International Journal of Pharmaceutics 443 (2013) 154–162

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Ultrasound enhanced release of therapeutics from drug-releasing implants based on titania nanotube arrays

Moom Sinn Aw*, Dusan Losic**

School of Chemical Engineering, Engineering North Building, The University of Adelaide, Adelaide, SA 5005, Australia

ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form
15 December 2012
Accepted 2 January 2013
Available online 8 January 2013

Keywords:
Local drug delivery
Titania nanotubes
Stimulated drug-micelles release
Drug carrier
Polymeric micelles
Ultrasound

ABSTRACT

A non-invasive and external stimulus-driven local drug delivery system (DDS) based on titania nanotube (TNT) arrays loaded with drug encapsulated polymeric micelles as drug carriers and ultrasound generator is described. Ultrasound waves (USW) generated by a pulsating sonication probe (Sonotrode) in phosphate buffered saline (PBS) at pH 7.2 as the medium for transmitting pressure waves, were used to release drug-loaded nano-carriers from the TNT arrays. It was demonstrated that a very rapid release in pulsatile mode can be achieved, controlled by several parameters on the ultrasonic generator. This includes pulse length, time, amplitude and power intensity. By optimization of these parameters, an immediate drug-micelles release of 100% that spans a desirable time of 5–50 min was achieved. It was shown that stimulated release can be generated and reproduced at any time throughout the TNT-Ti implant life, suggesting considerable potential of this approach as a feasible and tunable ultrasound-mediated drug delivery system *in situ via* drug-releasing implants. It is expected that this concept can be translated from an *in vitro* to *in vivo* regime for therapeutic applications using drug-releasing implants in orthopedic and coronary stents.

1. Introduction

Local drug delivery systems (DDS) are recognized as promising alternative solutions to address problems in conventional drug therapies, such as limited drug solubility, low drug efficacy, high drug potency, poor bioavailability and biodistribution, lack of selectivity and unfavorable pharmacokinetics (Allen and Cullis, 2004; Langer, 2001; Fahr and Liu, 2007). Advantages of local drug delivery (DD) are particularly recognized in bone therapies and orthopedics to prevent bone infections, reduce inflammation, improve fracture healing, tissue integration, or to treat primary and secondary bone cancers (Zilberman et al., 2010; Gulati et al., 2011). Several strategies for localized drug administration in bones using polymer gels, bone cements and collagen sponges have been explored and clinically approved. However, they contain many drawbacks, namely, unpredictable and unsatisfactory drug release, due to their non-uniform porosity, structural inconsistency and possible degradation (Mandal et al., 2010; Ruszczak and Friess, 2003). Contrarily, titania nanotube (TNT) arrays synthesized on the surface of Ti implants *via* a self-ordering process using electrochemical anodization; by virtue of their well-defined, highly ordered and controllable pore dimensions, biocompatibility, high surface-to-volume ratio, high aspect ratio, adjustable surface chemistry, mechanical and chemical stability, were recognized as a remarkable platform for the development of advanced drug- and drug carrier-releasing devices and implants in bone therapies (Losic and Simovic, 2009; Aw et al., 2011a; Gulati et al., 2012). Previous studies have also shown that TNTs can be used not only for DD in a diverse range of drugs or bioactive agents, namely, water insoluble drugs, anti-cancer drugs, proteins, drug carriers, but they can also improve osseo- and tissue integration, cell adhesion, osteoblast functionality, and provide adequate bone support (Tran and Webster, 2009; Popat et al., 2007; Gulati et al., 2012; Paulose et al., 2008; Park and Webster, 2005).

In our previous work, we have demonstrated several approaches to modulate the drug release of water insoluble drugs from drug-releasing TNT-Ti implants, by means of controlling nanotube dimensions, surface chemistry, plasma and biopolymers coatings, and using polymeric micelles as drug carriers (Aw et al., 2011b, 2012a,b; Simovic et al., 2010). It was shown that by a combination of polymeric micelles as drug carriers and TNT arrays as drug-releasing surfaces in implants, this strategy is a particularly attractive solution to design advanced DDS for bone therapies, where the local delivery of sensitive or degradable drugs, proteins, potent anti-cancer drugs and bone growth factors is required (Aw et al., 2011a). A variety of release patterns including extended,

* Corresponding author. Tel.: +61 4 3047 8246/+60 5321 5910; fax: +61 8 8313 4373.
** Corresponding author. Tel.: +61 8 8313 4648; fax: +61 8 8313 4373.
E-mail addresses: moom.aw@alumni.adelaide.edu.au, moom.aw@adelaide.edu.au, awymy001@mymail.unisa.edu.au (M.S. Aw), dusan.losic@adelaide.edu.au (D. Losic).

0378-5173/$ – see front matter.
http://dx.doi.org/10.1016/j.ijpharm.2013.01.004

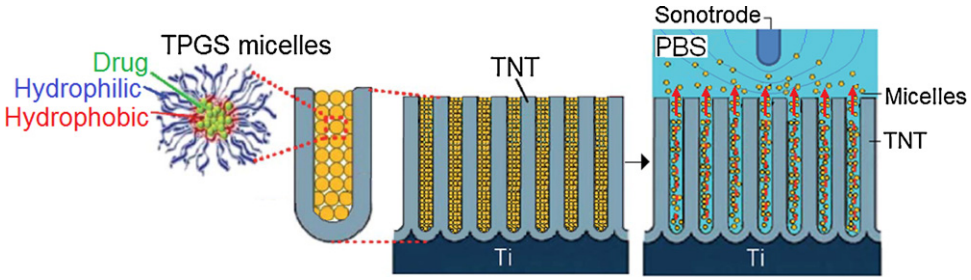

Fig. 1. The scheme of ultrasound-stimulated drug delivery based on titania nanotubes (TNTs) arrays as drug–drug carrier releasing platform and polymeric micelles as drug carriers.

