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Graphene-based device to fight catheter-related infections

Ana Margarida Oliveira Ferreira

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Supervisor: Inês Gonçalves (i3S)
Co-supervisors: Orlando Frazão (INESC-TEC), Patrícia Henriques (i3S)

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I3S/INEB - Instituto de Investigação e Inovação em Saúde,
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Biomédica, Universidade do Porto, Portugal



INESC-TEC - Instituto de Engenharia de Sistemas e
Computadores, Tecnologia e Ciência, Porto, Portugal



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“Great things are done by a series of small things brought together.”

Vincent van Gogh

Resumo

As infecções associadas a cateteres, frequentemente originadas da colonização bacteriana do *hub* do cateter, são uma causa significativa de morbidade e mortalidade entre os pacientes de hemodiálise e uma fonte de despesas para os sistemas de saúde a nível mundial. Para além disso, o uso de antibióticos para combater estas infecções leva ao desenvolvimento de resistência por parte das bactérias, o que é um problema de saúde pública que a Organização Mundial de Saúde considera de elevada urgência resolver.

Existem alguns dispositivos médicos, particularmente tampas de cateter desinfetantes, no mercado que procuram a prevenção de infecções associadas a cateteres através da esterilização do *hub* do cateter. No entanto, estas tampas falham na desinfecção do interior do *hub* do cateter ou são economicamente pouco viáveis. Portanto, é de extrema importância desenvolver-se uma solução alternativa que seja eficaz e económica para a prevenção destas infecções.

O grafeno e os seus derivados têm propriedades antimicrobianas intrínsecas, que atuam por mecanismos físicos (ex., rompimento da membrana) e químicos (ex., stress oxidativo provocado por transferências de carga). Para além destes, a sua boa absorção de radiação infravermelha próxima (NIR) faz deles agentes para aplicações de fototerapia, incluindo eliminação de bactérias. Quando interage com a luz, o grafeno gera calor (terapia fototérmica) e espécies reativas de oxigénio (terapia fotodinâmica), ambos contribuindo para o dano e morte bacteriana.

Este trabalho pretende desenvolver um protótipo de uma tampa de cateter de hemodiálise à base de grafeno e ativada por luz para esterilização do *hub* do cateter. Esta tampa, a SmartCap, consiste numa tampa de plástico onde uma fonte ótica está acoplada a um guia de onda revestido por óxido de grafeno que se estende para o interior do *hub* do cateter. Quando a fonte ótica ilumina o guia de onda, a luz chega ao óxido de grafeno na superfície, desencadeando os efeitos fototérmico e fotodinâmico que atuam no interior do *hub* do cateter.

Duas fontes óticas - um laser díodo e um díodo emissor de luz (LED) - e vários guias de onda - filamentos de polimetilmetacrilato (PMMA) e polietileno tereftalato modificado com glicol (PETG), tubos capilares de PETG e fibra de vidro - foram caracterizados. O laser emite luz monocromática a 851 nm, enquanto o LED emite luz com um pico a 861 nm e com largura a meia altura de 30 nm. A potência ótica do laser é aproximadamente o dobro da potência ótica do LED. Entre os guias de onda poliméricos, os filamentos de PMMA apresentam menor perda de propagação (0,18 dB/cm), seguidos dos filamentos de PETG (0,42 dB/cm) e dos tubos capilares de PETG (0,96 dB/cm). As fibras de vidro têm uma perda de propagação muito menor de 12 dB/km (informação da ficha técnica). Os guias de onda foram revestidos com GO por revestimento por imersão, e um revestimento homogéneo foi obtido com 4 imersões numa

suspensão de GO de 12,5 mg/mL. A morfologia da superfície dos revestimentos foi avaliada por microscopia eletrônica de varrimento e mostra ter rugosidades típicas do GO. A hidrofilicidade da superfície foi avaliada pela medição do ângulo de contacto e revela ser hidrofílica (51°). Testes preliminares da estabilidade do revestimento mostraram que esta pode ser melhorada usando uma temperatura de 60 °C para secar o revestimento e após esterilização com óxido de etileno e irradiação com NIR. Protótipos da tampa de plástico foram desenhados em software de desenho assistido por computador e produzidos por impressão 3D, usando acrilonitrila butadieno estireno (ABS). Depois da montagem dos componentes, as propriedades físicas dos protótipos foram avaliadas e todos mostraram ter efeito fototérmico que aumenta com a quantidade de GO e potência ótica. Um efeito antibacteriano contra *Staphylococcus epidermidis*, com 99,4% de morte bacteriana, foi obtido por um protótipo constituído pelo laser de 850 nm a emitir 150 mW e um guia de onda de PMMA com 17 mm revestido com 4 camadas da suspensão de GO de 12,5 mg/mL, após 4 h de irradiação.

O trabalho desenvolvido nesta tese mostra que a SmartCap é uma tecnologia promissora, com uma produção simples e escalável.

Abstract

Catheter-related infections (CRIs), often originating from bacteria colonization of the catheter hub, are a significant cause of morbidity and mortality among hemodialysis patients and a huge economic burden to the healthcare systems worldwide. Besides, the use of antibiotics prescribed to fight those infections leads bacteria to develop resistance, which is a global public health problem that the World Health Organization considers to be of utmost urgency to tackle.

There are some medical devices on the market aiming at CRI prevention by sterilization of the catheter hub, particularly disinfecting catheter caps. However, they either fail to disinfect the interior of the hub or are economically less viable solutions. Therefore, it is of utmost importance to develop an effective and economical alternative solution for CRI prevention.

Graphene-based materials (GBMs) have intrinsic antimicrobial properties, by physical (e.g., membrane piercing) and chemical (e.g., oxidative stress by charge transfer) mechanisms of action. Besides, their good absorption of near infrared (NIR) light makes them suitable agents for phototherapy applications, including bacterial elimination. In combination with light, GBMs generate heat (photothermal therapy) and reactive-oxygen species (photodynamic therapy), both contributing to bacteria damage and death.

This work aims to develop a prototype of a graphene-based light-activate hemodialysis catheter cap for catheter hub sterilization. This cap, the SmartCap, consists of a plastic cap in which an optical source is coupled to a graphene oxide (GO)-coated optical waveguide that extends into the catheter hub. When the optical source illuminates the optical waveguide, the light reaches the GO on the surface, triggering the photothermal and photodynamic effects that act inside the catheter hub.

Two optical sources - a laser diode and a light-emitting diode (LED) - and several optical waveguides - polymethyl methacrylate (PMMA) and polyethylene terephthalate glycol-modified (PETG) filaments, PETG capillary tubes and glass fiber - were characterized. The laser emits single-wavelength light at 851 nm, whereas the LED emits light with a peak of 861 nm and a full width at half maximum of 30 nm. The optical power of the laser is approximately double of the optical power of the LED. Among the polymeric optical waveguides, PMMA filaments present the lowest propagation loss (0.18 dB/cm), followed by PETG filaments (0.42 dB/cm) and PETG capillary tubes (0.96 dB/cm). The glass fiber has a much lower propagation loss of 12 dB/km (information from datasheet). The optical waveguides were GO coated by the dip-coating method, and a homogeneous coating was achieved with 4 dips of a 12.5 mg/mL GO suspension. The surface morphology of the GO coatings was evaluated by SEM and shows to have wrinkles typical of GO films, and its wettability was assessed by optical contact angle and reveals to be hydrophilic (51°). Preliminary tests of coating stability showed that this can be

increased by using a temperature of 60 °C to dry the coating during the dip-coating process and after sterilization with ethylene oxide and NIR irradiation. Plastic cap prototypes were designed by CAD software and produced by 3D printing using acrylonitrile butadiene styrene (ABS). After assembly of the components (plastic cap, optical source and GO-coated optical waveguide), the physical properties of the SmartCap prototypes were evaluated, and all of them show a photothermal effect that increases with the amount of GO and optical power. A bactericidal effect towards *Staphylococcus epidermidis*, with 99.4% of bacteria death, was achieved by a SmartCap prototype composed of the 851 nm laser emitting at 150 mW and a 17 mm long PMMA optical waveguide coated with 4 dips of the 12.5 mg/mL GO suspension, after 4 h of irradiation.

The work developed in this thesis proves that the SmartCap is a promising technology, with simple and scalable production.

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Abbreviations and Symbols

ABS	Acrylonitrile butadiene styrene
AFM	Atomic force microscopy
AVF	Arteriovenous fistula
AVG	Arteriovenous graft
CAD	Computer-aided design
CFU	Colony forming unit
CKD	Chronic kidney disease
CNM	Carbon nanomaterial
CRBSI	Catheter-related bloodstream infection
CRI	Catheter-related infection
CVC	Central venous catheter
ESRD	End-stage renal disease
EtO	Ethylene oxide
FLG	Few-layer graphene
FLGO	Few-layer graphene oxide
FWHM	Full width at half maximum
G	Graphene
GBM	Graphene-based material
GO	Graphene oxide
GQD	Graphene quantum dot
GSH	Glutathione
Gt	Graphite
HAI	Healthcare associated infection
HD	Hemodialysis
HHD	Home hemodialysis
ICHD	In-center hemodialysis
IPA	Isopropyl alcohol
LED	Light-emitting diode
MLG	Multi-layer graphene
MLGO	Multi-layer graphene oxide
NIR	Near-infrared
OCA	Optical contact angle
OSA	Optical spectrum analyzer
PDT	Photodynamic therapy
PETG	Polyethylene terephthalate glycol modified

PMMA	Polymethyl methacrylate
PTT	Photothermal therapy
rGO	Reduced graphene oxide
ROS	Reactive-oxygen species
RRT	Renal replacement treatment
RT	Room temperature
SEM	Scanning electron microscopy
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UV	Ultraviolet
VA	Vascular access
XPS	X-ray photoelectron microscopy

θ_c	Critical angle
$\varnothing$	Diameter
Φ_c	Contact angle
σ_L	Liquid surface tension
σ_s	Solid surface energy
σ_{SL}	Solid-liquid interfacial energy

Chapter 1

Introduction

1 - Motivation

Catheter-related infections (CRIs) occur when microorganisms can access the extra- or intraluminal part of the catheter, by different routes, being the colonization of the catheter hub the most common in long-term use catheters. CRIs are the first cause of hospitalization and the second most common cause of mortality among hemodialysis patients. Besides the social impact of CRIs, they are a huge economic burden to the healthcare systems worldwide. Additionally, the use of antibiotics prescribed to fight those infections leads bacteria to develop resistance, which is a global public health problem that the World Health Organization considers to be of utmost urgency to tackle. This way, it is of great importance to develop strategies to prevent CRIs without using antibiotics.

Currently in clinics, after each dialysis session, the catheter hub is filled with a lock solution and covered with a standard plastic cap that acts as a physical barrier between the catheter hub and the external environment. On the market, other caps are available aiming at catheter disinfection and thus CRIs prevention. However, these caps either fail to disinfect the interior of the catheter hub or are economically less viable solutions. Therefore, there is still a need for an efficient and economical alternative solution.

Graphene-based materials (GBMs) revealed to have intrinsic antibacterial properties mediated by physical and chemical mechanisms. The interaction with light enhances the bactericidal action of GBMs, either through photothermal therapy, in which heat is generated, and photodynamic therapy, with reactive-oxygen species generation. Thus, the combination of graphene with light is a promising technology for antibacterial applications.

This project proposes to translate that technology into a prototype of a medical device capable of preventing infections, through eliminating bacteria. This medical device is a graphene-based light-activated hemodialysis cap for catheter hub sterilization, the SmartCap.

The SmartCap consists of a plastic cap in which an optical source is coupled to a graphene oxide (GO)-coated optical waveguide that extends into the catheter hub. When the light emitted by the optical source interacts with the graphene oxide deposited on the surface of the optical waveguide, a photothermal and photodynamic effects are triggered, acting against bacteria that managed to colonize the interior of the hub. Figure 1 shows a schematic representation of the SmartCap prototype and its components.

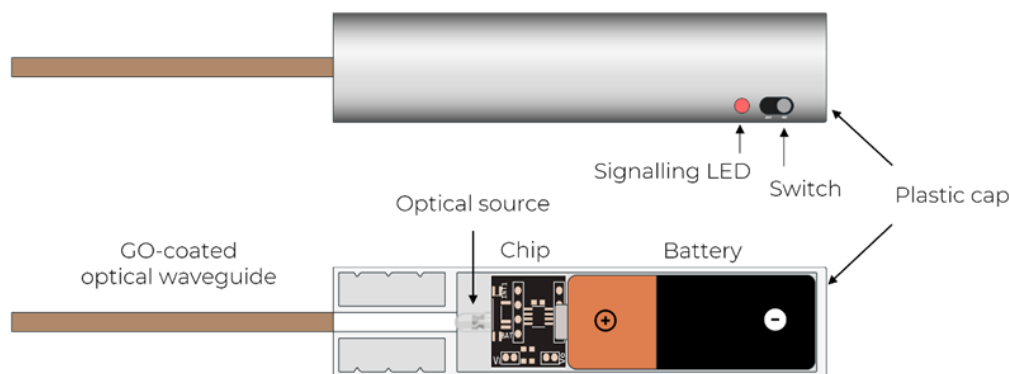


Figure 1. SmartCap prototype and its components.

2 - Objectives

This thesis aims to develop a SmartCap prototype. For that purpose, it is divided into specific objectives, namely:

- Explore and characterize different optical devices, namely optical sources and optical waveguides.
- Coat optical waveguides with a commercial graphene oxide and characterize the coating.
- Design and produce models for the plastic cap, compatible with commercial catheters, to couple the optical devices.
- Assemble the components and validate the prototype.
- Study the optical and thermal properties of the prototypes produced.
- Perform preliminary antibacterial assays to assess the antibacterial action of the prototypes.

3 - Dissertation structure

The present dissertation is divided into five chapters.

Chapter 1 includes the motivation and the objectives of this dissertation as well as its structure.

Chapter 2 comprises a review on relevant topics for this dissertation. Initially, a contextualization is made about renal disease, hemodialysis treatment and vascular accesses,

followed by the problem of catheter-related infections and the current solutions existing in clinics. Then, the phototherapy is described, and an historic introduction to optical devices is presented. Finally, an overview of graphene-based materials is presented, focusing on their antimicrobial properties, and literature exploring graphene as a phototherapy agent for antimicrobial applications is reviewed.

Chapter 3 describes the materials and methods used during this dissertation.

Chapter 4 presents the results obtained throughout the work along with the discussion of those results.

Chapter 3 and Chapter 4 are divided into four subsections based on the main topics of this dissertation. The first subsection regards the characterization of the optical devices (optical sources and optical waveguides) explored in this work. The second relates to the graphene oxide and coating of the optical waveguides. The third includes the production of the cap, the assembly of the SmartCap prototype and the evaluation of its physical properties. The fourth subsection is dedicated to the antibacterial performance of the SmartCap prototype.

Chapter 5 summarizes the main conclusions of the developed work and briefly discusses about future work.

4 - Outputs

Oral communication

- M. Ferreira, C. S. Monteiro, P. C. Henriques, O. Frazão, and I. C. Gonçalves, “Lighting graphene oxide: the guide to the end of catheter-related infection?”, at 15º Encontro de Investigação Jovem da Universidade do Porto (IJUP), May 2022

Poster communication

- M. Ferreira, C. S. Monteiro, P. C. Henriques, I. C. Gonçalves, and O. Frazão, “Study and development of an optical waveguide cap for biomedical application”, accepted for presentation at European Optical Society Annual Meeting, Sept 2022

Conference proceeding

- M. Ferreira, C. S. Monteiro, P. C. Henriques, I. C. Gonçalves, and O. Frazão, “Study and development of an optical waveguide cap for biomedical application”, Journal of the European Optical Society, Rapid Publications, 2022 [accepted for publication]

Chapter 2

Literature review

1 - Hemodialysis catheters

1.1 - End-stage renal disease

Chronic kidney disease (CKD) is characterized by persistent structural or functional modifications of the kidney [1]. The disease's prevalence has been increasing and affected 697.5 million people worldwide in 2017, leading to 1.2 million deaths [2]. CKD can be classified into different stages with increasing severity regarding kidney damage (Figure 2). End-stage renal disease (ESRD) corresponds to the final stage of CKD, in which one or both patients' kidneys stop working, and affects around 5.32 million people worldwide [2]. To survive, ESRD patients need renal replacement therapy (RRT), which includes kidney transplantation and two types of dialysis, peritoneal dialysis and hemodialysis (HD), being the latter the most common RRT. Worldwide, there are around 4 million people under RRT, of which 69% perform hemodialysis [3]. These numbers are expected to increase significantly mainly due to population aging and the increasing prevalence of diabetes and hypertension, which are the two most common causes of ESRD [4].

