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Technical Note

Formation of microcapsules by ultrasound stimulation for use in remote-controlled drug-eluting stents

Wei Yao[a,*], Yan Bao[a], Yu Chen[b]

[a] Wolfson Centre, Department of Biomedical Engineering, The University of Strathclyde, Glasgow G4 0NW, United Kindgom
[b] Department of Physics, The University of Strathclyde, Glasgow, United Kindgom

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ABSTRACT

Coronary Heart Disease (CHD) is the leading cause of death globally. The placement of drug-eluting stents (DESs) in diseased coronary arteries is the most successful minimally-invasive intervention to treat CHD. The key limitations of such interventional therapy are the risk of in-stent restenosis (ISR) and late stent thrombosis. This paper investigates a new drug-release system by formatting nanoparticles as drug carriers, which are later subjected to an external ultrasonic stimulus for controlled drug release remotely for DESs. The drug delivery could delay smooth muscle cell growth whilst enabling effective regeneration of a functional endothelium. Microcapsules were produced by employing a layer-by-layer technique, encapsulated with Rhodamine 6G dye used in place of anti-restenotic drugs. Gold nanoparticles were employed as a shell in the microcapsules. The presence of gold nanoparticles significantly enhanced the efficiency of the ultrasonically induced dye release from the microcapsules and increased the sensitivity of the microcapsules to ultrasonic stimulation compared to those without gold nanoparticles.

1. Introduction

Coronary Heart Disease (CHD) is a global health challenge, resulting in around 17.3 million deaths annually [1]. The placement of stents in diseased coronary arteries is the most successful minimally-invasive intervention to treat CHD. The most advanced stents are drug-eluting stents (DES), which release a drug to inhibit the excessive smooth muscle proliferative process responsible for the inconsistent results achieved with bare metal stents. However, there is evidence that DES prevent regrowth of the endothelial cell layer (endothelium) that lines the innermost layer of the artery and it is increasingly clear that regeneration of this layer is crucial to securing positive long term outcomes [2]. There is thus a need for a stent that inhibits smooth muscle cell growth whilst enabling effective regeneration of a functional endothelium. Current DES drug release profiles lead to high initial anti-proliferative drug concentrations within the artery wall, which slowly decay over weeks and months [3]. However, the process of endothelial regeneration is thought to occur within the first few days following bare metal stent implantation [4], whilst the excessive smooth muscle cell proliferation response is significantly delayed and occurs over weeks and months [5]. In this context, it is perhaps not surprising that existing DES have been associated with incomplete regeneration of the endothelium. Any drug delivery which could delay drug release until after the endothelium had fully healed would therefore be a significant advance. The aim of the research is to investigate ultrasound activated nanoparticles as a means of achieving this, with drug being released into the artery wall when it is most needed.

However, in-stent restenosis (ISR) remains a serious problem following implantation. The major reason for ISR is the injured arterial wall causing smooth muscle cell (SMC) proliferation and scar tissue accumulation [6]. Meanwhile, drug release happens spontaneously after implantation and often is uncontrolled [7]. To cope with this problem, nanocapsules combined with a drug delivery system can enable drug release in a specific site, as requirements [8–10].

Stimuli-responsive nanocapsules can release the drug in a controlled manner and the non-invasive nature of the technique has advantages in therapeutic application, such as reduced possibility of infection, avoided damage to surrounding tissues by devices. Most of the literature on nanoparticulate carrier systems is based on the employment of lipid, polymeric, different types of nanoparticulate carriers, and self-assembling carriers [10]. In general, the layer-by-layer assembly of nanocapsules shows advantages: it achieves the integration of component materials from different nature within the films, and it makes the incorporation of various biomolecules into the films. The layer-by-layer technique

* Corresponding author.
E-mail address: w.yao@strath.ac.uk (W. Yao).

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consists of renaturation of polyion adsorption, allowing the alternation of the terminal charge after each subsequent layer deposition. Furthermore, it achieves a defined control over the thickness, structure, mechanical characteristics and composition of assembled materials [11].

Gold nanoparticles (AuNP) have been studied for many years in applications such as cancer treatment [12]. They have high biocompatibility, ease of surface modification, facile synthesis, and tuneable optical characteristics [13]. Based on previous research, the release efficiency was investigated by coating gold nanoparticles in the microcapsules shell [14]. When subjected to ultrasound, the microcapsule wall undergoes a morphological change due to shear forces due to the ultrasonic oscillations. If the ultrasonic wavelength correlates to the microcapsule's size, it can maximize the effect. At 1 MHz, the wavelength closes to the size of PE microcapsules [15]. With the introduction of a rigid material such as the AuNP, the higher amount of the embedded nanoparticles leads to a decreased Young's modulus and microcapsule shell elasticity [16]. This can influence the fracture rate under ultrasonic triggering, making them more vulnerable to ultrasound. In addition, the gold nanoparticles are inert and are non-toxic, so they are a feasible material for medical applications. Moreover, the mechanical properties of gold nanoparticles also perform well making the microcapsules relative stable [17,18].

