure and quality and has been used to study metabolic bone diseases such as osteoporosis (Figure 3), to evaluate preclinical models of disease, and to test the efficacy of anti-resorptive and anabolic therapeutics, such as bisphosphonates. One emerging technique for microCT-based evaluation of bone fragility induced by loading, aging, or osteoporotic disease is the use of contrast agents to detect and quantify bone microdamage.

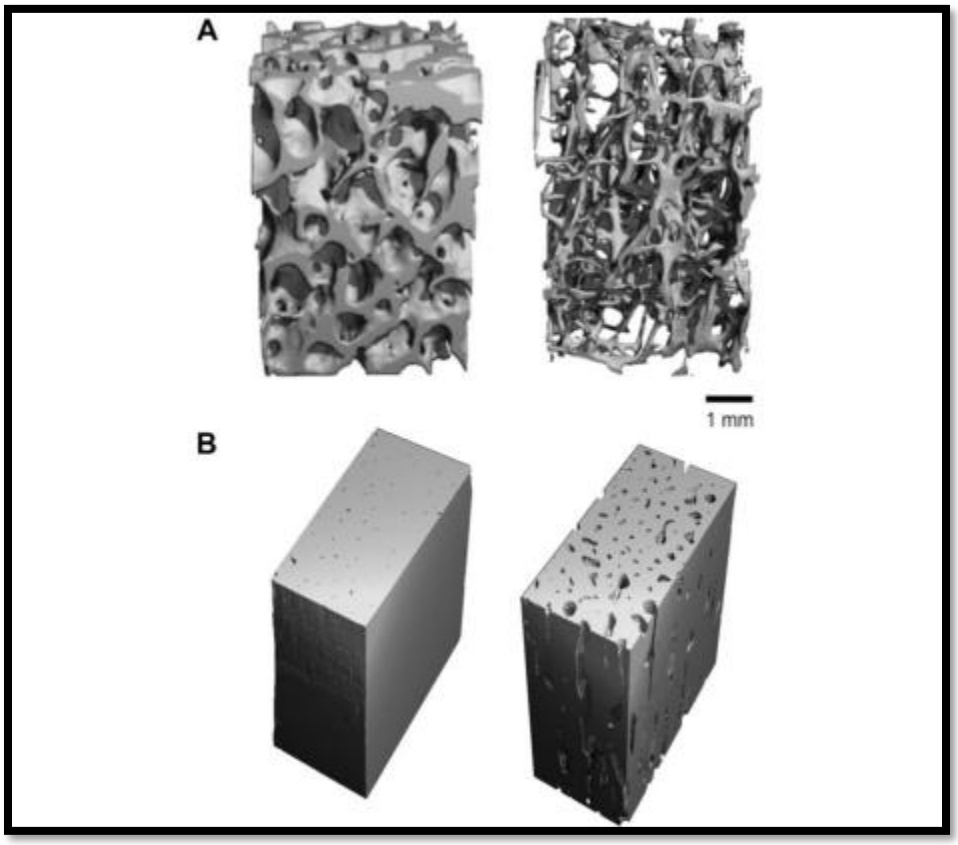

Figure 3 Microcomputed tomography (microCT) reconstruction of cortical and trabecular bone. MicroCT enables high-resolution three dimensional reconstruction of microstructural characteristics from trabecular architecture to cortical porosity. (A) Trabecular bone from femoral neck of 51-year-old male (left) and 84-year-old female (right). (B) Diaphyseal femoral cortical bone of 18-year-old male (left) and 73-year-old female (right). Age, gender, disease, and other factors influence the microstructural properties of both cortical and trabecular bone, and these can be evaluated quantitatively by microCT.

Porosity being the percentage of void space in a solid, is a morphological property independent of the material. Porosity assessment via porosimetry is based on the study of the flow of gases or liquids (or both), across a porous structure.

This method, therefore, is only suitable for the detection of open pores that allow fluid transport. Consequently, standard porosimetry methods cannot be used to assess total pore volume.

Liquid intrusion methods (e.g. mercury intrusion porosimetry) are based on the pressurized penetration of a liquid into a porous structure and are capable of determining the total pore volume exposed to the outside of a structure. As with flow porosimetry, closed pores are hidden from the test. Other strategies used for scaffold characterization include gas pycnometry, gravimetry, gas adsorption, Archimedes principle, and molecular diffusion studies.

It is recognized that part of the difficulty in identifying a suitable measurement strategy is the fact that no single investigative technique is able to fully characterize the porous nature of scaffolds if they exhibit porosity in different scales; e.g. in some cases from nano- to microporosity.

2-PERMEABILITY

Successful bone TE depends on the scaffold's ability to allow nutrient diffusion to and waste removal from the regeneration site, therefore, permeability is a key parameter for the design of scaffolds. Permeability is directly related to the degree of pore interconnectivity.

Studies have found that cell growth into a scaffold depends on how well nutrients can permeate through the porous structure during the cell migration process.

Several permeability measurement systems have been developed for determining the permeability of scaffolds.

- Gravity-induced systems have been applied using oil and water as fluid, respectively, or compressed air.

- used a high-resolution MRI methodology for characterizing the permeability and fluid velocity or used dry air as the fluid medium allowing rapid measurement operations.

-The permeability of a commercial bioceramic scaffold has been evaluated through numerical modelling.

The physical principle used for the measurement of permeability is based on the measurement of the pressure drop caused by the introduction of the scaffolds in a fluid (e.g. water or cell culture medium).

Thereby, the scaffold permeability can be easily determined by applying simple mathematical relations. Based on the relationship between the pressure drop gradient and the fluid flow velocity given by Darcy's law, the intrinsic permeability k [m^2] can be obtained as the linear relationship between flow rate and pressure gradient by the following equation :-

$$Q = \frac{-kA}{\mu}\frac{(P_b - P_a)}{L}$$

where Q is the flow rate (m^3 /s), A is the cross-sectional area (m^2),

P_b - P_a is the drop pressure between two points spaced L, μ is the dynamic fluid viscosity (Pa s), and L is the scaffold thickness.

The effects that scaffold permeability has on bone tissue regeneration have not been studied in depth using accurately controlled porous architectures with reproducibly designed permeability.