Formation of Microcapsules by Ultrasound Stimulation for Use in Remote-controlled Drug-eluting Stents

Authors

Wei Yao\textsuperscript{a}, Yan Bao\textsuperscript{a}, Yu Chen\textsuperscript{b}

\textsuperscript{a}Department of Biomedical Engineering, The University of Strathclyde, Glasgow, United Kingdom

\textsuperscript{b}Department of Physics, The University of Strathclyde, Glasgow, United Kingdom

Corresponding Author

Wei Yao

Wolfson Centre

Department of Biomedical Engineering

University of Strathclyde

Glasgow G4 0NW

UK

Email: w.yao@strath.ac.uk

Word count: 5000
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.

Keywords: Drug-Eluting Stent, Remote Control, Nano-particle
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 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 maximise 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 (1MHz, 1 W/cm2) 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 hours 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 hours. 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.
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.

Figure 1 The layering processes of microcapsules.

The grey sphere represents the PLA microcores; 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 hours. The solution was centrifuged for 15 minutes at 13000 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 hours. 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 hours. Following centrifugation and re-suspension, the last layer of PAH was assembled in the same way. Finally PLA-Rh6G/PAH/PSS/AuNP/PAH core-shell structure composite microcapsules were obtained after centrifugation and re-suspension, ready for the ultrasonic stimulation experiments.

2.2. Ultrasonic stimulation

In this research, the trigger mechanism was remotely stimulated with ultrasound at a frequency of 1MHz, which poses no harm for the human body within a safe range of frequency. In this research, a hand-held ultrasound device with a frequency of 1 MHz was employed for the stimulation of PLA-Rh6G/PAH/PSS/PAH/AuNP/PAH microcapsules. The technology of therapeutic ultrasound is a high frequency sound vibration which cannot be felt by humans, and can stimulate tissue up to 5 cm beneath the skin’s surface. The transducer used in the experiment is a therapeutic ultrasound device ULTRALIEVE made by Actegy Ltd UK. The effective intensity that represents the amount of energy transferred to the tissues is 2.4W/cm². Power supply input is at AC 100-240V 50/60Hz, 0.35A. The size of the transducer is 34mm diameter × 10mm high and the effective radiating area is 4cm².

It is generally tissues or microcapsules which contain components that are sensitive to the effects of heat. Abnormalities in biochemical processes may appear following the increase of temperature above the
normal basal levels. Normally, the heat generated by ultrasound is mostly absorbed by blood circulation in vivo, and some heat is distributed through adjacent tissues. Therefore, the heat imparted onto body tissue should be given further attention, otherwise, some tissues or cells will be destroyed. The ultrasonic stimulation process in this project was not active for a long period of time to avoid overheating the microcapsules. In this project, the frequency of medical device was 1MHz, which meets the safety requirement in human body application.

The duty cycle contains three different levels including Low (30% duty cycle, 0.75W/cm2), Medium (40% duty cycle, 1.0W/cm2) and High (50% duty cycle, 1.2W/cm2). The duty cycle is defined as the percentage during one period in which the signal is active [36-37]. A schematic diagram of the ultrasonic stimulation imparted onto the microcapsules is shown in Figure 2.

\[
D = \frac{T}{P} \times 100\% \tag{1}
\]

In Equation 1, D represents duty cycle, T(μs) represents the duration of active signal, and P(μs) represents total duration of signal. For example, a 30% duty cycle pulsed waveform would have ultrasound on for a total of 30% of the entire treatment, and off for a total of 70%. A 100% duty cycle is the same of “continuous”. The device used in this research, the duty cycle is fixed which includes 30%, 40% and 50%.
The PLA-Rh6G/PAH/PSS/PAH/AuNP/PAH microcapsules were suspended in the tube and the tube was fixed in water. The ultrasound device was fixed and kept working, which is shown on the left of figure 2. In the microcapsules details shown on the right of figure 2, the gray spheres represent the PLA microcapsules, the red particles represent Rhodamine 6G dye, the blue curves represent the PAH layer (positive charged), the black curves represent the PSS layer (negative charged), and the yellow curves represent the AuNP shell. The dye is released by the stimulation of ultrasound.

In the future, these drug-loaded microcapsules will be planned to apply to the stents, allowing for a stimuli-response to ultrasound.

3 Results

3.1 SEM analysis microcapsules
PLA-Rh6G /PAH /PSS /PAH /AuNP / PAH/ microcapsules were prepared by an alternating deposition of cationic polymers (PAH) and anionic polymers (PSS) onto the PLA cores doped with Rh6G. An SEM image of the microcapsules is shown in Figure 3.

![SEM image of microcapsules](image)

Figure 3 shows that the majority of the microcapsules have dispersed spherical shapes with an average size of 165 ± 25 nm (200 microcapsules were accounted).