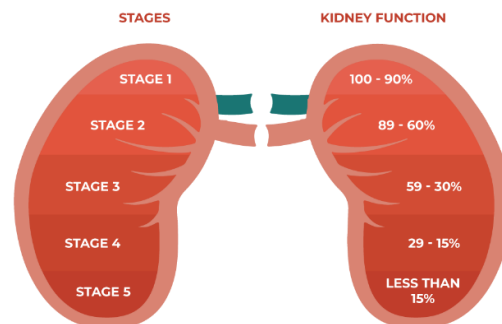


Figure 2. Stages of CDK with the corresponding kidney function.

1.2 - Hemodialysis

In hemodialysis, blood is removed from the patient's body towards a dialysis machine, or dialyzer, where it is filtered. Inside the dialyzer, some solutes, such as urea, are removed from the blood, and other molecules, such as bicarbonate, are transported into it. Then, the filtered blood returns to the body. By doing so, hemodialysis acts as an artificial kidney, restoring the intracellular and extracellular fluid environment characteristic of a healthy kidney [5]. Depending on the place where the treatment is performed, hemodialysis divides into in-center HD (ICHHD), requiring the patient to go to a dialysis facility each session, usually three times a week, and home HD (HHD), in which the patient receives the treatment at home. Home modality is associated with a lower risk of cardiovascular hospitalizations and deaths when compared to ICHHD, but it also has a higher risk of infection-related hospitalizations. In European countries, as well as in the US, HHD is used in less than 2% of dialysis patients [6], [7].

Access to the bloodstream can be achieved using three different types of vascular access (VA): native arteriovenous fistula (AVF), which is a connection of an artery and a vein, prosthetic arteriovenous graft (AVG), similar to AVF but with an artificial connection, and central venous catheter (CVC), which is a tube inserted into a peripheral or a proximal central vein (the internal jugular, subclavian and femoral veins are the most accessed). A representation of these three VA can be found in Figure 3. AVFs are the preferable choice for VA, due to their lower rate of thrombotic and infectious complications compared with AVGs and CVCs [8]. Indeed, the risk of death from infection of a patient using a CVC is 41% higher than that using an AVF [9]. However, AVFs cause extra pressure and blood flow into the vein, patients with weak blood vessels cannot withstand a fistula and end up staying with CVC for the rest of their HD treatment. Besides, AVF requires a maturation time of at least six weeks, whereas CVC does not, thus allowing the immediate start of HD, which makes it the chosen VA for urgent cases [10]. Therefore, despite the high prevalence of AVFs among HD patients against that of CVCs (from 15% in Finland [11] to 37% in Switzerland [12]), most new patients starting HD treatment use a CVC for first VA (from 54% in Finland [11] to 81% in the US [7]). Although the percentages vary significantly from country to country, the same trend occurs in all of them.

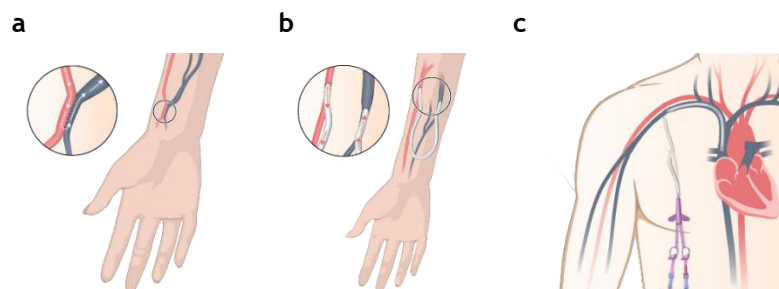


Figure 3. Vascular accesses used in hemodialysis: (a) AVF, (b) AVG and (c) CVC [13].

1.3 - Central venous catheters

There are four types of CVCs: tunneled, non-tunneled, peripherally inserted and totally implantable CVCs, being the first two the most relevant for HD. Tunneled CVCs pass under the skin from the vein insertion site to a separate skin exit site, whereas non-tunneled CVCs are inserted directly into the vein [8]. The first ones are preferred for long-term therapies, due to their higher stability and lower risk of associated infections. Generally, non-tunneled CVCs are only used in case of urgent RRT and end up being replaced by a tunneled CVC or by a more permanent VA [8], [14]. Regarding their configuration, CVCs have a hub at the end of the device, outside the patient's body. The hub connects the catheter to the external environment and is where other medical devices are attached. During HD sessions, the dialyzer is connected to the hub to allow blood flow through the system. After HD sessions, the hub is filled with a lock solution and a cap is attached to it to provide a physical barrier between the catheter and the external environment. Besides the hub, CVCs contain clamps on their non-tunneled portion, externally to the body, which prevent the backflow of fluid. Additionally, CVCs used in HD have two lumens, which are channels through which blood passes on their way in and out of the body [14]. Figure 4 shows a non-tunneled CVC and a tunneled CVC inserted in the jugular vein.

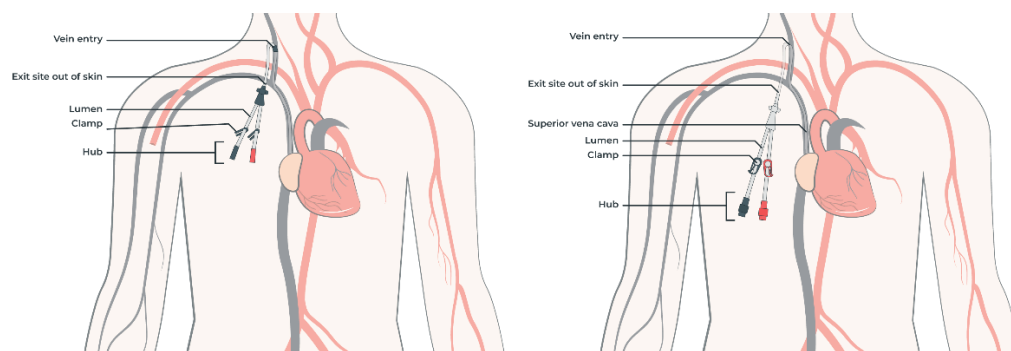


Figure 4. Non-tunneled (left) and tunneled (right) CVC.

2 - Catheter-related infections

Catheters are the type of VA most likely to bring complications, with infection being the most common one. In fact, 30-60% of CVC removals are due to infections [8]. Catheter-related infections (CRIs) can be localized, when they just affect a portion of the CVC, or systemic, in case they spread to the subcutaneous route [8]. The most severe type of infection occurs when pathogens reach the bloodstream, leading to a bacteremia [8], [15] that can eventually cause a metastatic infection [15]. These are distal infections that are not anatomically related to the primary site infection and include infective endocarditis, vertebral osteomyelitis, iliopsoas abscess, and septic arthritis [16]. The severity of the CRIs as well as their management are highly dependent on the originating infectious agent.

Most of CRIs (40-80%) are caused by the Gram-positive organisms *Staphylococcus epidermidis* and *Staphylococcus aureus* (within which 40% correspond to methicillin resistant (MSRA)), being the remaining infections originating from Gram-negative pathogens, such as *Pseudomonas aeruginosa*, and Gram-positive *Enterococci* [15], [17]. Despite more rarely, fungal species, particularly *Candida albicans*, also lead to CRIs (around 2%) [18]. Regarding the symptoms presentation and clinical outcomes, *S. aureus* and Gram-negative bacteria lead to common symptoms as fever and rigors, whereas *S. epidermidis* is more subtle [19]. It is more likely that patients require hospitalization when CRIs are caused by *S. aureus*, followed by *S. epidermidis* and Gram-negative bacteria [19]. *S. aureus* presents the highest risk of developing bacteremia [20] and a subsequent metastatic infection, being the prevalence of those during a *S. aureus* bacteremia of 5.7% to 75.3% [16]. Therefore, in CRIs caused by *S. aureus*, the catheter should be removed immediately to avoid severe complications, contrary to CRIs caused by other pathogens, in which catheter salvage may be attempted. Also, in CRIs caused by *C. albicans* catheter removal is unavoidable, as systemic antifungal agents are ineffective in eliminating *C. albicans* catheter biofilms [18].

Microorganisms cause infectious complications when they access the extra- or intra-luminal surface of the CVC and merge to form a biofilm. This access of pathogens can occur by different routes (Figure 5): (1) migration of microorganisms from the patient's skin through the catheter tract at the exit site; (2) pathogen colonization of the CVC hub or connector; (3) hematogenous seeding; and (4) contamination of infused fluids, being the first two the most common. The first mentioned of the two most common is more often the cause of short-term CVC infections, while the latter occurs mainly in long-term CVCs [8].

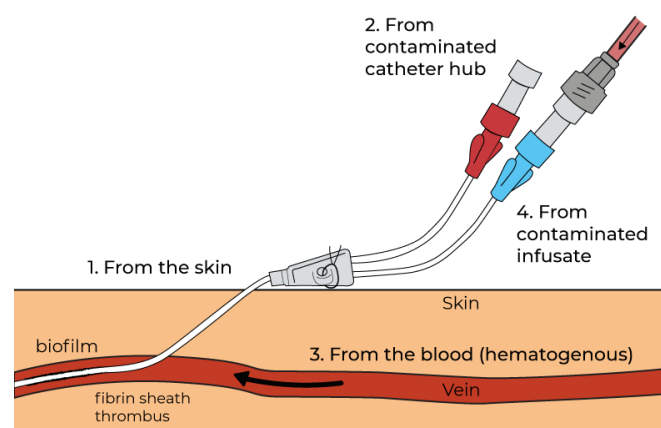


Figure 5. Potential routes of CRBSIs (adapted from [21]).

Catheter-related bloodstream infections (CRBSIs) are the leading cause of hospitalization and the second most common cause of mortality among HD patients [22]. Besides having a huge impact on patients' quality of life, CRBSIs represent a significant economic burden to the healthcare systems worldwide. In fact, a meta-analysis study performed in 2013 regarding the economic impact of healthcare associated infections (HAI) for the US healthcare system

revealed that CRBSIs are the costliest type of HAI. Per case, it costs an average of \$45,814 (\$30,919 - \$65,245) and implies a hospital stay of 10.4 days on average, being 6.9 days at the intensive care unit. When the CRBSI is caused by MRSA, the cost is even higher (averaging \$58k) as well as the hospital stay duration (averaging 15.7 days) [23].

3 - Strategies to prevent CRIs in hemodialysis

3.1 - Standard in clinics: prophylaxis and management

Centre for Disease Control and Prevention has recommended core interventions to reduce the risk of occurrence of a CRBSI. These include, among others, good hand hygiene practices, education for both patients and clinical personnel on catheter care and catheter hub disinfection, as well as a “scrub-the-hub” protocol for preparing catheter hubs prior to accessing [24], [25].

Currently, after each dialysis session, the catheter hub is filled with a lock solution, frequently heparin and sodium citrate solutions, avoiding thrombosis [26], and covered with a plastic standard cap, acting only as a physical barrier. In between HD sessions, both the lock solution and the cap remain in the hub, being removed in the following session. Besides, to reduce the handling of the hub during the session, as this is a significant risk factor for microorganisms' entrance into the hub, needleless connectors have been adopted in clinical practice, existing a wide variety of these devices on the market [27].

When a CRBSI occurs associated with any local or systemic inflammation or immunocompromised condition, the catheter should be removed and systemic antibiotic therapy for CRI is initialized empirically [10]. However, the use of antibiotics leads bacteria to develop resistance, which is a global public health problem that the World Health Organization considers to be of utmost urgency to tackle [28].

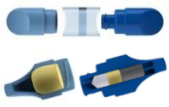
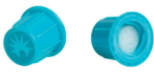
3.2 - Existing and emerging technologies

Throughout the years different strategies have been developed aiming the prevention of CRIs by killing microorganisms in the catheter hub. One approach is the modification of the surface of the catheters, usually made of silicone and polyurethane [29], by impregnating or coating them with antibiotics (rifampicin and minocycline) and disinfecting agents (chlorohexidine and silver sulfadiazine) [30].

A different strategy, which does not involve modifying the catheters, is the development of disinfecting caps that would replace the standard non-disinfecting caps used in clinics. Contrarily to the standards, the disinfecting caps would not only act as a physical barrier to prevent bacteria from entering the hub but also kill bacteria through the release of antiseptic agents. Most of the already available caps on the market (Curos™ from 3M [31]-[33], DualCap®

from MeritMedical [34], SwabCap™ from ICU Medical [35] and BD PureHub™ from BD [36]) contain a sponge with 70% isopropyl alcohol (IPA) that disinfects the outer surfaces of the catheter hub and/or catheter connector. On the other hand, ClearGuard™ HD from ICU Medical [37] uses chlorohexidine as antiseptic agent which is release from a rod that extends into the catheter hub allowing for a disinfection inside the hub. Table 1 includes a small description of all the disinfecting caps found on the market, as well as their main features.

Table 1. Disinfecting caps commercially available on the market.

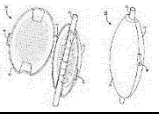
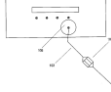
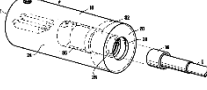
Technology	Description	Features	Refs.
3M™ Curoc™ Disinfecting Cap 	Cap for catheter hubs or Tego™ needle-free connectors, containing 70% isopropyl alcohol (IPA), disinfecting the surfaces of the hub/connector.	- Disinfects external surfaces of the catheter hub. - Disinfects in 1 min. - Kills 99.99% of the most common pathogens. - Provides a physical barrier for up to 7 days. - Single-use. - Antibiotic-free.	[31]-[33]
DualCap® Disinfection and Protection System 	Cap for male luer connectors and needle-free valves that contain medical-grade sponges saturated with 70% IPA, disinfecting the surfaces of the device.	- Disinfects external surfaces of the connector or valve. - Disinfects in 30 s. - Cannot be attached to an open catheter hub. - Blocks 70% IPA from entering the patient's bloodstream. - Provides a physical barrier for up to 7 days. - Single-use. - Antibiotic-free.	[34]
SwabCap™ Disinfecting Cap 	Disinfecting cap for needle-free connectors. It contains a 70% IPA sponge that disinfects both the top and thread of the connector.	- Disinfects the connector surface, both the top and threads. - Disinfects in 30 s. - Provides continuous disinfection for up to 7 days, if not removed. - Reduces in 34% hospital-associated central-line associated infections (HA-CLABSI). - Single-use. - Antibiotic-free.	[35], [38]
BD PureHub™ Disinfecting Cap 	Disinfecting cap for swabbable needle-free luer connectors. It disinfects with a sterilized 70% IPA solution.	- Disinfects in just 1 min. - Kills 99.99% of bacteria. - Provides a physical barrier for up to 7 days. - Reduces in 41% the risk of CLABSI - Single-use. - Antibiotic-free.	[36]
ClearGuard™ HD 	HD catheter cap for hub disinfection. It features a rod that extends into the hub. Both the rod and the cap thread are coated with chlorhexidine that elutes from them to the lock solution, killing bacteria.	- Disinfects inside and outside the hub. - Kills >99.99% of the most common pathogens. - Provides a physical barrier (recommended use of max 3 days). - Compatible with different lock solutions (heparin, citrate and saline solution). - Single-use. - Antibiotic-free. - Cannot be used in individuals allergic to chlorohexidine.	[37], [39]

Most of the mentioned caps only disinfect the outer surface of the catheter hub, so they are not able to kill pathogens that manage to enter and colonize the hub during catheter manipulation and dialysis session. Additionally, all the caps currently available are single use, requiring them to be replaced after every session. This makes some of them (for instance,

ClearGuard™ HD costs 4.42 €/pair) expensive solutions for the healthcare systems comparing to the standard caps already used in clinics (0.2 €/pair). Therefore, there is still need for a cost-effective sterilization device capable of disinfecting inside the catheter hub.

