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Pore size distribution of bioresorbable films using a 3-D diffusion NMR method

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ABSTRACT

Pore size distribution (PSD) within porous biomaterials is an important microstructural feature for assessing their biocompatibility, longevity and drug release kinetics. Scanning electron microscopy (SEM) is the most common method used to obtain the PSD of soft biomaterials. The method is highly invasive and user dependent, since it requires fracturing of the sample and then considers only the small portion that the user had acquired in the image. In the current study we present a novel nuclear magnetic resonance (NMR) method as an alternative method for estimation of PSD in soft porous materials. This noninvasive 3-D diffusion NMR method considers the entire volume of the specimen and eliminates the user's need to choose a specific field of view. Moreover, NMR does not involve exposure to ionizing radiation and can potentially have preclinical and clinical uses. The method was applied on four porous 50/50 poly(DL-lactic-co-glycolic acid) bioresorbable films with different porosities, which were created using the freeze-drying of inverted emulsions technique. We show that the proposed NMR method is able to address the main limitations associated with SEM-based PSD estimations by being non-destructive, depicting the full volume of the specimens and not being dependent on the magnification factor. Upon comparison, both methods yielded a similar PSD in the smaller pore size range (1–25 μm), while the NMR-based method provided additional information on the larger pores (25–50 μm).

1. Introduction

Porosity and the distribution of pore sizes play an important role in the characterization of materials – in particular, biomaterials. These characteristics have been shown to affect the biocompatibility of biomaterials [1] and their longevity [2], and govern the controlled release of drugs and other bioactive agents from them [3]. Since many biomaterials undergo structural changes in the body, by degradation or by biological response, such as tissue infiltration, there is a clear need to develop a method to track these changes in vivo. Thus, the use of noninvasive and nondestructive nuclear magnetic resonance (NMR) holds clear advantages in the biomedical field, where the ongoing surveillance of changes to a biomaterial or the biological reaction to it necessitates noninvasive monitoring over time.

Depending on the length scale of the pores, different methods can be used to gather microstructural information from porous materials. Mercury porosimetry is often used to estimate the pore size distribution (PSD) in porous media, and is sensitive to a wide range of pore sizes (3 nm–500 μm) [4]. In this method, mercury is injected into the sample and its volume/mass is measured as a function of the applied pressure [5]. One of the key limitations of this method is that it measures the size of the entrance to the pore rather than its actual inner volume [5]. It therefore cannot be used to analyze materials which contain closed pores, as the mercury has no way of entering them. Another limitation of this method is the use of high pressure, which may be unsuitable for soft materials. Lastly, mercury porosimetry is invasive, and can only be applied on dry samples. It is therefore not suited for the investigation of biomaterials in their natural environment.

Microcomputed tomography (micro-CT) is a noninvasive X-ray imaging method that provides a 3-D image of the internal architecture of a sample. 2-D images are acquired, each representing a slice, and then stacked together to form the 3-D structure. Micro-CT can be used to image porous polymers and scaffolds [6–8]. This method is primarily efficient when the materials are dry, and is based on a 3-D image-processing algorithm to provide the necessary structural features [9]. The ionizing radiation level plays a significant role when considering clinical applications, such as structural characterization of polymer implants in humans. The impact of X-ray exposure on tissue has been widely investigated and results in direct damage to nearby molecules, specifically DNA [9]. In micro-CT, the X-ray radiation exposure to the subject

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increases with the fourth power of the voxel size dimension [9]. While acceptable in preclinical studies on small animals, ultrahigh-resolution micro-CT radiation levels make it impossible for clinical use.

The most common way to obtain local microstructural features of biomaterials is by using scanning electron microscopy (SEM) images [10]. While it can provide a very high spatial resolution, this method is highly invasive and requires fracturing of the sample to expose a cross-section of its inner bulk, and in certain materials an electrically conducting coating is also needed.

The microstructural information from both micro-CT and SEM is obtained directly from an image. As opposed to spectroscopic methods such as NMR, the acquired image has a finite spatial resolution. In addition, before applying an image-processing algorithm to obtain the structural information, a field of view (FOV) must be chosen by the user. This choice, as will be demonstrated later on, predetermines the extracted geometrical properties of the sample. Furthermore, the automated image-processing algorithm can introduce additional error to the estimation.