delayed, sequential and simultaneous release of several drugs were demonstrated on TNTs (Aw et al., 2011b, 2012a,b). These strategies were specifically designed to address the limitations of conventional DD in bones, to improve existing bone therapies, or to mitigate the risks of rejection in orthopedic bone implants (Losic et al., 2011; Gulati et al., 2011). Nevertheless, these approaches do not have the capacity to change the release characteristics and drug dosage during the process of their elution from TNTs, as the release is governed by a diffusion-controlled process. Without an external force, this pattern remains the same. A stimulus-responsive release of drugs is important for many applications in bone therapies and orthopedics, where the local delivery of drugs *in situ* during emergencies is required, with an adequate dosage under well-controlled times and within surgical/medical constraints (Hall et al., 2007; Howell and Howell, 2012).

Therefore, to address stimulus-responsive requirements of local DD devices, in our previous work, we successfully demonstrated magnetically stimulated drug release from TNTs using dopamine-conjugated iron oxide nanoparticles (Aw et al., 2012c). One disadvantage of this system is that an accidental release of drugs can be caused by unexpected magnetic interference from any foreign source in close proximity to the TNTs. This could result in unwanted exposure of external magnetic field, which leads to drug leakage at unsolicited times. To address this shortcoming, we explore several DDS by adopting different external fields as the triggering source for drug or drug carrier release, *i.e.* using radiofrequency (RF) and ultrasound waves (USW). The aim of this work is to present the concept of stimulated drug-micelles release from TNT-Ti implants, using USW induced in buffer from an external ultrasound generator. Ultrasound technology has a long history in medicine for imaging. However only in recent years, the cavitations of ultrasonic microbubbles have been proposed as an innovative method for low-invasive and tissue-specific delivery of genes and drugs to cancer cells or tissues of interest (Schlicher et al., 2006; Kim et al., 2006; Wu and Nyborg, 2008). Several approaches using microparticles and systemic drug delivery carriers suitable for ultrasonically enhanced DD have been suggested, such as polymeric micelles, microbubbles, gas-filled liposomes, and modified lipospheres (Huang and MacDonald, 2004; Rapoport, 2012; Unger et al., 2004). Studies have indeed shown that micellar delivery for drugs with localized ultrasonic tumor irradiation could lower tumor growth rates (Gao et al., 2005; Pruitt and Pitt, 2002).

In this work, the application of ultrasound-mediated drug-micelles release for local DDS is demonstrated for the first time. The principle of the proposed concept using TNTs as drug-releasing implants is shown in Fig. 1. The ultrasound generator was used to exert oscillating pressure waves from the probe (Sonotrode) inserted in the medium (phosphate buffered saline, PBS at pH 7.2) close to the drug-micelles loaded TNTs. Indomethacin, a non-steroidal anti-inflammatory drug with analgesic and antipyretic activity, was used as the model for water insoluble drugs and encapsulated in polymeric micelles, *i.e.* D-α-tocopheryl polyethylene glycol succinate 1000 (TPGS) which functioned as drug carriers (Sriamornsak et al., 2012; Zhang et al., 2012). Several USW parameters to control the drug-micelles release, including pulse length, pulsation time, amplitude and power intensity were explored. The amount of released drug carriers from TNTs into PBS during the generation of USW was monitored closely *in vitro* and measurements were obtained using UV–vis spectroscopy and thermogravimetry analysis (TGA).

2. Materials and methods

2.1. Materials

High purity (99.997%) titanium (Ti) foils supplied by Alfa Aesar (USA) were used as the substrate for TNTs fabrication. The ammonium fluoride/ethylene glycol ($NH_4F/C_2H_6O_2$), phosphate buffered saline (PBS) tablets and acetone were obtained from Sigma–Aldrich Co., Australia, and used without further purification. D-α-Tocopheryl polyethylene glycol succinate 1000 (TPGS) used for the synthesis of amphiphilic polymeric micelles as drug carrier; and indomethacin (Ind) as water insoluble drug model, were also supplied by Sigma–Aldrich Co. (Australia). The purchased PBS tablets were reconstituted with 200 ml Milli-Q water, which yielded 0.01 M PBS, 0.0027 M potassium chloride and 0.137 M sodium chloride at pH 7.2 and 25 °C. High purity ultra-grade Milli-Q water (18.2 MΩ cm) (pH = 6.5 ± 0.5) with a final filtering step through a 0.22 μm filter was used for the preparation of all reagents and solutions.

2.2. Methods

2.2.1. Fabrication of TNT-Ti drug-releasing implant

The Ti foil was cut to size (1.2 cm × 1.2 cm), mechanically polished, ultrasonically cleaned and washed with acetone and Milli-Q water. TNT arrays were prepared *via* a two-step electrochemical anodization process using ammonium fluoride/ethylene glycol electrolyte at 20 °C and a constant voltage of 100 V for 2 h as described previously (Paulose et al., 2008; Kant and Losic, 2009). The voltage/current, voltage–time and current–time signals were continuously recorded (Labview, National Instruments) during the anodization period to ensure reproducibility of this fabrication process. The anodization voltage and time were used to control the nanotube diameters (70–180 nm) of TNTs and their lengths (thickness), ranging from <5 μm to >250 μm (Losic et al., 2011).

2.2.2. Synthesis of TPGS micelles and drug encapsulation inside the drug carrier

TPGS polymer – a water-miscible vitamin E derivative was used to formulate amphiphilic micelles as drug carriers to encapsulate indomethacin, using a simplified lyophilization technique (Aw et al., 2011a). 15 mg micelles was weighed and dissolved in