Besides the already available caps, several other authors developed medical devices aiming at catheter disinfection through radiation. Table 2 presents some examples of these medical devices that have been patented despite none having reached the market.

Table 2. Patented medical devices for catheter sterilization.

Title	Description	Features	Refs
1. Portable photodynamic disinfection light delivery device for catheter disinfection 	Portable light delivery device that provides circumferential illumination at desired wavelength(s) for photodynamic disinfection of catheters and related components (e.g., connectors, hubs, tubings), by activation of a photosensitizer.	- Disinfects different components, depending on where the photosensitizer is placed. - More than one biocompatible photosensitizer can be used. - Separated photosensitizer and portable device. - Kills the most common pathogens. - Antibiotic-free.	[40]
2. UV-C catheter hub sterilization and data acquisition system 	Handheld device for hub sterilization. It comprises a housing with an opening for a medical device, a sterilization chamber with a source of ultraviolet (UV) radiation and RFID scanner.	- Uses UV-C radiation. - Antibiotic-free.	[41]
3. Method and apparatus for UV radiation catheter sterilization system 	Apparatus for light sterilization of catheter lumen. It includes an external UV radiation source to which an optical fibre cable is coupled. The optical fibre is inserted into the lumen and emits UV light through its wall and/or end, disinfecting the interior of the catheter.	- Uses UV radiation. - Requires an external light source. - Antibiotic-free.	[42]
4. Method and apparatus for sterilizing intraluminal and percutaneous access sites 	Handheld device, including a cap for the access site and an apparatus within which a UV-C light source and the circuitry necessary to make it function are placed. The light source controllably emits UV-C radiation towards the cap, reaching the lumen of the access site, disinfecting it.	- Uses UV radiation. - Used in hospital or home. - Allows disinfection at any time (before and after treatment). - Sterilization apparatus reusable from patient to patient. - Cap replaced each dialysis session. - Antibiotic-free.	[43]
5. Assembly and method for disinfecting lumens of devices 	Apparatus for lumen disinfection, containing a light (UV or visible) source (LED) within a housing and a middle piece with an optical window that fits on a catheter connector. The light source emits radiation towards the transparent optical window of the middle piece reaching the lumen.	- Uses UV radiation. - Disinfection at home (no extra time in clinics). - Adaptable for multiple-use. - Light doses controlled remotely. - Rechargeable during HD session. - Requires filling the lumen with a light guiding fluid. - Antibiotic-free.	[44]

The major disadvantage of these medical devices is the use of UV radiation, known to be carcinogenic, which compromises their safety.

Although UV light continues to be the most common radiation used for disinfection purposes, other wavelengths have been proved to have bactericidal effects [45]-[47]. The use of light to treat medical conditions, as infectious diseases, is called phototherapy.

4 - Phototherapy

Phototherapy, which comprises photothermal therapy (PTT) and photodynamic therapy (PDT), is being increasingly studied and adopted to treat different diseases, including infectious complications. In PTT, light stimulates a photothermal photosensitizer that converts light into heat, resulting in a local temperature increase. As a result, cells' membrane permeability increases and cellular components denature, potentially causing cells' death. In PDT, light stimulates a photosensitizer that generates reactive-oxygen species (ROS). ROS trigger oxidative stress in cells, causing intracellular lipid peroxidation, DNA and protein damage, which can lead to cells death [48]. Figure 6 shows the mechanisms of phototherapy by a photosensitizer. Over antibiotic therapy, phototherapy has the major advantage of preventing bacteria from developing resistance [49].

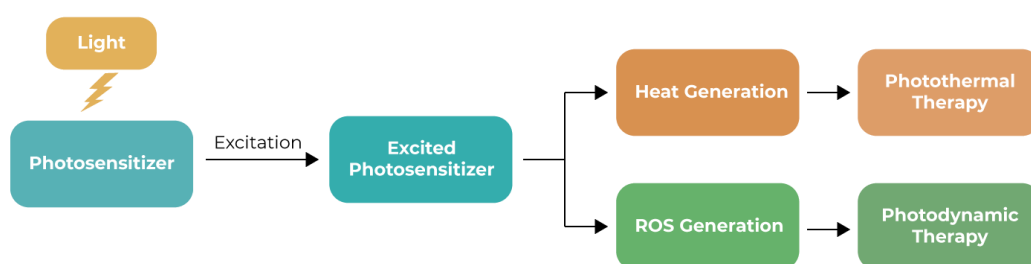


Figure 6. The mechanism of photothermal and photodynamic therapies by a photosensitizer. Adapted from [49].

In the past few years, near-infrared (NIR) radiation has shown high efficiency in the conversion of light into thermal energy [50]. Besides, as NIR light belongs to the first and second biological windows, which are spectral ranges extending from 700 nm to 980 nm and from 1000 nm to 1400 nm, respectively [51], it can penetrate deeply into the body and the absorption by tissues is minimized, causing minimal damage [50]. Therefore, NIR radiation has great potential in PPT and PDT applications, assuming to be an efficient and safe solution.

Different optical sources are used in the medical field to deliver light, particularly lasers and light-emitting diodes (LEDs). Besides optical sources, optical waveguides are optical devices used in light systems in medicine to guide the light.

5 - Optical devices

5.1 - Optical sources: Lasers and LEDs

The first laser was developed in 1960 by T. Maiman [52], at Hughes Research Laboratories, using a cylindric ruby crystal, coated on two parallel faces with silver, as the laser's active medium (solid laser), and a high-power flash lamp as the laser's pump source (Figure 7). In the same year, A. Javan, W. R. Bennett, Jr., and D. R. Herriott [53], researchers at Bell Labs, constructed the helium-neon (HeNe) laser, the first gas laser and the first continuous-wave laser, whose strongest beam was achieved at a wavelength of 1153 nm emitting with an optical power of 15 mW. In 1962, R. N. Hall [54], at the GE Research Laboratory, created the first gallium-arsenide (GaAs) diode laser, a semiconductor device that converts electrical energy into infrared light, although other research groups also worked successfully on the stimulated emission in the GaAs p-n junction in the same year [55], [56]. To operate, this laser had to be cryogenically cooled to liquid nitrogen temperature and then driven above the threshold by powerful current pulses [57]. In the same year, at the GE Research Laboratory, N. Holonyak [58] developed the first practical visible (red light) LED, using GaAs phosphide (GaAsP). Since then, LED technology evolved exponentially: in the 1980s, there were already high-efficiency red, orange and yellow LEDs, made of indium gallium phosphide (GaInP); in the 1990s, high-efficiency green, blue and violet LEDs, made of indium gallium nitride (GaInN), were also developed; and in 1996, several publications emerged reporting the development of white LEDs and the Nichia Corporation announced the full-scale production and commercialization of white LEDs, based on a blue LED chip and Yttrium Aluminum Garnet (YAG) phosphor [59].

The use of lasers in the medical field started in 1961, at the Columbia Presbyterian Medical Center in Manhattan when Dr. Charles J. Campbell used a ruby laser to destroy a tumor on a patient's retina [60]. The medical applications of lasers have been expanding throughout the years. These optical devices are used nowadays both for diagnostic (e.g., fluorescence, optical tweezers) and treatment (e.g., ophthalmology, cardiology, dermatology, nephrology, gynecology, surgical oncology) purposes [61]. LEDs have also been growingly applied in the medical context, for example for phototherapy [62].

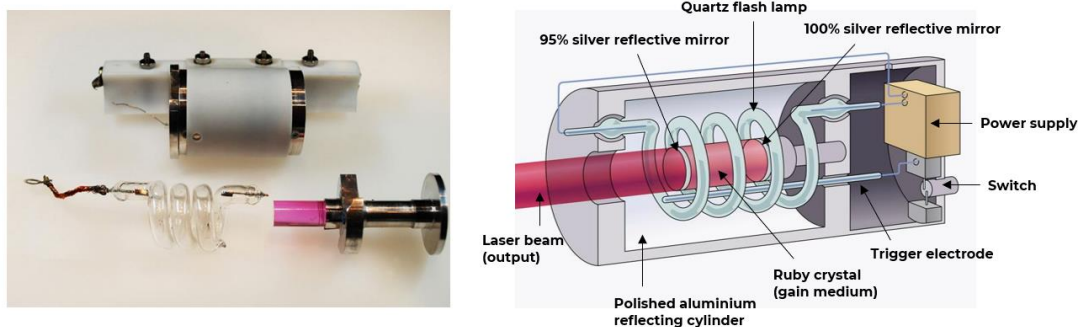


Figure 7. The first laser developed by T. Maiman in 1960 using a ruby crystal (left) (reprinted with permission from ref. [63], copyright 2020 Springer Nature) and schematic representation of its components (right) (adapted from [64]).

5.2 - Optical waveguides

The first reference to optical waveguiding occurred in 1880 when Wheeler used a glass pipe medium to transmit light [65]. The research on optical waveguiding systems continued the next years, and in 1954, van Heel [66] reported a method of carrying optical images without aberrations, which consisted of fibers coated with a layer of lower refractive index material that allowed total internal reflection. The coating was isolated from the surroundings by a thin coating of black paint. In this work, van Heel reported both glass and plastic fibers, obtaining better transmission results with the latter. The invention of lasers and the development of coherent optics boosted the need for optical fibers and guiding structures to integrate into optical circuits [67]. In 1964, Schlosser initiated a theoretical study about rectangular dielectric waveguides [67], and, in 1965, Anderson [68] developed the first film waveguides and other planar components for application in the infrared part of the spectrum. Also in 1965, the first working optical fiber was reported by Manfred Börner. He used a typical optical fiber, composed of a fiberglass core, a fiberglass cladding and a protective layer, and a laser as the optical source, giving rise to the first optical data transmission system [69]. Since then, both fibers optics and dielectric transparent waveguides have evolved exponentially, and there are available optical waveguides made from a wide range of materials including a large variety of polymers [70]. Figure 8 represents the total internal reflection principle in a solid core optical fibre. The relation between the refractive indices of the cladding and core allow to determine the limit angle, by Eq. (1), which derives from Snell's law, in which θ_c is the critical angle and n_1 and n_2 are the refractive indices of the core and cladding, respectively. Total internal reflection occurs when the incidence angle of the light beam is greater than the critical angle.

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right) \quad (1)$$

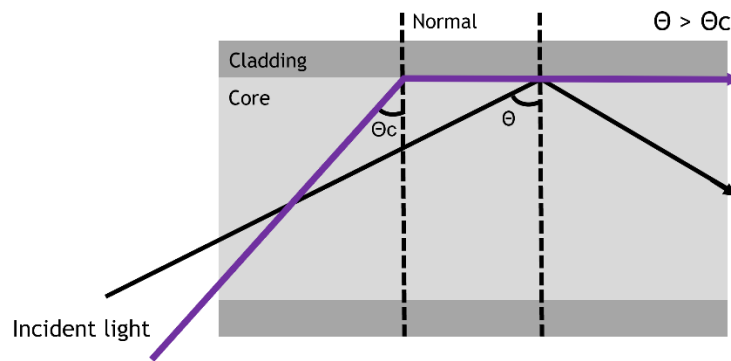


Figure 8. Total internal reflection in an optical fibre.

The first use of an optical waveguide for medical purposes happened in 1888 when the Austrian doctors Roth and Reuss used bent glass rods to illuminate body cavities. Nowadays, there are already biocompatible optical waveguides, made of glass, natural and synthetic polymers and hybrid materials, that are widely used for medical applications, such as

photothermal and photodynamic therapies, optical imaging, physiological signal detection, and optogenetics [71].

6 - Graphene-based materials

Carbon nanomaterials (CNMs) are complex structured materials composed of carbon atoms covalently bonded with other atoms and elements. They can be characterized based on their dimension (0D- to 3D-CNMs), crystallinity and hybridization state (only sp^2 or containing sp^3 atoms) [72].

Graphene (G) is a 2D crystalline carbon allotrope in which sp^2 hybridized carbon atoms form a flat hexagonal honeycomb lattice structure (Figure 9a) [73]. Despite earlier attempts to produce G [74], [75], it was only in 2004 that G was firstly isolated from graphite (Gt) through mechanical stripping by A. Geim and K. Novoselov [76], [77] - an achievement that led them to win the Nobel Prize award in 2010.

Since its first isolation, different fabrication methods have been developed to obtain high quality, defect-free and stable G with high performance [78]. Among others, these fabrication methods include micromechanical exfoliation, consisting of peeling G layers from Gt using adhesive tape [79]; electrochemical exfoliation, which uses an applied voltage to generate ionic species that form gaseous substances inside Gt, expanding and exfoliating G layers [80]; liquid-phase exfoliation, in which a solvent is used to exfoliate G from Gt through ultrasonication [81]; chemical vapor deposition, which uses metal substrates, usually nickel and copper, to catalyse the reaction between the deposited carbon radicals, previously decomposed from hydrocarbon precursors, to form single-layer G and few-layer G (FLG) [82]. The latter is a bottom-up production method, whereas the other three mentioned are top-down approaches.

Graphene-based materials (GBMs) include single-layer G, FLG, consisting of 2 to 5 layers packed together, and multi-layer G (MLG), with up to 10 layers. All these GBMs can be chemically oxidized, originating graphene oxide (GO), few-layer GO (FLGO) and multi-layer GO (MLGO). GO can be further reduced, originating reduced GO (rGO). Besides those, graphene quantum dots (GQDs) are another GBM, consisting of G blocks with 2D transverse size (less than 100 nm) [83]. Figure 9 shows the basic structure of G, as well as its chemically modified derivatives, GO, rGO and GQD.

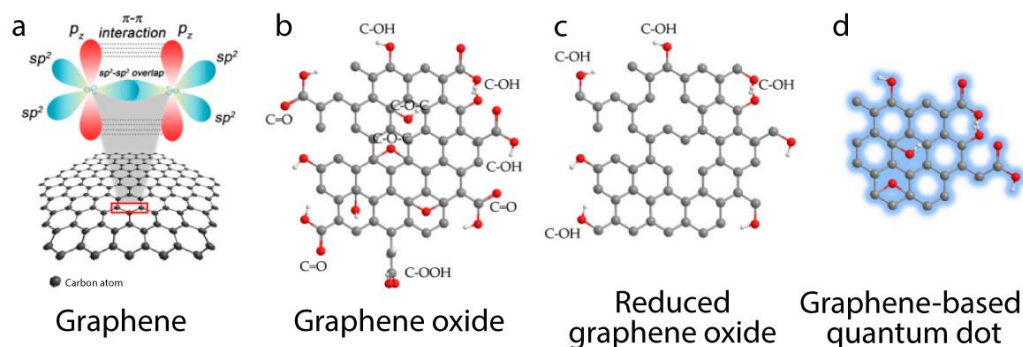


Figure 9. Structures of graphene-based materials: (a) graphene with a highlighted covalent bond between two sp^2 -hybridized carbon atoms; (b) graphene oxide; (c) reduced graphene oxide; and graphene-based quantum dot. Adapted from [84].

G presents outstanding and unique mechanical, optical, electrical, thermal, chemical and biological properties. Due to the short bonds, G has exceptional mechanical strength, being the strongest as well as the lightest and thinnest material in the world. Optically, G is almost transparent, transmitting 97.7% [85] of the incident light. Besides, it shows high thermal and electrical conductivities, reaching up to $5000 \text{ Wm}^{-1}\text{K}^{-1}$ and $200\,000 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, respectively [86]. The conductivity of G decreases with the increase of the number of layers and the oxidation state, as the oxygen functional groups scatter the charge carriers in G [87], destroy the lattice symmetry [88] and cause local strain [89], thus making GO a poor electrical and thermal conductor [87], [90]. Moreover, G has a large surface area, presents high stability in a physiological environment and can be easily modified [91]. Despite the controversy, G has been reported as a biocompatible material [91], with antimicrobial properties.

