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# Indocyanine Green Loaded PLGA Film Coated Coronary Stents for Photo-Triggered *in situ* Biofilm Eradication

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ABSTRACT

Biofilm formation is a major impeding factor for successful angioplasty. Bacteria dwelling in the biofilm are resistant to most systemically administered antibodies necessitating stent removal which is often not a suitable option for patients suffering from coronary artery disease. In the quest to develop a suitable system for combating biofilm development on stents, we have formulated a stable titanium surface modification based on electrostatic interactions and investigated *in situ*. For this purpose, surface activated titanium was modified with amine rich (3-Aminopropyl)-triethoxysilane (APTES) to enable coating with a photosensitising drug containing polymer (Indocyanine green (ICG)/ poly(lactic-*co*-glycolic acid) [PLGA]) solution. The surface modifications were characterised using scanning electron microscopy and confocal microscopy together with antibacterial studies were used to qualitatively and quantitatively determine the antibacterial effect respectively. IR (810 nm) laser was used to trigger ICG leading to a dramatic reduction (99.96%) in bacterial viability inside the biofilm. The polymer, PLGA used in this study is a widely explored biocompatible and biodegradable polymer thereby making it an excellent choice for coating materials intended for use in biological systems. Indocyanine green on the other hand is a well-documented diagnostic agent and has gained much popularity in the recent past as a photosensitising agent for use in photodynamic therapy. Using this novel technique the photosensitising ICG could be triggered using IR laser leading to the generation of bacteriostatic reactive oxygen species at the site of irradiation. Due to the inertness of the photosensitising ICG in the absence of IR trigger, risk of antibacterial resistance could be ruled out. In the absence of biofilm formation following stent insertion/angioplasty, the ICG containing PLGA degrades with time. This first of its kind minimally invasive method would help avoid/treat bacterial infections and biofilm formation on coronary stents.

## 1. Introduction

Aortic or coronary stent insertion is associated with a serious risk of bacterial infection [1]. The high risk of biofilm formation within 24 h after attachment of the bacteria to the surface is extremely dangerous for the patient due to reduced capabilities to treat the bacteria within the biofilm by systemically administrated antibiotics [2]. The bacteria build a stable matrix comprising polysaccharides and proteins leading to an increase in antibiotic resistance [3]. Even patients with a good immune response often fail to eliminate the bacteria in biofilms. Stent removal is the most common practice following biofilm development and is associated with a mortality rate of 18% [4]. Biofilm formation on stent surfaces is extremely dangerous and may lead to persistent infections or sepsis. It is therefore necessary to find better ways to avoid or treat a bacterial infection on stent surfaces. In this study, we have developed a novel method using photodynamic therapy (PDT) to eradicate bacteria in biofilms wherein a thin film of biocompatible and biodegradable poly (lactic-co-glycolic acid) (PLGA) containing indocyanine green (ICG) is attached onto the titanium surface. PLGA is a biocompatible and biodegradable polymer which also has antiadhesive properties to reduce the count of attached bacteria qualifying PLGA as an excellent choice for biodegradable carrier systems [5–8]. Due to the decrease in pH at the site of bacterial infection, the pH sensitive PLGA readily hydrolyses releasing its cargo. Several kinds of drugs such as antithrombogenic agents, anti-inflammatory agents, anti-infective agents or a combination thereof could be used in combination with PLGA. The drug of choice, ICG, used in this study, is an FDA approved diagnostic fluorescent dye and a proven photosensitiser used for several forms of photodynamic therapy [9,10]. Irradiation of ICG at 810 nm leads to the production of reactive oxygen species (ROS) and singlet oxygen [11]. The generated singlet oxygen interacts with unsaturated phospholipids to form hydroperoxides [12]. Since photosensitisers are

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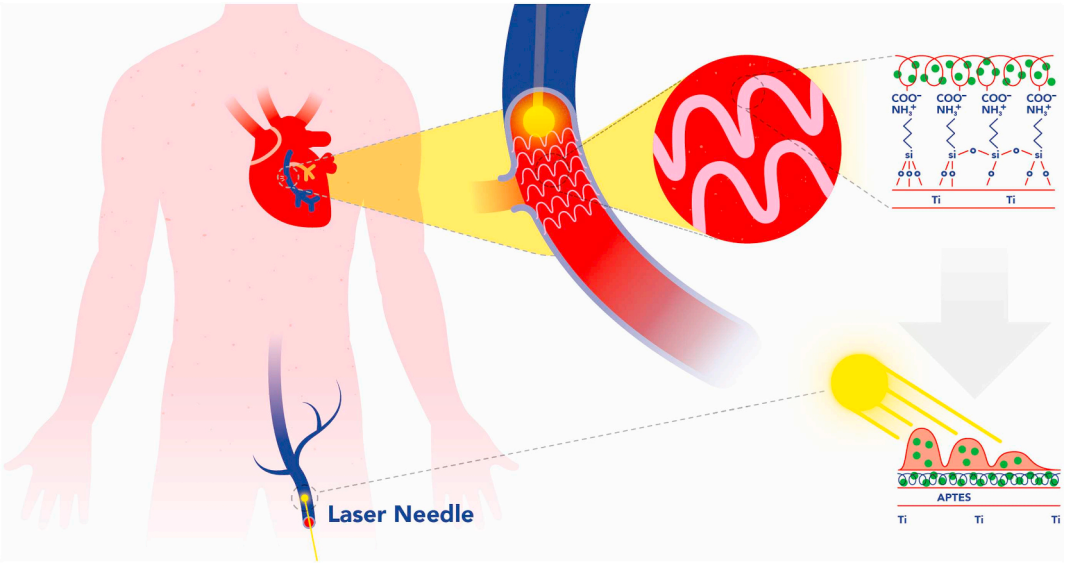


**Fig. 1.** Illustration of *in situ* photo-triggered activation of ICG in coronary stents.

only active in their excited state, development of antibiotic resistance is reduced. This study, titanium has been used as a model stent material and *Staphylococcus saprophyticus* subsp. *bovis* as biofilm creating model organism to study the effect of ICG mediated PDT.