3.2 Characterisation of the microcapsule assemblies

In this research, the microcapsules were synthesized with four polymer layers and one AuNP layer, which contained opposite charges, via layer-by-layer self-assembly processes. To examine the layer-by-layer assembly of PAH, PSS and AuNP on the PLA microcapsules, the sequential assembly procedures were monitored by means of zeta potential of the microcapsules. The variation of zeta potential with the sequential adsorption of the polyelectrolyte layer for the PAH/PSS/PAH/AuNP/PAH coatings is shown in Figure 4.
The zeta potential value of the pristine PLA microcapsules was negative (-34.5 mV). This is believed to be due to the carboxylic groups present at the surface of the PLA microcapsules [38]. It was observed from the zeta potential measurements that the adsorption of a positively charged PAH layer on the PLA-Rh6G microcapsules changed the zeta potential from -34.5 mV to +30.4 mV. Subsequently, the deposition of a PSS layer led to another potential reversal from +30.4 to -50 mV. Further alternating deposition of the PAH, AuNP and PAH led to continuous reversals in zeta potentials. This revealed a stepwise layer assembly during the fabrication of the composite microcapsules.

The UV-vis spectrum of the PLA-Rh 6G/PAH/PSS/PAH/AuNP microcapsules is shown in Figure 5. An absorption band due to the surface plasmon resonance of gold nanoparticles was observed indicating the presence of gold nanoparticles in the microcapsules. A red-shift of the surface plasmon band from 519 nm shown in Figure 5(b) to 556 nm in figure 5(a) may possibly be due to the aggregation of the gold nanoparticles when they were assembled onto the microcapsules. This absorption overlaps with a slope
of scattering from the large microcapsules. The absorption of Rhodamine 6G (526 nm) is not visible here because it overlaps with the strong scattering of the micro-capsules and also the absorption band of the gold nanoparticles.

![Graph](image.png)

Figure 5. (a) Extinction spectrum of the PLA/Rh6G/PAH/PSS/PAH/AuNP/PAH micro-capsules (556nm). (b) UV-vis extinction spectrum of colloidal gold nanoparticles (519nm).

### 3.3 Ultrasonic stimulation of the microcapsules

The release of Rhodamine 6G dye from the microcapsules after each ultrasonic stimulation was examined via the measurement of concentration of Rh6G in the supernatants. Both the samples and the control samples (microcapsules without gold nanoparticles) undergo ultrasonic stimulation at three different duty cycles. Figure 6 shows the fluorescence intensity change of the dye released from the PLA-Rh6G/PAH/PSS/PAH/AuNP/PAH microcapsules against the ultrasonic stimulation times of the different duty cycles. The measurement was taken after each 15 minutes of stimulation.
Figure 6. Rhodamine 6G concentration released from the capsules with gold nanoparticles against the ultrasonic stimulation time of the different duty cycles. The orange line represents the total concentration of Rhodamine 6G contained in the capsules.

The measurement was repeated twice independently in the collection of the presented in vitro data. The graph represents means with error bars for six times. Graph markers denote the data mean. The bars represent one standard deviation of the mean. Rhodamine 6G concentration in Figure 6 showed that samples after high-duty cycle ultrasonic stimulation carried out 6 times, 15 minutes each time, released more of the dye than under medium or low-duty cycle treatment. Little dye was released from these microcapsules after the first 15 minutes of the stimulation. Clear increases of Rhodamine 6G were observed after the second stimulation, although no significant differences were found among the different duty cycles. With the increase of the ultrasonic stimulation times, the Rhodamine 6G concentration further increased. This indicates that the ultrasonic treatment has a promoting effect on the dye release from the microcapsules with the gold nanoparticles. After the fourth (total 60 minutes) ultrasonic
treatment, the dye was still released from the samples. However, the rate of the Rhodamine 6G concentration changes was reduced, particularly for the medium and low-duty cycle treatments. After the sixth ultrasonic stimulation (90 minutes), the Rhodamine 6G concentration of the dye released from the sample under the high-duty cycle treatment was close to the initial total intensity of the dye adsorbed onto the microcapsules. This implies that most of the dye contained in the samples had been released. In comparison, the Rhodamine 6G released from the samples with the medium or low-duty cycle of ultrasound was lower than with the high-duty cycle. This means that there was still some dye in the microcapsules. This is not surprising as the medium or low-duty cycle of ultrasound presented lower power intensities than the high-duty cycle of ultrasound, causing a lower dye to be released from the microcapsules. Nevertheless, the dye released from the microcapsules with ultrasonic stimulation was still presented.