The expectational properties of GBMs make them suitable for a wide range of biomedical applications [92], including drug delivery [93], biosensors [84], tissue engineering [94], theragnostic [83], phototherapy [95], and antimicrobial applications [96].

6.1 - Antimicrobial properties of GBMs

The antimicrobial properties of GBMs were firstly reported by W. Hu et al. [97] in 2010. In their study, GO and rGO nanosheets showed to decrease microbial viability of *Escherichia coli* to 1.5% and 15%, respectively, being dependent on the GBM concentration. They also produced freestanding GO and rGO films by vacuum filtration that eliminated the airborne bacteria on contact [97]. Among all GBMs, GO and rGO generally present the highest antimicrobial capacity, due to the oxygen-containing groups impacting the membrane and oxidative stress [96].

The antimicrobial activity of GO is mediated by both physical and chemical mechanisms. The physical mechanisms include membrane piercing due to the sharp edges of GO sheets that damage bacteria membrane, eventually causing lysis [98]. Bacteria wrapping also contribute to the antimicrobial activity of GO [98]-[100]. After wrapping up the bacteria, GO sheets can damage their membrane which may deform and collapse [98]. On the other hand, O. Akhavan

[100] showed that GO sheets trap bacteria disconnecting them from the external environment, preventing their proliferation. The extraction of phospholipids from bacteria membranes has also been reported as an antibacterial mechanism of GO. This phenomenon is allowed by the strong dispersion interaction between GO and lipid molecules and leads to low membrane lipid density and loss of membrane integrity [101]. Generally, the antimicrobial action increases with increasing lateral size and concentration [101].

The chemical action is mainly associated with the oxidative stress caused by charge transfer, reactive-oxygen species (ROS) generation and glutathione (GSH) depletion [96], [102], [103]. Some authors divide the oxidative stress mediated into ROS-dependent, when it is caused by ROS generated by GO, and ROS-independent, when GO oxidizes a cellular structure or component without ROS intervention. The oxidation of GSH, which is an antioxidant synthesized by cells and bacteria to protect them from oxidative damage [104], is an example of a ROS-independent path and has shown to contribute to the antibacterial effect of GO [103]. The induced oxidative stress leads to lipid peroxidation, DNA and protein damage, which can cause cell death [96]. Catalysis of extracellular metabolites has also been reported as another chemical mechanism underlying the antimicrobial activity of GO [102].

Despite the different mechanisms underlying the antimicrobial activity of GO, they are dependent on the form of the material: colloid, free-standing materials, composites and coatings. For instance, GO coatings do not act by wrapping up bacteria as GO is adhered to a substrate, so bacteria entrapment only occurs in colloids or when GO is released to the bacteria medium. In fact, the antibacterial action of GO coatings is mainly due to oxidative stress and membrane damage by GO sharp edges.

There are several studies reporting the antibacterial activity of GO-coated surfaces and the factors influencing that activity against different pathogens. Perrault et al. [105] showed that the smaller the size of GO nanosheets, the higher the bactericidal effect on deposited bacteria. However, the GO nanosheets size did not affect the viability of bacteria in the suspension around the GO-coated surface (planktonic bacteria) [105]. The orientation of GO sheets has also been reported as impacting the antibacterial action of GO-coated surfaces. Vertically aligned GO sharp edges that lead to severer membrane damage than planar GO sheets [106]-[108]. N. Yadav et al. [109] studied the influence of the GO coating morphology/topography in bacterial biofilm inhibition and found that rougher surfaces promote bacteria adhesion and biofilm formation. They also showed that the increase the amount of GO increases the bactericidal effect up to certain level [109]. Besides, GO coatings exhibit different antibacterial effects against different pathogens. O. Akhavan [106] showed that Gram-negative *E. coli* suffered less physical membrane damage by GO sheets than Gram-positive *S. aureus*, due to the outer membrane. Contrarily, Y. Liu et al [107] reported higher antibacterial effect against *E. Coli* than *S. aureus*, because ROS penetrated more easily on the thinner membrane

of Gram-negative bacteria than on the thicker wall of Gram-positive species, making *E. coli* more susceptible to oxidative stress.

6.2 - GO as a photosensitizer for antimicrobial applications

Either GO and rGO have shown to present strong NIR optical absorption [110], making them suitable agents for phototherapy applications, such as bacteria elimination.

Upon NIR irradiation, the bactericidal ability of GO is enhanced due to both photothermal and photodynamic effects [111]-[113]. R. Kurapati et al. [113] showed that NIR irradiation, using a laser emitting at 1064 nm and with an optical power of 85 mW, boosted the antibacterial action of GO/polymer composite thin films against *E. coli*, by a synergetic effect of membrane damage by GO sharp edges and photothermal heating. After 15 min of irradiation, a ~100 °C local temperature increase was observed leading to >90% of bacteria killing. Besides, they reported an increase in NIR light absorption, and consequently in the heating and antibacterial effect, with an increase of GO amount [113]. Similarly, M. S. Khan et al. [114] used a 1064 nm laser to irradiate a GO dispersion, achieving an efficient bactericidal effect against different pathogens (*S. aureus* and *P. aeruginosa*) by photothermal treatment [114]. Synergistic photodynamic and photothermal antibacterial effects were reported by J. Sun et al. [112], who developed a nanocomposite membrane containing GO as a photosensitizer that efficiently kills bacteria (*S. aureus* and *E. coli*) upon 980 nm NIR irradiation for 5 min, by ROS generation and moderate temperature rise [112]. Despite some authors attribute the light-enhanced oxidative stress induced to the generation of ROS by GO, Y. Chong et al. [115] suggested that it is mainly ROS-independent. They showed that the acceleration of charge transfer between bacteria cellular components and GO upon NIR irradiation contributes more significantly to the oxidative stress of cells than the generated ROS [115].

The previously mentioned studies explored GO in the form of colloids or composites for antimicrobial applications. However, there are few studies addressing the antibacterial action of NIR irradiated GO coatings and films.

K. Nagao et al. [116] reported the antibacterial action of GO-coated dentin blocks against *Streptococcus mutans* upon NIR irradiation. Dentin blocks were dip-coated in GO dispersions with different concentrations and the coated blocks were irradiated for different time periods at different distances. The photothermal effect showed to be dependent on the GO concentration and the irradiation times, increasing with both factors. The irradiation distance also played a significant role in the photothermal effect, with higher distances leading to lower temperature rises. Upon 30 s of NIR irradiation (800-1000 nm), with an irradiation distance of 10 mm, 10 µg/mL GO-coated dentin blocks suffered a 35.5 °C temperature increase (from 23 °C to 58.5 °C). These conditions led to 98.3% of bacteria death [116].

On the other hand, L. Hui et al. [117] developed a NIR-activated antibacterial surface made of rGO capable of killing airborne bacteria on contact by photothermal effect. Upon 10 min of

simulated solar irradiation, a temperature rise of around 22 °C (from 20.8 °C to 42.5 °C) was achieved, leading to a >90% bacteria death (namely *P. aeruginosa*, *E. coli*, *S. aureus* and *B. subtilis*). To evaluate the effect of the NIR light, a 3 mm thick pork tissue was used as a shield between the disinfecting surface and the light source, and similar antibacterial effects were observed. In parallel, the authors used an 850 nm laser emitting at 0.1 Wcm⁻² to irradiate the same rGO-based surface and obtained a temperature increase of <4 °C, indicating that a wide range of wavelengths is better to promote the photothermal effect on the rGO surface than a single-wavelength NIR source [117].

More recently, P. C. Henriques et al. [118] achieved a bactericidal action against methicillin-resistant *S. aureus* and *S. epidermidis* through irradiation of FLG and FLGO films with NIR-emitting (812 nm) LEDs at 0.150 Wcm⁻². After 45 min of irradiation, FLGO and FLG films caused the death of 99% and 90% of the bacteria in the supernatant, respectively, and 85% and 65% of the adhered bacteria, respectively. The underlying mechanism of action was a synergistic photothermal and photodynamic effect (Figure 10).

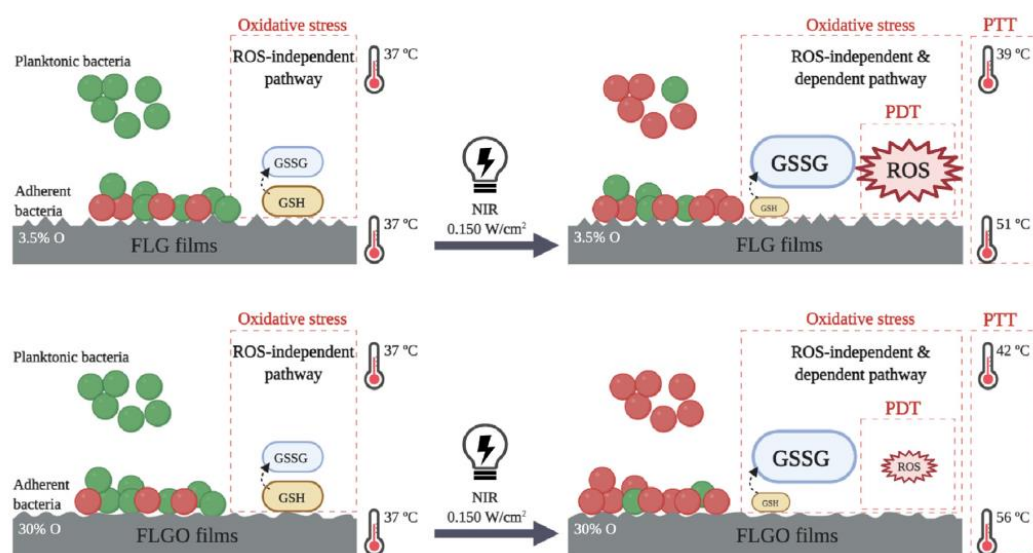


Figure 10. Mechanism of action of FLG and FLGO films towards bacteria. Bacteria death (red bacteria) occurred both without and with NIR irradiation, despite significantly higher in the latter case. GSH - glutathione, GSSG - glutathione disulfide (oxidized glutathione) (reprinted with permission from ref. [118], copyright 2021 Elsevier).

The temperature of the FLGO films increased from 37 °C to 56 °C, after the 45 min of irradiation, leading to a slight temperature rise in the supernatant (from 37 °C to 42 °C). On the other hand, ROS were generated and GSH was almost completely depleted. The photothermal effect of FLG films was lower than that of FLGO films, however the ROS generation was higher in FLG than FLGO films, despite the similar GSH depletion. This indicates that FLGO films act predominantly by ROS-independent pathways, although the photodynamic effect boosts the oxidative stress caused in bacteria [118].

Chapter 3

Materials and Methods

1 - Optical devices

1.1 - Optical sources

Several optical sources were characterized, including different laser diodes and light-emitting diodes (LEDs). The ones that presented the best performance are herein reported, namely a laser diode (RLD85PZJ4, ROHM Semiconductor) and a LED (SFH 4550, OSRAM).

1.1.1 - Optical sources spectral characterization

The spectra of the optical sources were acquired using an Optical Spectrum Analyzer (OSA, AQ6370, Yokogawa), which can measure in a wavelength range of 600-1700 nm, with a resolution of 20 pm and a maximum optical power of 20 dBm. Figure 11 shows a schematic representation of the setup used for the spectra acquisition. The spectrum of the laser was acquired in a wavelength range of 825-875 nm and the spectrum of the LED in a range of 800-900 nm, both with 100 pm of resolution. The acquired signals were then normalized and plotted using Spyder (open-source scientific environment written in Python).

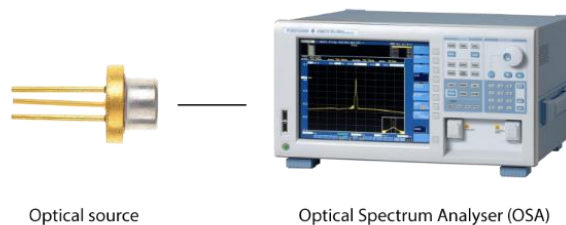


Figure 11. Experimental setup used for the acquisition of the optical sources' spectra.

1.1.2 - Optical sources optical power characterization

The optical power characterization aims to assess the optical power curve, which relates the applied voltage, or current, to the output optical power, and the optical power stability over time for different applied voltages to the circuit. The optical power curves allow to easily modulate the output optical power of the optical sources by controlling the applied voltage.

For the first-mentioned, the output optical power of the optical sources was measured using a power sensor (PM300F-50, Coherent) paired with a power meter (FieldMaxII-TO, Coherent). The output optical power was acquired for 10 s, and the average value, given by the power meter, was recorded, for five different applied voltages. Using Spyder, the experimental points were scattered, and the correspondent trend lines were estimated and plotted to obtain the optical power curve.

For the optical power stability assessment, the output optical power was acquired and recorded over 20 min, every 0.5 s, for five different applied voltages. The same power sensor and power meter were used for the optical power measurements, and a computer with FieldMaxII software was used for the data recording, as shown in Figure 12. The acquired signals were plotted using Spyder.

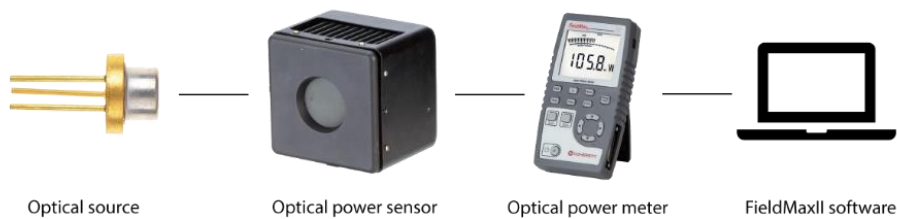


Figure 12. Experimental setup used for the optical power measurements. The FieldMaxII software was only used for the optical power stability assessment.

1.2 - Optical waveguides

Four different materials were explored as optical waveguides: polymethyl methacrylate (PMMA) and polyethylene terephthalate glycol modified (PETG) transparent filaments, PETG transparent capillary tubes and glass fibres. Table 3 presents the referred optical waveguides and their references, as well as their composition and dimensions.

Table 3. Optical waveguides explored in the experimental work, and respective references, compositions and dimensions.

Optical waveguide	Reference, Company	Composition	Dimensions (mm)
PMMA filaments	5200615, Herz	- PMMA	Ø 1.75
PETG filaments	Z-GLASS, Zortrax	- PETG: 80% - Fiberglass filings: 8 ~ 12% - Additives and colorants: 0 ~ 8%	Ø 1.75
PETG capillary tubes	51680, Globe Scientific	- PETG	OD: 1.6 ID: 0.9
Glass fibre	FP1500URT, Thorlabs	- Core: pure silica - Cladding: hard polymer	Ø 1.50

1.2.1 - Optical waveguides preparation

The preparation of the polymeric optical waveguides, namely the PETG and PMMA filaments, includes the reduction of their curvature, as there are purchased in spools, and the polishing of their end faces, to reduce the light loss caused by the diffusion of light due to the defects on the end face.

To reduce the curvature, the filaments were placed inside a metallic hollow tube, with a slightly larger diameter (Ø 2 mm) than the filament and heated up to a temperature that softens the polymer. The tube with the filament was then cooled at room temperature before taking the filament out of it. For the PETG and PMMA filaments, temperatures of 65 °C and 80 °C were used, respectively. Then, the filament was cut into smaller filaments, from 20 to 50 mm, resulting in imperfect end faces. For the polishing, 2000 and 5000 grit water sandpapers were used sequentially. In the end, the polished faces were evaluated using a stereomicroscope.

The PETG capillary tubes do not require the same preparation of the filaments as they are already purchased as linear and 75 mm length tubes. Thus, they were only cut in one side, which is not the side that couples to the optical source.

The glass fibre was cut using a ruby fibre scribe (S90R, Thorlabs), allowing a clean cut that does not require further polishing.