Ultrasound is an effective external stimulation that can induce encapsulated drug delivery in vivo [19]. Therefore, ultrasound can act upon biomolecules. Ultrasound-responsive polymers for drug delivery systems have been studied in medical diagnostics and treatment [20]. Some polymeric systems that respond to ultrasound are mainly polymeric micelles, gels or other layer-by-layer (LbL) coated nanoparticles. Langer et al., have studied the release rate of incorporated components through the stimulation of ultrasound from polymers, including polylactides, biodegradable polyglycolides and ethylene-vinyl acetate copolymers [21–27]. It has been shown that ultrasonic stimulation can facilitate the permeation through some polymers with no erosion and enhance the decomposition rate in some biodegradable polymers [28,29]. Miyazaki et al. studied the ethylene-vinyl alcohol (EVAL) copolymer and insulin in diabetic rats and were able to control insulin release through the ultrasonic stimulation [30]. Receiving implants encapsulating insulin produced an ultrasonic stimulation (1 MHz, 1 W/cm^2) and a significant decrease in the level of blood glucose was observed. The results demonstrated a rapid rate of release of insulin in the targeted region. In previous research of this group, they also demonstrated that the release rate of 5-fluorouracil from an EVAL copolymer can rise at desired times upon the ultrasonic stimulation in vivo [31]. Ultrasonic stimulation can induce the collapse of drug carriers and achieve payload release for the uptake of target cells. The site specificity can be promoted by incorporating a surface ligand on the carrier, which is able to bind to specific receptors for specific targeting [32,33].

2. Methods

2.1. Synthesis of polylactic acid (PLA) microcapsules

The work in this research is an attempt to develop a smart drug nanostructured delivery system that is controllable using ultrasonic stimulation. This research focused on the polyelectrolytes like Polylactic acid (PLA), Poly(allylamine hydrochloride) (PAH), and Poly(styrene sulfonate) (PSS) because of their feasibility and their ability to function as a drug carrier. Rhodamine 6G, a kind of fluorescein dye, loaded in microcapsules was used as a drug surrogate in this model system for the controlled release studies.

The core of the microcapsule was made up of PLA microparticles. PLA microcapsules were achieved by using the nanoprecipitation method and this method has been introduced in previous research [34]. The main procedure to generate PLA/Rhodamine 6G microcapsules was to dissolve PLA in acetone and equilibrate for 12 h at room temperature. Next, Rhodamine 6G was added to the acetone solution. Gradually, the colour changes of an acetone solution were observed from colourless to bright red due to the Rhodamine 6G becoming dissolved. The organic solution was stirred to allow the PLA and the Rhodamine 6G to become completely attached. After stirring, the organic solution was added drop by drop into distilled water containing no surfactant, with appropriate stirring for 2 h. After injecting the organic phase into the aqueous phase, a conspecific microdispersion was obtained. The PLA microcapsules were suspended in water through centrifugation. The PLA/Rhodamine 6G capsules served as the cores of the microcapsules for the next layer-by-layer assemblies. The Rhodamine 6G dye served as a tracer material in place of anti-restenotic drugs. It can be easily detected using fluorescence spectroscopy.

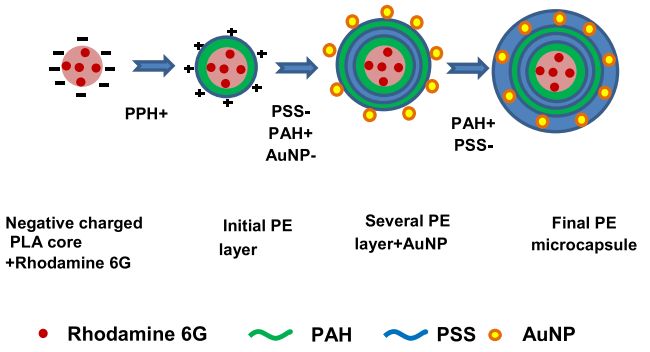

Fig. 1. The layering processes of microcapsules.

The layers coated on the PLA cores contain PAH polymers (positive charged), PSS polymer (negative charged) and citrate stabilized gold nanoparticles (negative charged). With the opposite charge on polyelectrolytes, the microcapsules can be formed and the number of layers can be varied as required. Zeta potential measurement was employed to assess the layer-by-layer assembly process. In terms of the remote ultrasonic stimulation process, the microcapsules suspended in liquid can be stimulated. In the further clinical application, the system aims to allow the drug release from DES to occur in a controllable manner (Fig. 1).

The grey sphere represents the PLA microo-cores; the pink dots represent the Rhodamine 6G which serves as a tracer material in place of anti-restenotic drugs; the green layer represents the PAH layer which is positive charged; the blue layer represents the PSS layer which is negative charged; the gold layer represents the gold nanoparticles which are negative charged. PLA microcapsules consist of a PLA core, PAH, PSS and gold nanoparticles shells fabricated by a layer-by-layer assembly technique. In brief, a 37.5 mL of PAH (1 mg/mL) aqueous solution was added to 25 mL of the PLA and stirred for 2 h. The solution was centrifuged for 15 min at 13,000 rpm and the supernatant was removed. The PAH coated microcapsules were resuspended in 31 mL of distilled water for the next layer assembly. For the second polyelectrolyte layer coating, 46.5 mL of PSS (1 mg/mL) was added to the above solution and stirred for 2 h. After centrifuging employing the same parameters, PAH/PSS coated microcapsules were re-suspended in 38 mL of distilled water. The third layer of PAH was assembled following the same process and the PLA-Rh6G/PAH/PSS/PAH microcapsules were again redispersed in 47 mL of water. Next 28.2 mL of citrate stabilized gold nanoparticles (25 mM, synthesized by the sodium citrate reduction method [35]), was added and was vigorously stirred for 2 h. Following centrifugation and re-suspension,