JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 34 (2014) 313–319

Available online at www.sciencedirect.com

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Research Paper

Nanomechanical properties of multi-block copolymer microspheres for drug delivery applications

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ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form
28 February 2014
Accepted 9 March 2014
Available online 19 March 2014

Keywords:
Nanoindentation
Mechanical properties
Hydrophilic multi-block copolymer
Monodisperse microspheres
Controlled release

ABSTRACT

Biodegradable polymeric microspheres are interesting drug delivery vehicles for site-specific sustained release of drugs used in treatment of osteoarthritis. We study the nanomechanical properties of microspheres composed of hydrophilic multi-block copolymers, because the release profile of the microspheres may be dependent on the mechanical interactions between the host tissues and the microspheres that aim to incorporate between the cartilage surfaces. Three different sizes of monodisperse microspheres, namely 5, 15, and 30 μm, were tested in both dry and hydrated (swollen) states. Atomic force microscopy was used for measuring nanoindentation-based force–displacement curves that were later used for calculating the Young's moduli using the Hertz's contact theory. For every microsphere size and condition, the measurements were repeated 400–500 times at different surface locations and the histograms of the Young's modulus were plotted. The mean Young's modulus of 5, 15, and 30 μm microspheres were respectively 56.1±71.1 (mean±SD), 94.6±103.4, and 57.6±58.6 MPa under dry conditions and 226.4±54.2, 334.5±128.7, and 342.5±136.8 kPa in the swollen state. The histograms were not represented well by the average Young's modulus and showed three distinct peaks in the dry state and one distinct peak in the swollen state. The peaks under dry conditions are associated with the different parts of the co-polymeric material at the nano-scale. The measured mechanical properties of swollen microspheres are within the range of the nano-scale properties of cartilage, which could favor integration of the microspheres with the host tissue.

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http://dx.doi.org/10.1016/j.jmbbm.2014.03.002
1751-6161/

1. Introduction

Osteoarthritis is the most common joint disorder (Arden and Nevitt, 2006) and the most frequently reported cause of long-term disability (Badley, 1995). Since disease-modifying drugs are not currently available for osteoarthritis, medicinal treatment often includes non-steroidal anti-inflammatory (NSAID) drugs (Lin et al., 2004) that are often used for pain management. The delivery of such drugs is a challenging task due to the chance of adverse reactions via conventional drug injections on the one hand (Lin et al., 2004; Mamdani et al., 2002) and fast drug clearance that causes the drug concentration to rapidly drop below the therapeutically effective levels on the other hand (Ayral, 2001). Recently proposed approaches for the delivery of NSAID include the use of liposomes, nanoparticles, and microspheres for sustained and adjustable drug release (Gerwin et al., 2006; Sandker et al., 2013). Among those, microspheres have the advantages of being easily adjustable in their release kinetic, allowing well-controlled sustained drug release varying from days to months (Sandker et al., 2013).

It is known that the mechanical properties of the microspheres used for drug delivery applications influence their performance (Chan et al., 2008; Mercadé-Prieto and Zhang, 2012). The effects of mechanical properties are even more important for skeletal diseases where strong mechanical forces are transferred through the tissues and the microspheres interact with the surrounding tissues both chemically and mechanically. The release kinetics of the microspheres may change due to those mechanical interactions and the modulations that mechanics may have with diffusion kinetics and biodegradation behavior of the microspheres.

In this paper, we study the mechanical properties of monodisperse microspheres composed of hydrophilic phase-separated multi-block copolymers that are developed for delivery of drugs used in the management of osteoarthritis. We use atomic force microscopy (AFM) for nanoindentation tests and the Hertz's contact theory to calculate the nano-mechanical properties of the microspheres. The mechanical properties of microspheres with different sizes are measured both in the dry and swollen states. In the vast majority of studies that report the mechanical properties of microspheres, a limited number of measurements are carried out and the obtained mechanical properties are averaged to calculate the mean values of the mechanical properties. In this study, we repeated the measurements several hundred times for every case to be able to measure the properties more accurately and to reveal nano-scale properties of the microspheres that cannot be identified using a few measurements.