1.2.2 - Optical waveguides characterization

For the characterization of the optical waveguides, the light losses along their length were assessed for all the optical waveguides tested. To achieve that, an optical source was used to illuminate the optical waveguide at its initial end. Then, the output optical power of the waveguide at the end was measured, using the power sensor and the power meter previously mentioned. Optical waveguides of different lengths were evaluated, from 20 to 50 mm. Due to the variability of the polishing, which affects the light entering the waveguide, for each length, six samples were prepared, thus resulting in six measurements for each length. Figure 13 shows the schematic representation of the experimental setup used.

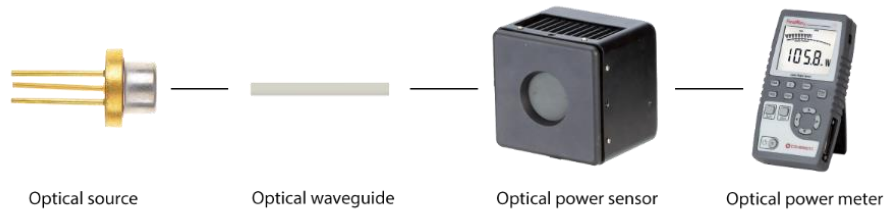


Figure 13. Experimental setup used for the light losses' characterization of the waveguides.

Using Spyder, the measured optical powers were converted from mW to dB, by Eq. (2), in which P_{out} is the measured output optical power and P_{in} is the optical power entering the optical waveguide, i.e., exiting the optical source.

$$Loss (dB) = -10 \log_{10} \frac{P_{out}}{P_{in}} \quad (2)$$

Then, the mean values of the output optical power for each length were calculated, and both the experimental points (length, optical power) and the mean values were plotted. The linear fit of the mean values was then estimated.

1.2.3 - Optical waveguides modification

The polymeric optical waveguides were modified, in different ways, aiming to increase the light reaching the surface and consequently the light interacting with the GO coating.

The modifications were performed after the optical waveguides were prepared as previously described. The first modification attempted was the depolishment of the waveguides surface using a P2000 grit sandpaper. Similarly, other filaments were gradually depolished, using P2000 grit and P100 grit sandpapers. In other filaments, cuts were made around the surface and along the length, approximately each 1 mm, using a blade. Other filaments were bent every 5 mm. Also, tapers were made by stretching the filaments after heating them up to 100 °C. In other filaments, instead of modifying the surface, the initial end was obliquely cut. Not all modifications were applied to the PMMA and PETG filaments. For instance, it was not possible to bend PMMA filaments without breaking them, so only PETG filaments were bent, and also tapers were only produced from PETG filaments, since PMMA has a higher soften temperature. The mentioned modifications are represented in Figure 14.

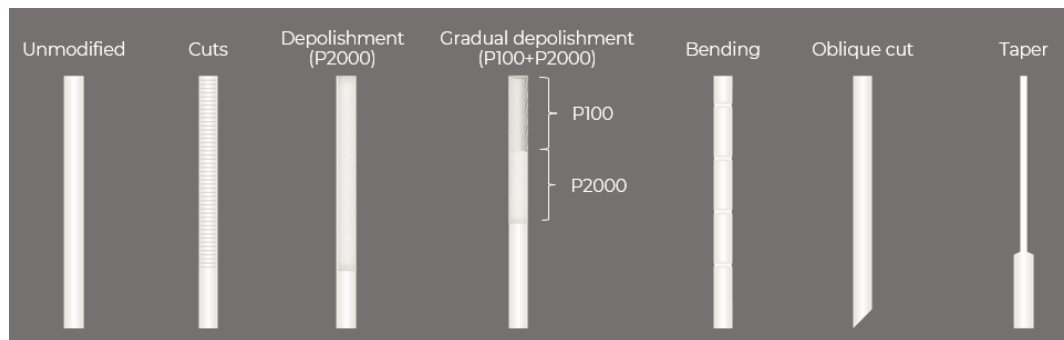


Figure 14. Representation of the modifications applied to the optical waveguides.

2 - GO coating of optical waveguides

2.1 - Graphene oxide

Graphene oxide from Graphenea was chosen to coat the optical waveguides. Two different graphene oxide were purchased, namely a GO water dispersion (4 mg/mL) and a highly concentrated GO paste (25 mg/mL). Figure 15 shows the characterization of the GO provided by the manufacturer.

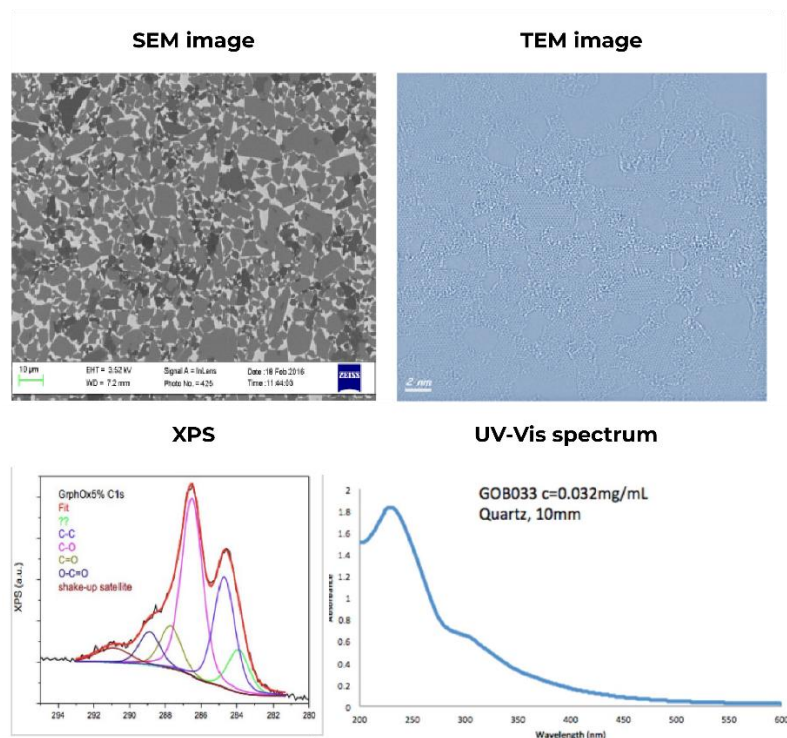


Figure 15. GO characterization provided by Graphenea, namely the scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images, X-ray Photoelectron Spectroscopy (XPS) analysis and UV-Vis absorption spectrum.

The manufacturer ensures a monolayer content of >95% for a 0.5 mg/mL dispersion, recommending a slight sonication treatment for more concentrated dispersions to achieve the percentage of monolayer GO flakes, as they tend to agglomerate. The particles size is <10 μm in the 4 mg/mL GO dispersion and up to 33 μm in the 25 mg/mL GO paste, being possible to decrease it also by a sonication treatment. The chemical composition is 49-56% carbon, 41-50% oxygen and residual percentages of hydrogen (1-2%), nitrogen (0-1%) and sulfur (2-3%). In a range between 200 and 600 nm, GO presents a peak in the absorbance at 230 nm.

2.1.1 - Graphene oxide absorption spectrum

The characterization of GO was obtained by its absorption spectrum, to complement the characterization provided by the manufacturer. First, the GO suspension (4 mg/mL) was diluted to a concentration of 0.25 mg/mL to reduce its opacity and allow spectrum acquisition. Then, a microplate reader (Synergy Mx, BioTek Instruments, Inc.), with a measurement range of 250-

950 nm and 1 nm of resolution, and the Gen5 Data Analysis software, were used to measure the absorbance of the GO suspension. The GO absorption spectrum was acquired for a range of 400-950 nm with a resolution of 2 nm. The measured values were plotted using Spyder to obtain the absorption spectrum.

2.2 - GO coating of optical waveguides

The optical waveguides were coated by dip coating technique. First the surface of the waveguide was cleaned with a kimwipe soaked in 70% (v/v) ethanol to remove surface residues and grease. The waveguide was then dipped in the GO suspension for 10 s. Then, the filaments were dried for 15 min in the drying oven at 60 °C to accelerate the drying process. Depending on the desired number of GO layers, this process can be repeated. In the end, the optical waveguide is partially dried at 60 °C for 30 min and left to dry completely at room temperature. Figure 16 shows schematically the described dip coating method.

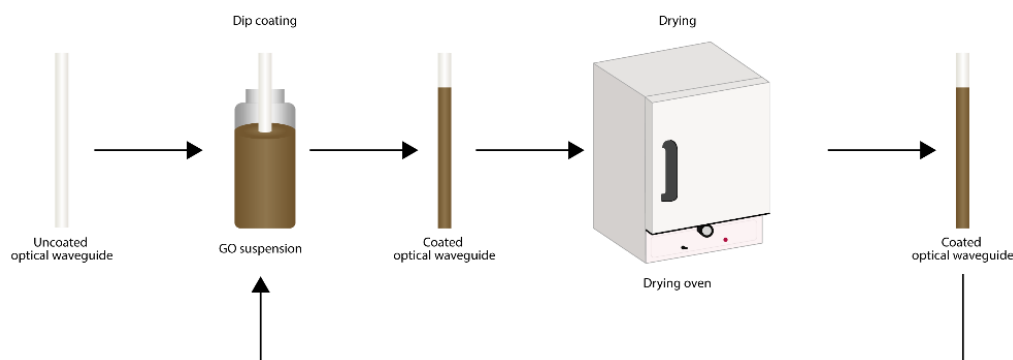


Figure 16. Dip coating technique used for coating the optical waveguides.

Two GO water dispersions were used to coat the optical waveguides, namely a 4 mg/mL GO suspension, as originally purchased, and a 12.5 mg/mL GO suspension, which is a dilution (1:1) of the highly concentrated GO paste (25 mg/mL).

The influence of the time that an optical waveguide stay immersed in the GO suspension has on the amount of GO on its surface was evaluated, so a dipping time could be selected. For that purpose, PMMA optical waveguides were dip coated with the 12.5 mg/mL GO suspension, varying the dipping times: 10 s, 30 s and 1 min. After each dipping cycle, the samples were dried, to eliminate completely the water, and weighted with an analytical balance (AS 60/220/C/2, Radwag). For each dipping time, six samples were evaluated. The presentation of the results and statistical analysis were performed using the statistics software GraphPad Prism (GraphPad Software Inc.).

2.3 - GO-coated optical waveguides characterization

The GO coating was characterized by different techniques, namely stereomicroscopy, scanning electron microscopy and optical contact angle measurement. Additionally, the stability of the coating was also assessed.

2.3.1 - Stereomicroscopy

GO-coated optical waveguides were observed using a stereomicroscope (Stereomicroscope SZX10, Olympus) with an illumination system (KL 1500 LCD, Olympus) to evaluate the homogeneity of the coating. Digital images were acquired with a magnification of 0.63x.

2.3.2 - Scanning electron microscopy

The surface of the GO-coated optical waveguides was observed by scanning electron microscopy (SEM, Phenom XL, Phenom World) analysis in Secondary Electron Detector (SED) mode with an acceleration voltage of 15 kV, at UPTEC. The images were acquired with magnifications of 500x, 2000x and 5000x. For the preparation of the samples, the GO-coated filaments were cut into smaller pieces to fit the SEM pins and adhered onto carbon tape. Before SEM analysis, all the samples were coated with a thin film of gold (Au) by sputtering to enhance their conductivity.

2.3.3 - Optical contact angle

The wettability of the GO-coated optical waveguides was evaluated through the optical contact angle measurement using water as the liquid.

The measurements were performed at room temperature using an optical contact angle measuring equipment (OCA 15, DataPhysics Instruments). In each sample, seven water drops (seven replicas) of 2 μL were placed on the optical waveguide in the horizontal plane, as schematically represented in Figure 17a. After image acquisition, the analysis of the optical contact angle was performed in the image processing SCA20 software (DataPhysics Instruments). The optical contact angle Φ_c at the three-phase contact line of the deposited drop is dictated by an equilibrium of vectorial forces, schematically represented in Figure 17b. This equilibrium can be described by the Young's equation, Eq. (3), in which σ_L is the surface tension of the liquid that acts tangential to the drop surface, σ_s is the surface energy of the solid acting along the solid surface and σ_{SL} is the solid-liquid interfacial energy acting in the opposite direction to the latter.

$$\sigma_L \cos \Phi_c = \sigma_s - \sigma_{SL} \quad (3)$$

In the SCA20 software, the drop contour and the base line at the solid-liquid interface are recorded and the Θ_c is automatically calculated. The presentation of the results and statistical analysis were performed in GraphPad Prism software.

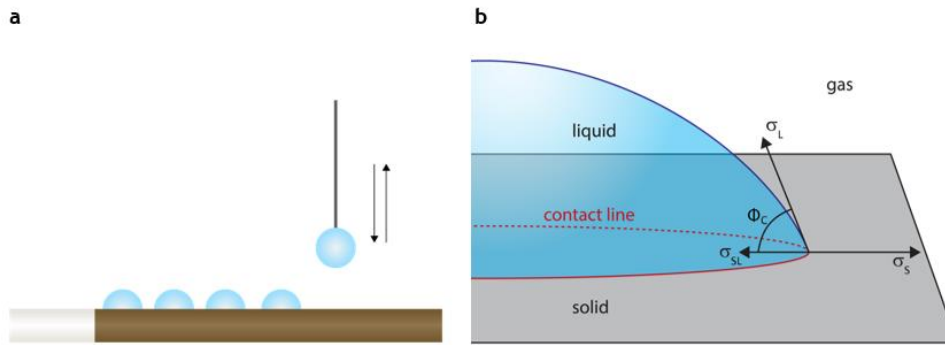


Figure 17. Optical contact angle measurement. (a) Deposition of water drops on the GO-coated optical waveguides. (b) Vectorial forces at the three-phase contact line of the deposited drop (adapted from [119]).

2.3.4 - Coating stability test

The detachment of GO from the surface of GO-coated optical waveguides, when immersed in water, was assessed to evaluate the coating stability. For that purpose, the GO-coated optical waveguides were immersed in eppendorfs filled with water for six hours and then, immediately after removal, they were pushed against a kimwipe. The coating stability evaluation was qualitative, by observation of the GO-coated optical waveguides and the kimwipe, and the results were presented through photographs.

3 - Assembly of the SmartCap prototype

3.1 - Design and production of the plastic cap

For the design and production of the plastic cap, first, the measurements of the different components to be integrated into the cap were performed, namely the thread of a commercial hemodialysis catheter, the optical waveguides and the optical sources. Then, based on the measured dimensions, the cap prototype was designed in Fusion 360 from Autodesk, a commonly used computer-aided design (CAD) software. The CAD file was converted into a stereolithography (.stl) file, which was then used in the slicing software (Z-SUITE, Zortrax) to generate a sliced printing file (.zcode) containing information regarding the printing settings. The cap was 3D printed using an extrusion printer (Zortrax M200, Zortrax) and acrylonitrile butadiene styrene (ABS) filament. Finally, the printing supports were removed, and the cap was manually finished. Features were adjusted and redesigned in the CAD software and the process was repeated until the final cap is achieved. The main steps for the prototype production are schematically represented in Figure 18.

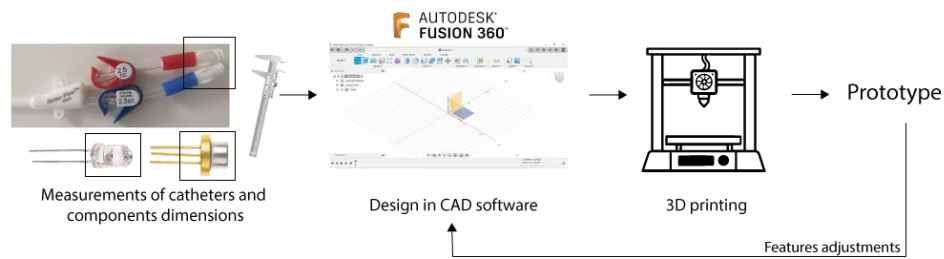


Figure 18. Main steps for the design and 3D printing of the plastic caps.