To study the influence of gold nanoparticles on the efficiency of the dye release, microcapsules without gold nanoparticles in their shells were also synthesized and analyzed. Their results are shown in Figure 7. The graph represents means with error bars for six times. Graph markers denote the data mean. The bars indicate data range.
Figure 7. Rhodamine 6G concentration of the dye released from the capsules without gold nanoparticles measured after various ultrasonic stimulation times of different duty-cycles.

It shows that there is no obvious changes in the concentration and R6G concentration is below 3% of the total concentration after six cycles of stimulation. However, the change of fluorescence intensity with the increase of the ultrasonic stimulation time was small. After the 6th stimulation, the concentration of Rh6G was just 10% of the total initial concentration of Rh6G. Moreover, there was no significant difference in the efficiency of the dye released by employing different duty cycles. It is thus clear that gold nanoparticles in microcapsules play an important role in the ultrasonic induced release of dye.

Finally, the release of dye from samples (1.5 ml) stored at room temperature at 200 without ultrasonic treatment during a period of four weeks was also studied as a comparison. Both microcapsules with and without gold nanoparticles were used in this study. Figure 8 shows the Rhodamine 6G concentration changes of the dye released from microcapsules after a long period of storage at room temperature at 20 degrees.
Figure 8. Rhodamine 6G concentration of dye released from capsules (a) with and (b) without gold nanoparticles after room temperature storage (without any ultrasonic stimulation). The red line represents the Rhodamine 6G contained in the samples.

Figure 8 (a) shows that after an initial small release in the first week, a large amount of dye was released from microcapsules with gold nanoparticles in the following three weeks. The graph represents means with error bars for five times. Graph markers denotes the data mean. The bars indicate data range. In contrast, no obvious changes in the Rhodamine 6G concentration was observed in the case of microcapsules without gold nanoparticles for the first few weeks. The intensity of released dye after 4 weeks was significantly weak compared to that from microcapsules with gold nanoparticles. This finding suggests that microcapsules containing gold nanoparticles are less stable compared to those without gold nanoparticles.

4. Discussion

In this study, the presence of gold nanoparticles allowed the dye to be released from PLA-Rh6G/PAH/PSS/PAH/AuNP/PAH microcapsules more efficiently, compared to microcapsules without
gold nanoparticles. According to these results, several factors may play a role in the stimulation process. The presence of gold nanoparticles in the microcapsules makes the shells stiffer and lower in their elasticity, which may have an effect on how easily the shells will move when applying an oscillating force to them in liquid by means of ultrasonic stimulation. If they are moved, the rupturing may occur as expected. As illustrated by Fery et al., the presence of inorganic nanoparticles increases the density contrast of a microcapsule shell, which also decreases the elasticity of the shell. These are important for achieving high efficiency in the ultrasonically treated release of compounds encapsulated in capsules [39].

The effects of ultrasound as a release trigger may be attributed to acoustic cavitation in liquids under ultrasonic vibrations with a frequency of more than 20 kHz. As demonstrated by Maria N. Antipina et al., ultrasound energy produces pressure and can cause the shrinkage of gas-filled nanobubbles. Then, the cavitating nanobubbles implode, which produces local shock waves and destroy the nanocapsules assemblies [40-44]. Even at low input power, the collapse of microbubbles in liquid results in an enormous concentration of energy. When the capsules are subjected to ultrasonic stimulation, shear forces between the successive fluid layers occurs, which leads to the disruption of the capsule assemblies and the subsequent release of their payloads [39]. Therefore, acoustic cavitation resulted in dye release from the nanocapsules in this study is a possible factor.

As for the nanocapsules without gold nanoparticles, they presented little dye release in this research. It can be assumed that the capsules may be disrupted because of the absorption of intense energy of ultrasound over a longer time. In previous research by academics, ultrasonic stimulation operating on the capsules has been proven to produce heat. High temperature causes changes in permeability of the outer
shell and can even rupture the capsules. Subsequently, the release of capsules payload will be achieved [45]. The heat produced by ultrasound may also have an influence on the dye released from samples without gold nanoparticles.

5. Conclusions

This work aims to investigate a drug delivery system with stimuli-responsive polymeric microcapsules that can achieve a locally controlled drug release by means of remote ultrasonic stimulation. The microcapsules were synthesized with polyelectrolyte layers and gold nanoparticles by means of a layer-by-layer assembly technique, encapsulated with a model dye used in place of anti-restenotic drugs. The microcapsules with and without gold nanoparticles embedded in the shell were examined for their response to ultrasonic stimulation. The presence of gold nanoparticles significantly enhances the efficiency of the ultrasonically induced dye release from the microcapsules. It is shown that the microcapsules containing gold nanoparticles are more sensitive to ultrasonic treatment compared with the microcapsules without gold nanoparticles. Such a method will give the interventional cardiologist more control over the medical implants.

Declarations

Interests conflict: None

Funding: None

Ethical approval: Not required

6. References