2. Materials and methods

Monodisperse microspheres of different sizes, namely 5, 15 and 30 μm, were made of a biodegradable hydrophilic multi-block copolymer (SynBiosys®20LP10L20-LLA40, InnoCore Pharmaceuticals, Groningen, The Netherlands) using the Microsieve™ membrane emulsification equipment (Nanomi, Oldenzaal, The Netherlands) and were tested in both dry and swollen states. The hydrophilic 20LP10L20-LLA40 multi-block copolymer is composed of hydrophilic poly (DL-Lactide)-PEG-poly (DL-Lactide) and hydrophobic poly (L-Lactide) segments that are randomly chain-extended with 1,4-butanediisocyanate. The poly (DL-Lactide)-PEG-poly (DL-Lactide) segments form amorphous domains, whereas the poly (L-Lactide) segments form crystalline domains, thereby forming a phase-separated multi-block copolymer in which the crystalline domains act as physical crosslinks (Fig. 1). Differently sized 20LP10L20-LLA40 microspheres were tested in both dry and swollen states to determine their mechanical properties. Before starting the test with AFM, the microspheres were attached to a firm substrate. A thin layer of a water-resistant epoxy-based glue (Bison, Netherlands) was carefully applied to fix the microspheres on the microscope slide. For measurement under hydrated conditions, prior to testing, the microspheres were submerged in deionized water for three days to allow them reach their equilibrium swelling degree. The tests were carried out at room temperature.

An AFM with a Nanoscope controller (Bruker, Dimension V, Japan) and a standard fluid cell (Bruker) was used for performing nanoindentation tests on the microspheres (Fig. 2). A symmetric triangular AFM probe (Bruker, Camarillo, USA) with a nominal diameter of 2 nm and a nominal cantilever spring constant of 0.35 N/m was used for the measurements to find the more symmetric characteristics of the microspheres (Zhu et al., 2011). The actual cantilever spring constant was determined using the thermal fluctuations technique (Notbohm et al., 2011). The standard calibration process for finding the sensitivity factor for converting voltage to deflection was followed before the start of the measurements. Using around 30 individual microspheres, 400–500 indentation curves were obtained at a frequency of 1 Hz and an indentation depth of around 500 nm. The Nanoscope analysis software (Bruker, version 1.4) was used for analyzing the obtained force–displacement curves and calculating the Young's modulus according to the Sneddon theory (Zhu et al., 2011). According to the Sneddon theory, force and displacement are related to each other through the following relationship:

$$F = \frac{\pi \tan \varphi}{2\gamma^2} \frac{E}{(1-\upsilon^2)} h^2 \quad (1)$$

where F is force, h is displacement, φ is the half angle of the cone, $\gamma = \pi/2$, υ is the Poisson's ratio and E is the Young's modulus of the microsphere (Franke et al., 2007; Oyen and Cook, 2009; Zhu et al., 2011). A Poisson's ratio of 0.5 was assumed which is close to the value used in some other studies on cartilage nanomechanics (Loparic et al., 2010; Stolz et al., 2004). With such a value of the Poisson's ratio, the material behaves incompressibly.

The Young's moduli calculated for the swollen microspheres with different sizes were statistically assessed using

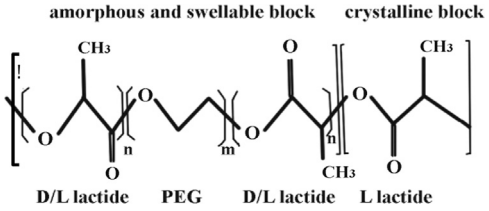

Fig. 1 – Molecular structure of SynBiosys 20LP10L20-LL40 multi-block copolymer.