Different caps were designed and printed throughout the work. In the initial phase, a simplified model of the cap was produced to explore the printing parameters and obtain an inner thread capable of screwing up a commercial catheter. Posteriorly, several prototypes were produced aiming to support the different optical sources and optical waveguides explored.

3.2 - Assembly of the SmartCap prototype

The optical devices, namely the optical source and the GO-coated optical waveguide, were coupled inside the plastic cap to obtain the SmartCap prototype. In each cap model, the optical source must be held in a position that ensures a centre alignment with the optical waveguide, so that the light enters it. Different prototypes were assembled throughout the work, so different conditions could be tested.

3.3 - SmartCap prototype physical characterization

Assembled prototypes were physically evaluated regarding their optical and thermal properties. In combination with the optical properties, the thermal properties provide information regarding the photothermal effect, since it measures the temperature increase of the GO on the surface of the optical waveguides that can be related to the NIR light interacting with the GO.

3.3.1 - Optical properties

The optical properties of the assembled prototypes were evaluated using an IR viewer (ABRIS viewer, irvi). To acquire the images, the IR viewer was fixed in a support and the prototype was placed on a 3D printed black support (Annex A), preventing light reflections from interfering in the visualized images.

3.3.2 - Thermal properties

The thermal properties of the assembled prototypes were evaluated using a thermal camera (TE-EV1, Thermal Expert) and the Thermal Expert Q1 software, which is the real time monitoring software associated to the camera. For the thermal images acquisition, the camera

was placed on a 3D printed support (Annex A), at 0.3 m height from the table, and the assembled prototype was fixed to the table with double-sided adhesive tape.

4 - Antibacterial effect of the SmartCap prototype

The antibacterial assay includes preparing the bacteria culture media, growing the bacteria and preparing the inoculum that is used during the antibacterial assay. These steps are described below and are partially represented in Figure 19.

4.1 - Culture medium preparation

Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) from Merck were used to prepare the agar plates and the liquid medium, respectively, before the antibacterial assays. For that purpose, 32 g of TSA powder and 24 g of TSB powder were separately dissolved in 800 mL of water type II and autoclaved at 121 °C for 15 min. The TSA medium was poured onto petri plates to form a solid medium, and the TSB medium was filtered and aliquot into 50 mL falcons. Both the TSA plates and the TSB medium were stored at 4 °C.

4.2 - Bacteria growth

To assess the bactericidal action of the SmartCap prototype, *S. epidermidis* (ATCC 35984) was used in the antibacterial assays. The bacteria, preserved at -80 °C, were firstly spread on an agar plate and incubated overnight at 37 °C in stationary conditions to grow subcultures. Then, to prepare the pre-inoculum, two colonies were collected from the agar plate and inoculated in 5 mL of TSB medium in a falcon. The bacterial suspension was incubated at 37 °C overnight at 150 rpm.

4.3 - Bacteria inoculum preparation

The bacterial suspension was washed twice with 5 mL of TSB through centrifugation at 2700 rpm for 10 minutes (5810R, eppendorf) and then resuspended in 5 mL of TSB. Then optical density at 600 nm (OD_{600}) was read, using a UV-Vis spectrophotometer (Lambda 35 UV/Vis Spectrometer, Perkin Elmer) at 1:10 to determine the concentration of the pre-inoculum. The concentration was adjusted to 0.5×10^4 - 2.65×10^7 Colony-Forming Units (CFU)/mL to obtain the initial inoculum, depending on the assay.

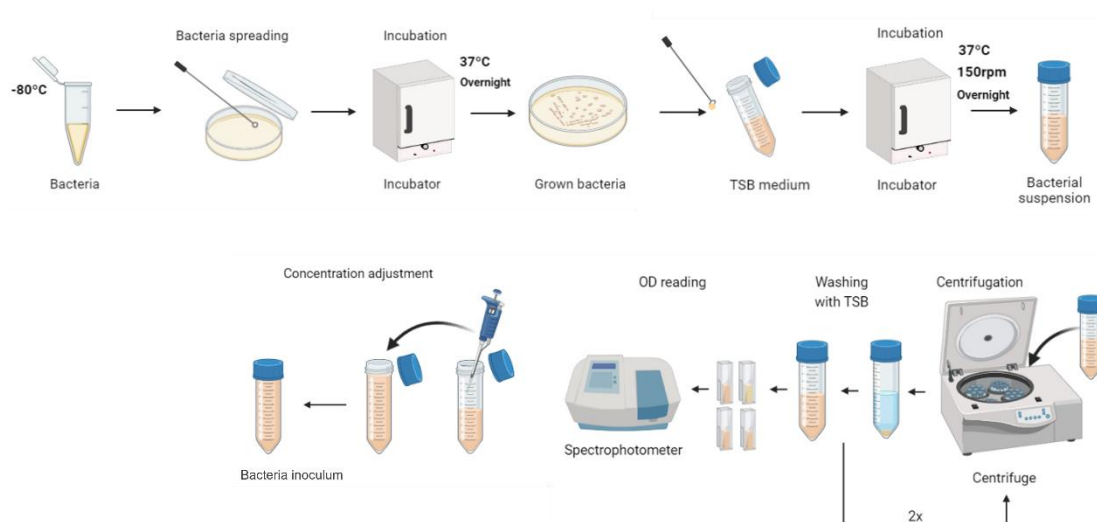


Figure 19. Main steps of bacteria growth (top) and inoculum preparation (bottom) in the antibacterial assay preparation phase.

4.4 - Antibacterial assay

The antibacterial assays were performed in two different setups, schematically represented in Figure 20. In the first (Figure 20a), the assembled SmartCap prototype was fixed in an eppendorf containing the bacterial suspension (varied volume between assays), so that the GO-coated optical waveguide stayed immersed. In the second (Figure 20b), the GO-coated optical waveguide was laterally placed in a well of a 48-well plate and the optical source was externally coupled to the optical waveguide. The 48-well plate was previously drilled (hole with 2 mm diameter) and the optical waveguide was externally glued to fix it and to isolate the hole preventing the bacterial suspension from leaking during the assay. This configuration for the antibacterial assay aims to bring the test conditions closer to those previously tested with graphene films [118], in which the volume of bacterial suspension was smaller.

During the assay, the setup (SmartCap + bacteria) was kept at 37 °C for a defined period of time, corresponding to the irradiation time in which the prototype is on. Besides the complete prototype, a SmartCap prototype without optical source was also evaluated as the GO control, and bacteria growing alone was used as controls of bacteria growth and viability. A NIR control, which consists of a SmartCap prototype with an uncoated optical waveguide, was not performed since only one optical source of each type was available.

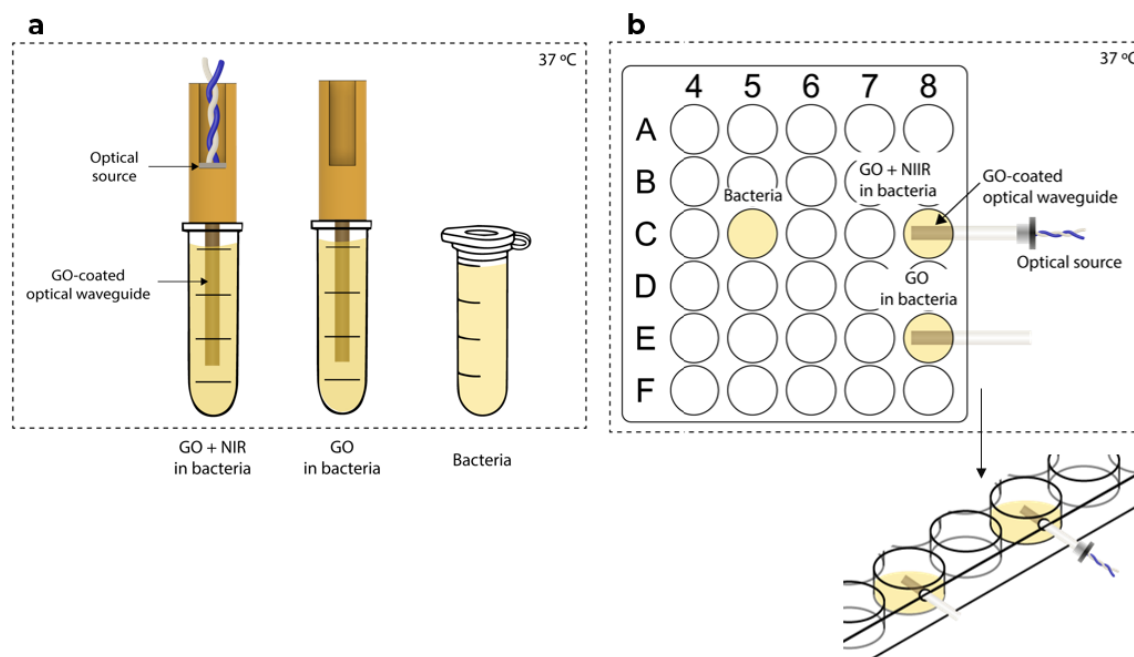


Figure 20. Experimental setups for the antibacterial assays. (a) GO-coated optical waveguide vertically immersed in an Eppendorf. (b) GO-coated optical waveguide laterally immersed in a 9 mm-diameter well.

4.5 - CFU counting

After the antibacterial assay, the bacteria solutions were homogenized, either by vortex or up and down with the pipette, and 10-fold serial dilutions with TSB were performed. The diluted solutions (10 μ L drops) were plated onto the agar plates, in triplicate. The TSA plates were then incubated at 37 °C overnight to yield visible colonies. In the dilutions that allowed the colonies to grow separately, CFU are counted. The bacterial concentration was calculated by $CFU_{counting} \times \frac{1}{0.01 \text{ mL}} \times 10^{DF}$ (CFU/mL), in which DF is the dilution factor.

4.6 - Data treatment

Data treatment was performed in GraphPad Prism software. The results were presented by the mean $\pm$ standard deviation of the bacterial concentration (CFU/mL) of the triplicates for each sample. The unpaired t-test with Welch's correction was performed to evaluate if the difference between conditions was statistically significant, after proving the normal distribution of the results.

Chapter 4

Results and Discussion

1 - Optical devices characterization

1.1 - Optical sources

In this subsection, the results of the characterization of the optical sources used in this experimental work are presented and discussed.

1.1.1 - Optical sources spectral characterization

The optical signals of the optical sources were acquired in the range of 825-875 nm (Laser@850nm) and 800-900 nm (LED@860nm) using an OSA. Figure 21 shows the normalized spectra of both optical sources.

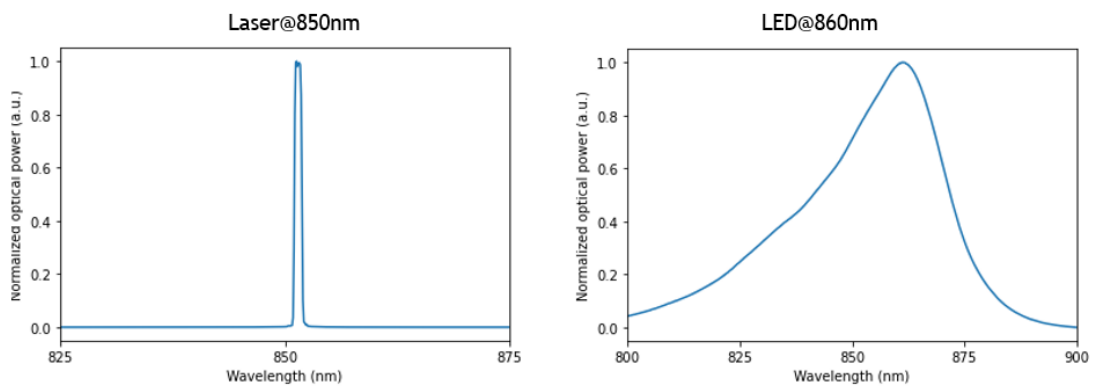


Figure 21. Normalized spectra of the optical sources: (left) Laser@850nm and (right) LED@860nm.

The spectrum of the Laser@850nm consists of a narrow emission range with a peak wavelength of 851 nm, while the LED@860nm emits light in a wide range of wavelengths with a peak wavelength of 861 nm. The full width at half maximum (FWHM) of the LED@860nm, which refers to the width of the optical signal at half its maximum intensity, is 30 nm, from

841 nm to 871 nm. These are expected results as lasers are optical sources characterized by emitting monochromatic light, i.e., light with a single wavelength, whereas LEDs emit wider ranges of wavelengths (polychromatic light).

It is relevant to mention that with the temperature increasing, originating from the heating of the optical source, there is an increase of the emission wavelength. Therefore, during the experimental work, the wavelengths of the emitted light will be higher than the reported here, depending on the optical power of the optical source that influences its temperature. To confirm this, the emission spectra of both optical sources were acquired 10 min after turning them on at high optical powers. The normalized spectra can be found in Figure 22.

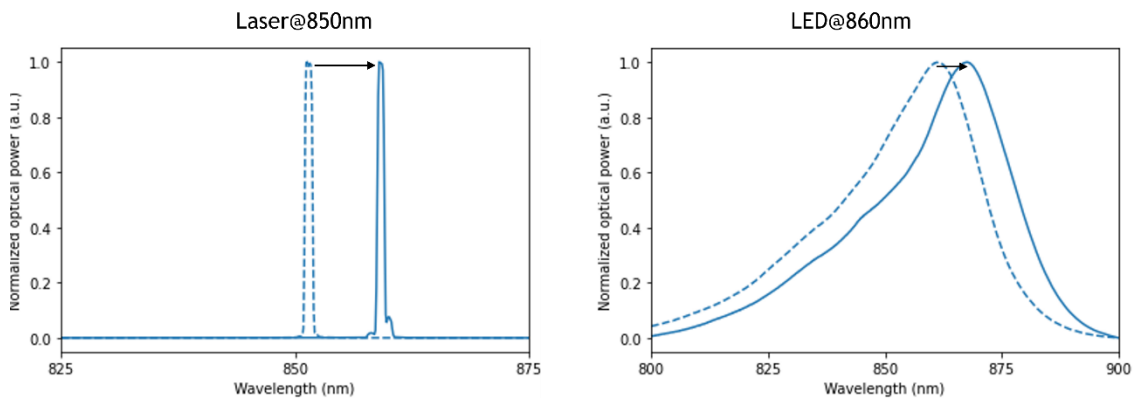


Figure 22. Normalized spectra of the optical sources immediately after turning them on (dashed line) and after 10 minutes (continuous line): (left) Laser@850nm and (right) LED@860nm.

Both spectra suffered a shift to the right, meaning that the wavelength of the emitted light increased with the temperature. The peak wavelength of the Laser@850nm moved to 861 nm and its temperature reached 80 °C. The peak wavelength of the LED@860nm was 868 nm, being the FWHM between 849 nm and 879 nm, and its temperature reached 60 °C.

1.1.2 - Optical power characterization

The optical power characterization of the optical sources was performed through their optical power curves, relating the output optical power to the applied voltage, and consequently current. The optical power curves of both optical sources are shown in Figure 23, through the experimental points of the output optical power and applied voltage and their tendency lines. The parameter of the linear fits in the range 1.65-3.32 V, namely the slopes and the coefficients of determination, can be found in Table 4.

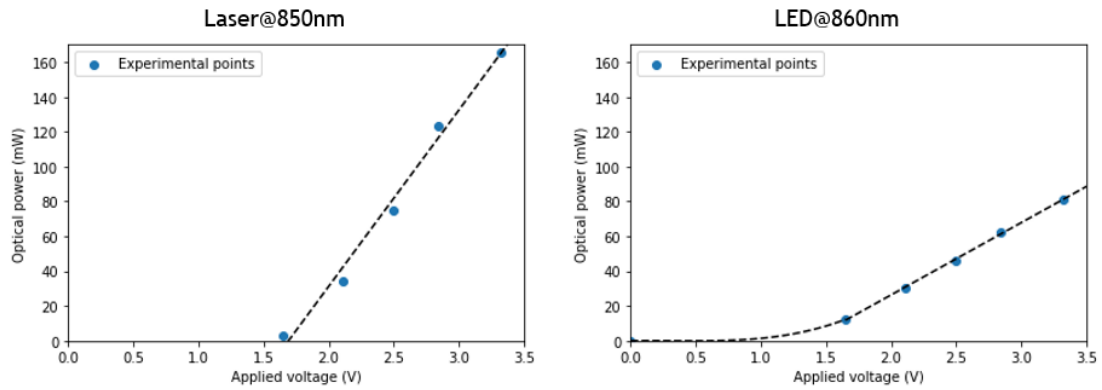


Figure 23. Optical power (in mW) as a function of the applied voltage (in V) of the optical sources: (left) Laser@850nm and (right) LED@860nm.