In NMR, nuclear spins in a magnetic field are put under resonance (using radio-frequency pulses that induce precession). The precession of such an ensemble of spins is detected as an electromagnetic induction, which is the NMR signal. Diffusion-weighted NMR (DW-NMR) is a sub-technique of NMR in which manipulation of the phase of the precessing spins quantifies the displacement of the molecules holding the spins. DW-NMR is a noninvasive method that is sensitive to the displacement of molecules. Typical DW-NMR experiments operate in the following way: an ensemble of spins of water hydrogen that precess synchronously and coherently (with the same phase) is assumed. Application of a momentary spatial perturbation in the magnetic field (e.g. applying a magnetic field gradient) induces a momentary deviation of the precession frequency. This deviation is "preserved" even after the momentary perturbation (gradient) has gone, as a residual difference in the accumulated phase of precession. Application of an opposite perturbation after a finite time (Δ) will "erase" this residual phase. However, spins that diffuse during the time Δ will encounter canceling perturbations, and will have a residual non-zero phase. The higher the diffusion of molecules in the ensemble, the higher their displacement in time Δ and the variance of the residual phase. Thus, experimentally, the degree of diffusion can be quantified by the phase variance that is detected, or as an attenuation of the superposed signal of the entire ensemble. A typical DW-NMR experiment consists of a series of experiments, as described above, in each of which a pulsed gradient of different value is applied.

However, molecular diffusion in porous media is not free, as molecules of the dispersed phase (usually water) interact with the boundaries of the continuous phase. When the environment for molecular displacement is not a free medium, the displacement is tightly linked to the specific microstructural barriers and hindering obstacles that surround the molecules. The detected displacement thus reflects the interactions of the moving molecules with the boundaries. The statistics of displacement of water in a spherical pore can be characterized analytically. Thus, if a material is composed of identical equal sized pores, the result of a DW-NMR experiment can be easily fitted, and the size of the pores can be found. Estimation of microstructural properties with diffusion weighted NMR-based techniques presents many benefits, the most important of which are its non-invasiveness and its ability to collect information from the entire volume of the sample. DW-NMR has been applied on many porous materials, and specifically on tissues [11]. The qualitative characterization of microstructures has already been demonstrated in many types of experimental models, ranging from biological tissues [12,13] to dairy products [14] and sandstone [15,16]. However, in samples that are composed of polydisperse pores, an exact quantitative NMR-based estimation of the PSD is a challenging task, since it involves the solution of a mathematically ill-posed problem (i.e. small noise in the data may cause large changes in the reconstructed pore size distribution). Despite this challenge, estimation of an emulsion's PSD using diffusion weighted NMR has been demonstrated [17]. Recently, this method was extended to include a second dimension in the parameter space [18], by applying a second magnetic gradient pair immediately after the first one. By doing so, the two dimensions in the parametric space are the gradient amplitude and the relative angle, φ, between the two gradient pairs. The method for reconstruction of the pore size distribution is applied as follows (for a mathematical description, see the Supplementary Information): (1) a series of DW-NMR experiments is performed, with variance of these two experimental values (gradient amplitude and relative direction). (2) The spectrum of possible pore sizes is discretized (binned) with values chosen between R_{min} and R_{max}. (3) The theoretical signal attenuation is calculated analytically for each of these discrete radii and for each set of experimental values. This process results in a library (a matrix) of known basis values calculated for each experimental set. (4) The observations are assumed to result from the superposition of contributions of signals from multiple pores with different radii, each weighted by its relative fraction (defined by the PSD). (5) A numeric fitting process is applied whereby iterations include trials with different PSDs. The solution is the PSD that gives the best fit to the experimental observations described in (1). The 2-D method was shown to improve the stability and reliability of the estimated PSD. It was further tested experimentally on calibrated microcapillary PSD phantoms, resulting in accurate size distribution estimation [19].

In the current study we present a novel noninvasive method of estimation of PSD in soft porous materials that considers the entire sample volume, and serves as an alternative to the traditional SEM method. The above-mentioned 2-D NMR method is further extended here to a 3-D parametric space (see the Supplementary Information for further details). Based on previous work [18], this extension is predicted to significantly improve the stability and reliability of the PSD estimation. The 3-D method was applied on four porous 50/50 poly(DL-lactic-co-glycolic acid) (PDLGA) bioresorbable films with different porosities, which were created using the freeze-drying of inverted emulsions technique. The microstructure of such films, specifically the overall pore volume, pore size and interconnection of pores, has been shown to greatly affect the release pattern of bioactive agents from such films in wound dressings, stents and bone scaffold applications [3,20]. The relevant size range of pores within these bioresorbable films is 1–50 μm, which is applicable to and representative of many other polymer biomaterials.

2. Materials and methods

2.1. Preparation of porous structure

50/50 PDLGA films were prepared utilizing the freeze-drying of inverted emulsions technique [3]. The aqueous phase of the inverted emulsion consisted of double-distilled water with 1% (w/v) bovine serum albumin as surfactant. The organic phase of the inverted emulsion consisted of 15% (w/v) of 50/50 PDLGA, which was dissolved in chloroform. Freshly prepared inverted emulsions were prepared by homogenization of the organic and aqueous phases (O:A ratio = 6:1) at four different stir rates (2500, 5000, 7500 and 10,000 rpm). The emulsions were then poured into an aluminum plate and immediately frozen in a liquid nitrogen bath before placing them in a pre-cooled (−105 °C) freeze-dryer. Freeze-drying the samples overnight to remove the solvents preserved the microstructure of the emulsion-based structures in the solid state. The different homogenization rates of the emulsion