## 2. Materials and Methods

Titanium plates (2.25cm$^2$) were cleaned with MilliQ® water followed by absolute ethanol and finally with chloroform in an ultrasonic bath with each cleaning step lasting for 10 min. After drying, the plates were incubated in a mixture of sulphuric acid (98%) and hydrogen peroxide (30%) at a ratio of 1:3 for 40 min. 30 titanium plates (2.25cm$^2$) were then thoroughly washed in MilliQ® water. After drying, the plates were treated with 5% (3-Aminopropyl)-triethoxysilane (APTES)/toluene (anhydrous, 99.8%) in 40 ml solution, under argon flow at boiling temperature (110 °C) for 4 h using the method of G.Tan with slight modifications [13]. The plates were then washed with absolute ethanol and dried under nitrogen flow and stored in a desiccator under argon atmosphere. The plates were incubated for 40 min in 20 ml PLGA/Indocyanine green (ICG) (PLGA 10 mg/ml; ICG 1 mg/ml) dissolved in acetone. Resomer 503 h ($M_w$ 24,000–38,000 g/mol) from Evonik Industries (Darmstadt, Germany) comprising lactide:glycolide 50:50 with acid terminated ends was used as a PLGA of choice. The plates were finally dried under normal atmospheric conditions and stored in the desiccator [14]. For the visualisation of the coating, the samples were sputtered coated with gold and examined in a scanning electron microscope under high vacuum ($4 \times 10^{-6}$mbar) at 15 kV accelerating voltages and 30 µA emission current [15]. Atomic force microscopy (AFM; JPK Nanowizard 3) was used to measure the coating thickness after scratching off a part of the coating. Measurements were performed using aluminium coated silicon cantilevers HQ:NSC16 with scan rates between 0.5 and 1Hz[14]. For the *in vitro* release study, 1cm$^2$ coated titanium plates were incubated in phosphate-buffered-saline (PBS, pH 7.4) at 37 °C under sink conditions. At 0, 1, 6, 24, 48, 72, 120, 168 and 240 h time points, 3 titanium plates were collected and dried under nitrogen flow in the absence of light and dissolved in Methanol:Acetonitril (1:3) and finally measured by UV–vis spectroscopy at 783 nm. For stability tests, the coated titanium plates were incubated in PBS (pH 7.4) at 37 °C for 3 h, 1, 2, 3, 5, 7, 10 and 14 days. After the incubation period, the plates were dried and evaluated by scanning electron microscopy (SEM). Elementary analysis using energy-dispersive X-ray spectroscopy was performed in a JEOL JIB-460F scanning electron microscope (Jeol, Tokyo, Japan) equipped with an XFlash Detector 5010 (Bruker Nano, Berlin, Germany).

For irradiation experiments, a Weber Endolaser (Weber Medical, Lauenförde, Germany) with a wavelength of 810 nm (infrared) and an irradiation intensity of 500 mW was used. Bacterial viability was assessed in *S. saprophyticus* subsp. *bovis* similar to previous studies done by Dayyoub et al. with slight modifications [14,16]. Bacterial suspension (in TSA broth) at optical density of 0.6 at 600 nm was used. PLGA/ICG coated titanium plates were incubated in 5 ml bacterial suspension for 48 h at 37 °C. Subsequently, the slices were gently rinsed with PBS (pH 7.4) and placed in 24 well plates containing 2 ml PBS. The samples were irradiated for 10 min at distance of 3 cm (corresponding to 78.2 J/cm$^2$). The samples were then incubated in 1 ml trypsin solution (5 mg/ml) under gentle stirring at 100 rpm/min for 20 min at 37 °C to remove the adhered bacteria. The trypsin solution was diluted 1:10, 1:100 and 1:1000 1:10000 with MilliQ® water. 50 µl of the dilutions were homogenously spread onto TSA agar plates (Ø90mm). The CFUs were counted after 24 h incubation at 37 °C under humidified atmosphere. For qualitative determination of viability, samples were prepared similar to the CFU counting method and reagents of the BacLight bacterial viability kit (Thermo Fischer Scientific, Darmstadt, Germany) containing SYTO9 and PI fluorescent dyes were added according to the manufacturer's protocol under light exclusion. Samples were mounted onto glass slides and visualised using a confocal microscope according to the manufacturer's recommendation regarding the wavelengths and filters.

For statistical analysis, Minitab 18 (Minitab Inc., USA) was used. The threshold for significance in significance test was set at *p*-values under 0.05. After performing variance, homogeneity and the corresponding robust test, the results were compared with Tukey post-hoc-test in ANOVA [17].

## 3. Results and Discussion

Through incubation in sulphuric acid and hydrogen peroxide, the activated surface exhibiting hydroxyl groups shows more affinity for silanisation with APTES. APTES contains positively charged amine groups on the surface which facilitate an interaction with the carboxyl groups of the PLGA. The APTES was covalently bound to the titanium surface by a condensation reaction[6]. In the final step, a PLGA/ICG film was created by dipping the titanium in PLGA/ICG solution as seen in Fig. 2B. By using this method to coat the titanium plates, an ICG loaded PLGA film was obtained with a median thickness of 336.67 ± 28.05 nm ($n$ = 6) measured by AFM scratch test and