Table 4. Parameters (slope and coefficient of determination) of the linear fits for the optical power curves of the optical sources.

Optical source	Slope (mW/V)	R ²
Laser@850nm	101.3	0.99
LED@860nm	41.5	0.99

For both the Laser@850nm and the LED@860nm, the optical power presents a linear response with a slope of 101.3 mW/V and 41.5 mW/V, respectively, in a voltage range of 1.65-3.32 V. For the same applied voltage, the optical power of the Laser@850nm is approximately the double of the optical power of the LED@860nm.

Besides, from the Laser@850nm optical power curve, it is possible to see that with an applied voltage of 1.65 V there is no light emission (null optical power), meaning that the current is lower than the threshold current of the Laser@850nm, which is around 50 mA for that applied voltage according to the datasheet. Thus, with applied voltage higher than 1.65 V, or current higher than 50 mA, the laser is mainly in stimulated emission, rather than spontaneous emission as occurs with current lower than the threshold current, and so there will be emission of light by the Laser@850nm. Contrarily, LED@860nm emits light with any applied voltage, as LEDs work in spontaneous emission instead of stimulated emission, so they do not have a threshold current. Shortly, spontaneous emission results from the transition of an atom from an excited state to a lower energy state, which occurs naturally, resulting in incoherent light, i.e., light beams in which the photons have different frequencies and are in different phases. On the other hand, in stimulated emission incident photons stimulate atoms in excited state to fall down to lower energy state, resulting in two photons (the incident and the emitted) with the same frequency, in phase (coherent light) and with the same direction (collimated beam) [120].

The optical power signals were acquired for 20 minutes to assess their stability for different applied voltages. The optical power signals over time of both optical sources are presented in Figure 24.

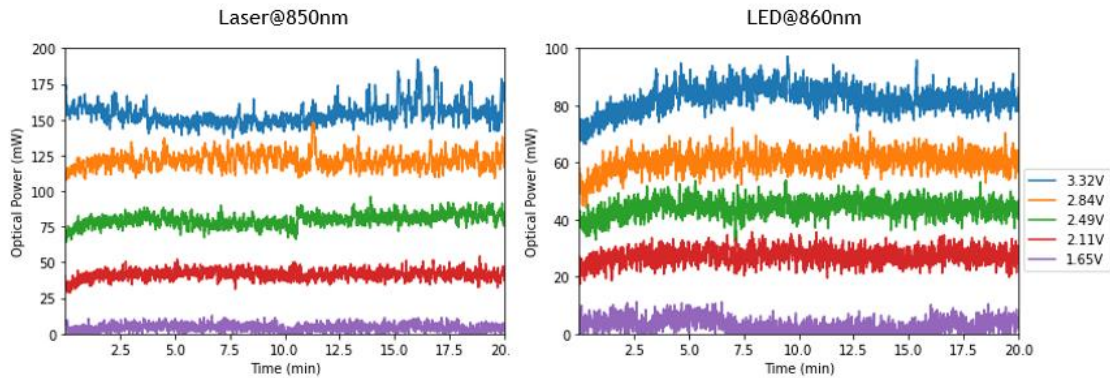


Figure 24. Optical power (in mW) measured over time for different applied voltages of the optical sources (left) Laser@850nm and (right) LED@860nm.

Regarding the optical power stability over time, both optical sources present stable output optical power for applied voltages above 2.11 V, although they take more time to stabilize with an applied voltage of 3.32 V. Some of the oscillation of the optical power signals can be justified by the fact that the power sensor used is based on thermopile technology, and so the heating of the optical sources causes fluctuation in the measured optical power. Besides, the external light sources (for instance, from windows) also contribute to a certain fluctuation of the measured optical powers.

As proposed by L. Hui et al. [117], the polychromatic nature of the light promotes the photothermal effect on GO and consequently its antibacterial action. Therefore, for the application in the SmartCap, the LED@860nm can be advantageous over the Laser@850nm. On the other hand, the Laser@850nm allows for a wider range of optical power to be explored, as it reaches higher optical power than the LED@860nm. Besides those differences, the numerical aperture of the light beams emitted by both optical sources - low in lasers (collimated beam) and high in LEDs - is another factor that may influence the performance of the prototypes, as a collimated beam allows for more light to enter the optical waveguide, whereas in a wider beam more light will be emitted out of the optical waveguide.

1.2 - Optical waveguides

In this subsection, the results of the characterization of the optical waveguides used in this experimental work are presented and discussed.

1.2.1 - Optical waveguides preparation

The preparation of the polymeric optical waveguides include their linearization, cut and polishing of end faces to reduce the imperfections that would contribute to light diffusion. The quality of the polishing was evaluated by stereomicroscopy. Figure 25 shows stereomicroscope images of the end faces of the four optical waveguides explored before and after polishing.

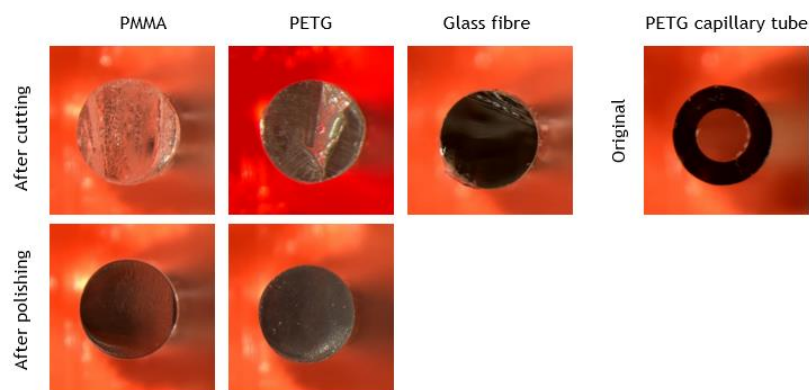


Figure 25. Stereomicroscope images of the end faces of the PMMA and PETG filaments and glass fibre before and after polishing, and of the original (without cutting) PETG capillary tube.

The cut of the PMMA and PETG filament leaves defects and roughness on their end faces that are eliminated by polishing. This allows for a lower loss of light entering the optical waveguide caused by diffusion. Contrarily, the cut of the glass fibre is a clean cut that does not leave imperfections on the end face. Finally, the PETG capillary tube is not cut in the end face that couples to the optical source and does not require polishing, since it has no defects.

1.2.2 - Optical waveguides characterization

The optical waveguides were characterized regarding their propagation losses. Figure 26 shows the graphical representation of the experimental points of the loss (dB) of the optical waveguides with different lengths (2-5 cm), their calculated mean values and the respective linear fit. In Table 5 are presented the parameters, namely the slope and the coefficient of determination, of the linear fits for each optical waveguide.

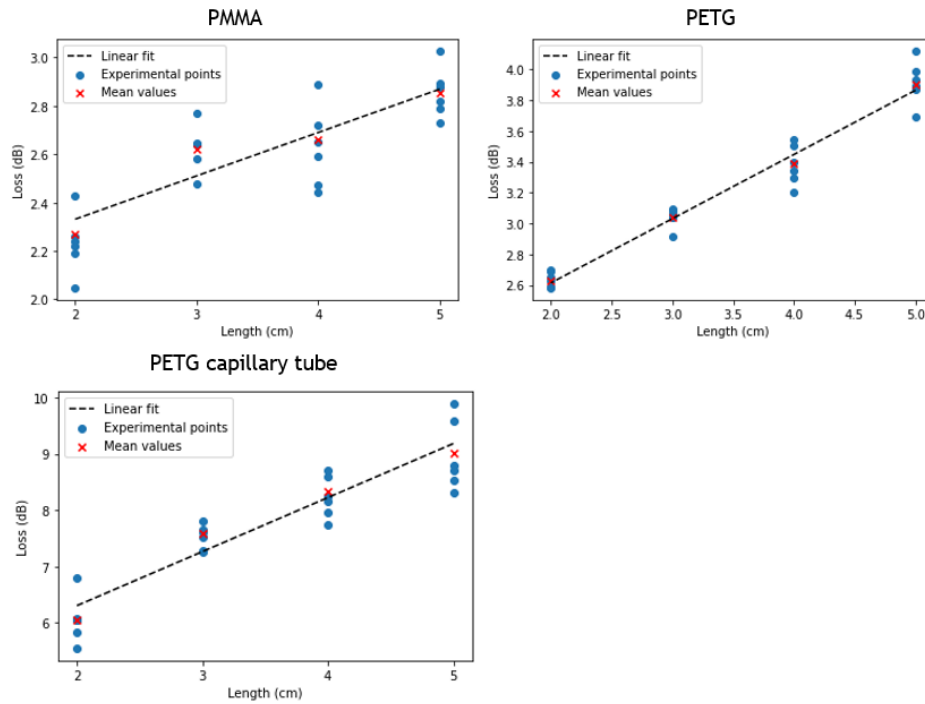


Figure 26. Propagation loss of the different polymeric optical waveguides: PMMA and PETG filaments and PETG capillary tubes. Experimental points (blue dots), mean values (red crosses) and linear fit of the latter (dashed line).

Table 5. Parameters (slope and coefficient of determination) of the linear fits of the optical power losses mean values for the explored optical waveguides.

Optical waveguide	Slope (dB/cm)	R ²
PMMA filaments	0.18	0.90
PETG filaments	0.42	0.99
PETG capillary tubes	0.96	0.96

The PMMA filaments present a propagation loss of 0.18 dB/cm at 850 nm, which is similar to the literature found (0.20 dB/cm at 850 nm [67]). The PETG filaments have a propagation loss of 0.42 dB/cm at 850 nm. The literature found reported propagation losses of 0.26 dB/cm at 543 nm and at 1550 nm for PETG coreless (Ø30 µm) optical waveguides drawn from 3D printers [121]. The higher propagation loss in the PETG optical waveguides used in this experimental work can be due to their composition, namely the 8-12% of fiberglass fillings and 0-8% of additives that may increase the light loss, either by scattering and absorption. The propagation loss of the PETG capillary tubes is 0.96 dB/cm at 850 nm, higher than that of the PETG filaments, which means that the hollow structure leads to higher light losses throughout the optical waveguide.

Among the polymeric optical waveguides explored in this experimental work, PMMA filaments are the best optical waveguides, presenting the lowest propagation loss. Nevertheless, the glass fibre presents the lowest propagation loss of 12 dB/km (from datasheet)

of all the optical waveguides, being insignificant for the dimensions used in this experimental work.

In the SmartCap prototype, the NIR light emitted by the optical source should be spread throughout the optical waveguide, so that it interacts with the GO deposited on its surface, promoting the desired photothermal and photodynamic effects. Therefore, the higher propagation losses of the polymeric optical waveguides can be advantageous for that purpose, as long as the light is lost by scattering, instead of absorption by the polymer. The light scattering, caused by the interaction between the light wave and a particle or by the roughness on the surface, consists of transferring the energy of a directional propagating wave to other directions, thus the light does not lose optical power. Contrarily, when the light is absorbed by a material, its energy is converted into heat and there is a decrease of the optical power. Thus, in the SmartCap prototype, scattered light will interact with GO, whereas absorbed light will not.

2 - Coating of optical waveguides

2.1 - Graphene oxide absorption spectrum

The absorption spectrum of the 4 mg/mL GO suspension diluted to a concentration of 0.25 mg/mL was acquired in the visible and NIR part of the electromagnetic spectrum allowed by the equipment, in a range of 400-950 nm with a resolution of 2 nm. The spectrum is shown in Figure 27 highlighting the range between 700 nm and 950 nm, where the NIR part of the electromagnetic spectrum belongs.

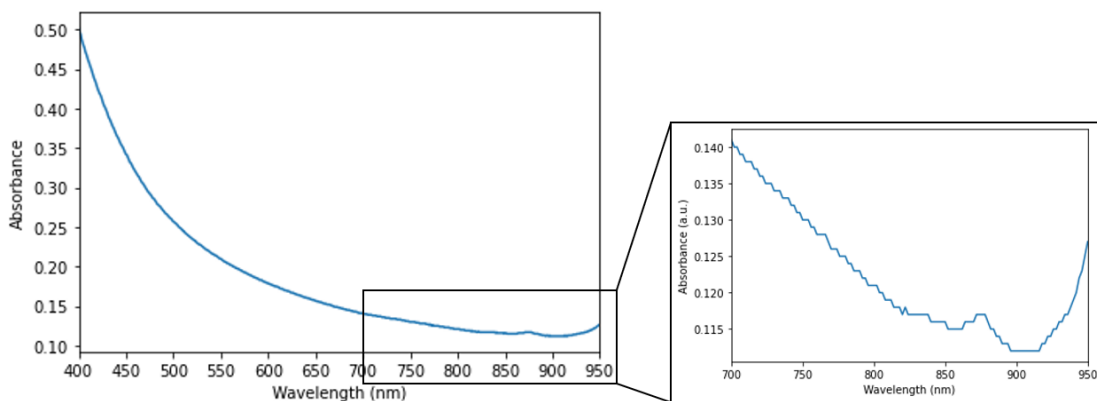


Figure 27. Absorption spectrum of GO from 400 nm to 950 nm.

The spectrum shows an exponential decrease of the absorption of GO with the wavelength, reaching the minimum in the NIR part of the spectrum at 900 nm. This spectrum is similar to GO spectra reported in literature [122].

As shown in the previous section, the optical sources explored in this experimental work emit NIR light at around 850 nm and 860 nm. Based on the results of a previous study by our group [118], NIR light (812 nm) promotes a photothermal and a photodynamic effect on graphene-based materials enough to eliminate bacteria almost completely, using low-power LEDs, despite the low absorption by GO. Therefore, herein, the SmartCap prototype was developed using NIR optical sources. Nevertheless, based on the GO absorption spectrum, it is possible to hypothesize that optical sources emitting at shorter wavelengths would require lower output optical powers to promote the same photothermal and photodynamic effects than NIR optical sources, as the absorption by GO is maximized. Thus, other optical sources can be considered in the future.

2.2 - GO coating of optical waveguides

The influence of the dipping time on the amount of GO on the surface of the optical waveguides was assessed by the mass of the GO coating of optical waveguides dipped for different dipping times, namely 10 s, 30 s and 1 min, in the 12.5 mg/mL GO suspension. Figure 28 shows the results of these measurements by the mean and standard deviation of six samples for each time and for each number of dips (GO layers).

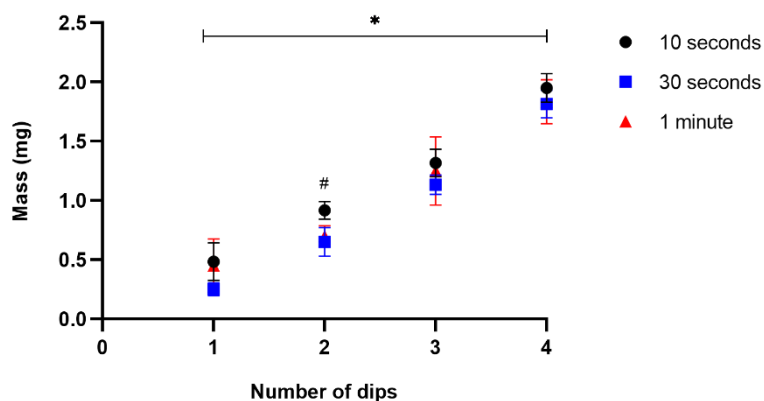


Figure 28. Mass (mg) of GO coating of PMMA optical waveguides dipped for different dipping times (10 seconds, 30 seconds and 1 minute) in the 12.5mg/mL GO suspension. *, # Statistical significance, $p < 0.05$: one-way ANOVA test to compare (*) optical waveguides coated with different number of GO layers (number of dips) and (#) GO-coated optical waveguides dipped for different dipping time.

Figure 28 show, as expected, that increasing the number of dips increases the amount of GO on the surface of the waveguide, for all dipping times. Particularly, with 4 dips (of 10 s) in the 12.5 mg/mg GO suspension, a GO coating of 1.95 ± 0.12 mg is achieved.

Besides, there is no significant difference in the mass of GO on the surface of the optical waveguide with the dipping time (except for the 2 dips in which the samples dipped for 10 s have more GO on the surface than those dipped for 30 s and 1 min). Thus, it seems that increasing the dipping time does not increase the amount of GO on the surface of the optical

waveguide, at least for dipping times above 10 s. Therefore, a dipping time of 10 s is enough to coat the optical waveguides used in this experimental work, which accelerates the process.

Besides, there are some variabilities between the samples (replicas) of the same group (same dipping time and number of dips). On the one hand, these may be due to the manual dip coating process that is not completely reproducible for all samples, particularly regarding the speed of immersion and removal of the optical waveguides and the movement of the optical waveguide during the dipping time. On the other hand, the relatively high concentration of the GO suspension in which the optical waveguides were dipped leads to heterogeneous deposition of GO between samples. Despite the agitation of the suspension, manually and by vortex, before the dip coating, it is difficult to ensure a homogeneous dispersion of GO in the suspension, due to the relatively high viscosity.

An automatic dip coating system, with velocity control and allowing static conditions during dipping time, along with an agitation system for the GO suspensions, should solve the problem of the lack of reproducibility between samples.

2.3 - GO-coated optical waveguides characterization

The GO coating of the optical waveguides was characterized by stereomicroscopy, scanning electron microscopy and optical contact angle. Stereomicroscopy was used to observe the surface of PETG and PMMA optical waveguides coated with different number of GO layers of different GO suspensions, while SEM and optical contact angle were performed in PMMA optical waveguides coated with 4 dips of the 12.5 mg/mL to evaluate the effect of different factors (drying temperature of the dip coating process, sterilization with EtO and NIR irradiation) on the GO coating. Additionally, a preliminary test to evaluate the coating stability of these last-mentioned samples was performed.

2.3.1 - Coating homogeneity and morphology

Stereomicroscope images of PMMA and PETG optical waveguides coated with the 4 mg/mL and 12.5 mg/mL GO suspensions, varying the number of GO layers (by varying the number of dips), were acquired to evaluate the homogeneity of the GO coatings. Figure 29 shows the stereomicroscope images acquired with a magnification of 0.63x.

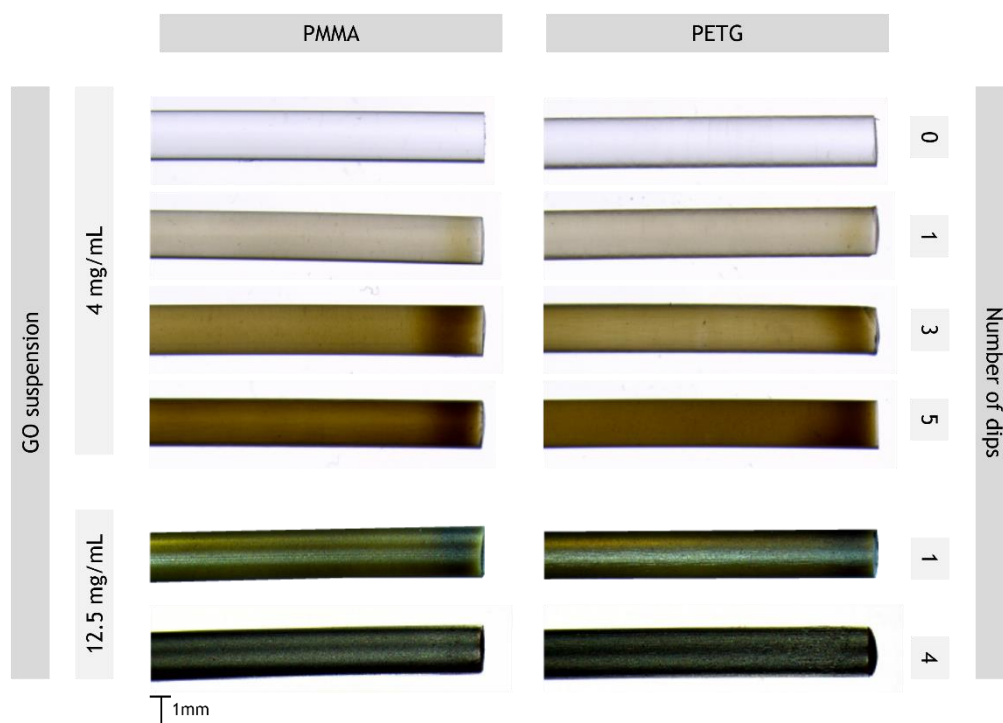


Figure 29. Stereomicroscope images (magnification $\times 0.63$) of PMMA and PETG optical waveguides dip-coated with the 4 mg/mL and 12.5 mg/mL GO suspensions, varying the number of dipping cycles.

The stereomicroscope images show that the optical waveguides were completely and uniformly coated with GO, not existing defects or uncoated parts. However, there is a visible accumulation of GO at the end of the optical waveguides, due to the dip coating process. Increasing the number of dipping cycles increases the amount of GO on the surface of the optical waveguide as well as the homogeneity of the coating. In fact, the most homogeneous coating was achieved with 4 dips in the 12.5 mg/mL GO suspension. Between the PMMA and PETG optical waveguides, there is no difference in the GO coated on their surface.

SEM images were acquired to assess the surface morphology of the GO coating of PMMA optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension. Aiming at verifying if the temperature of the drying step in the dip coating process has any impact on the GO coating surface morphology, GO-coated optical waveguides dried at 60°C and at room temperature (RT) were observed. Also, GO-coated optical waveguides (dried at 60°C) after sterilization and after NIR irradiation were also observed to verify whether the EtO sterilization and NIR radiation, respectively, cause any surface morphology changes. Figure 30 shows the SEM images of all optical waveguides with magnifications of 500x, 2000x and 5000x.

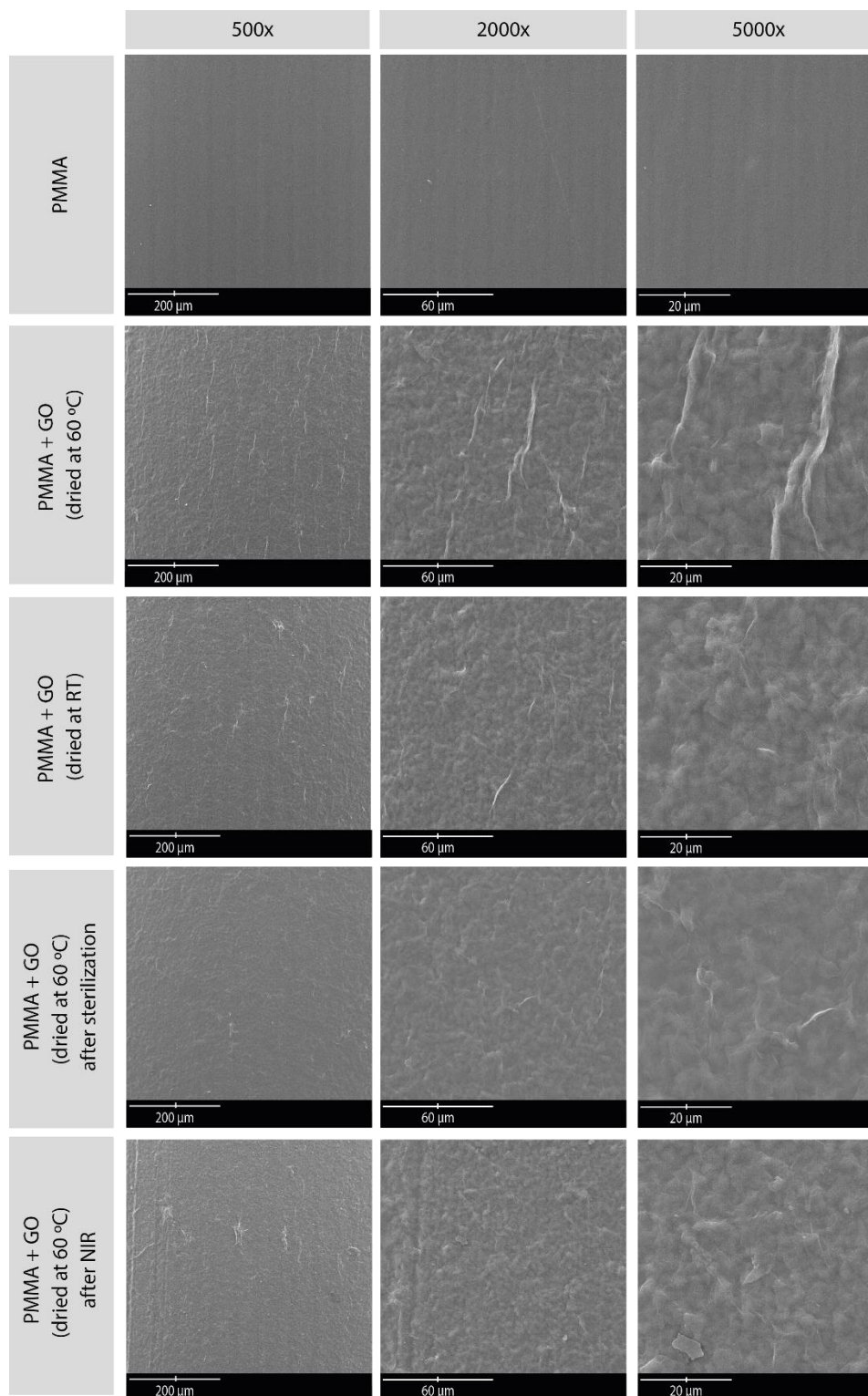


Figure 30. SEM images of PMMA optical waveguide uncoated and coated with 4 dips of the 12.5 mg/mL GO suspension: dried at 60 °C and at RT, after EtO sterilization and after NIR irradiation. Images acquired with magnifications of 500x, 2000x, 5000x.

SEM images of all coated optical waveguides show a continuous and homogeneous coating of GO when compared to the uncoated optical waveguide. The surface of the GO coating is characterized by the presence of wrinkles, more notorious in the images of the sample dried

at 60 °C (second row). In fact, wrinkling is common in 2D materials, due to the thermodynamical instability of 2D crystals that cannot exist in the 3D space at finite temperatures [123]. As a 2D material, graphene presents a microscopically corrugated structure (intrinsic wrinkle), originated from the different types of bonds that carbon form at finite temperatures [124], [125]. The functional groups of GO (hydroxyl, carbonyl and epoxy groups on the basal plane and carboxyl groups at the edges) cause atomic level corrugation, called extrinsic wrinkles, as they change the electronic structure of the carbon atoms (from sp^2 to sp^3 hybridization) [126]. When the GO sheets are assembled into a GO layered structure, more undulation, another form of wrinkling at micro- and macroscopic scales, emerges [127]. Although the mentioned wrinkles explain part of the undulated structure observed in GO coatings, the elevated amplitude of these wrinkles is related to other factors, as it is much higher than that observed in non-oxidized graphene materials. Particularly, the stronger interactions between GO and water causes extra stresses, due to the water surface tension, during water evaporation in the drying process. These GO-water interactions lead to the formation of crumpled GO sheets [128], [129].

Comparing the images of the GO-coated optical waveguides dried at 60 °C and RT, no significant differences are observed, indicating that a temperature of 60 °C during the drying process does not affect the surface morphology.

Similarly, comparing the images of the GO-coated optical waveguides subjected to EtO sterilization and NIR irradiation with the non-sterilized and non-irradiated sample (dried at 60 °C), no notorious differences can be observed in the surface morphology.

Overall, it seems that neither the drying temperature (up to 60 °C) nor the EtO sterilization or NIR irradiation processes cause significant changes in the surface morphology of the GO-coated optical waveguides.

2.3.2 - Coating wettability

The water contact angle of optical waveguides uncoated and coated with 4 dips of the 12.5 mg/mL GO suspension were measured to assess their surface wettability. The results of the contact angle measurements are presented in Figure 31, by the mean and standard deviation of the seven drops. Samples with the same conditions aforementioned were here evaluate to study the effect of the drying temperature, EtO sterilization and NIR irradiation on the wettability of the surface.

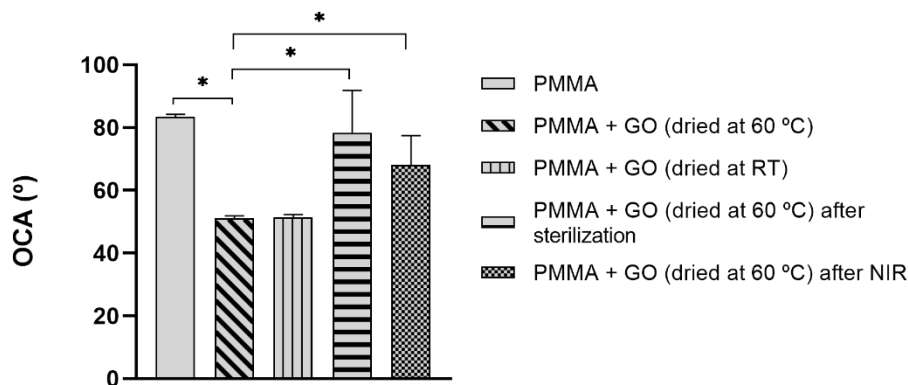


Figure 31. Optical contact angle, by the mean and standard deviation, of PMMA optical waveguide uncoated and coated with 4 dips of the 12.5 mg/mL GO suspension: dried at 60 °C and at RT, after EtO sterilization and after NIR irradiation. * Statistical significance, $p < 0.0005$ from the optical waveguide dried at 60°C by the one-way ANOVA test.

The GO coating decreases in more than 30° (from 83.5° to 51°) the water contact angle of the surface of PMMA optical waveguides, making it even more hydrophilic. The hydrophilicity of GO coating is due to the high content of hydrophilic groups of GO.

Regarding the drying temperature applied during the dip coating process, it has no effects on the wettability of the GO-coated optical waveguides, as the optical waveguides dried at RT and 60°C presented similar contact angles (51.5° and 51°, respectively).

Both the NIR radiation and the sterilization with EtO led to an increase in the contact angle, from 51° to 68.1° and 78.4°, respectively, despite both remain hydrophilic. In their study, P. Henriques et al. [118] had already observed an increase of the contact angle in FLGO films after NIR irradiation. Contrarily, the effect of EtO on the wettability of GO surfaces was not reported yet, although there are studies reporting an increase in the water contact angle of materials after EtO sterilization [130]. Besides, in both samples, the contact angle was very heterogeneous along the optical waveguide (high standard deviations), varying from 80.8° at the beginning to 58.2° at the end of the irradiated waveguide, and from 98.7° to 67.3° in the sterilized waveguide. To verify if the differences in the optical contact angle were related to differences in the surface morphology, SEM images of the surface of these two samples were acquired in three different positions (beginning, middle and end) of the optical waveguide. However, no notorious differences were observed in the morphology of the surface of the different images (Annex B).

Despite seven water drops were used as replicates, only one sample of each condition was used ($n=1$), and so more samples should be assessed in the future to validate these results. Other techniques can also be used to study the samples to understand the effect of the EtO sterilization and NIR irradiation that causes the increase in the contact angle. Particularly, X-ray photoelectron spectroscopy (XPS) to assess the chemical composition of the surface and atomic force microscopy (AFM) can be used to observe the 3D topography.

2.3.3 - Coating stability

The coating stability of the same samples evaluated by SEM and by OCA measurement (PMMA optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension) was evaluated through a preliminary test, consisting of immersing the samples in water for 6 hours and then push them against a kimwipe. Figure 32 shows the results of the coating stability test through photographs.

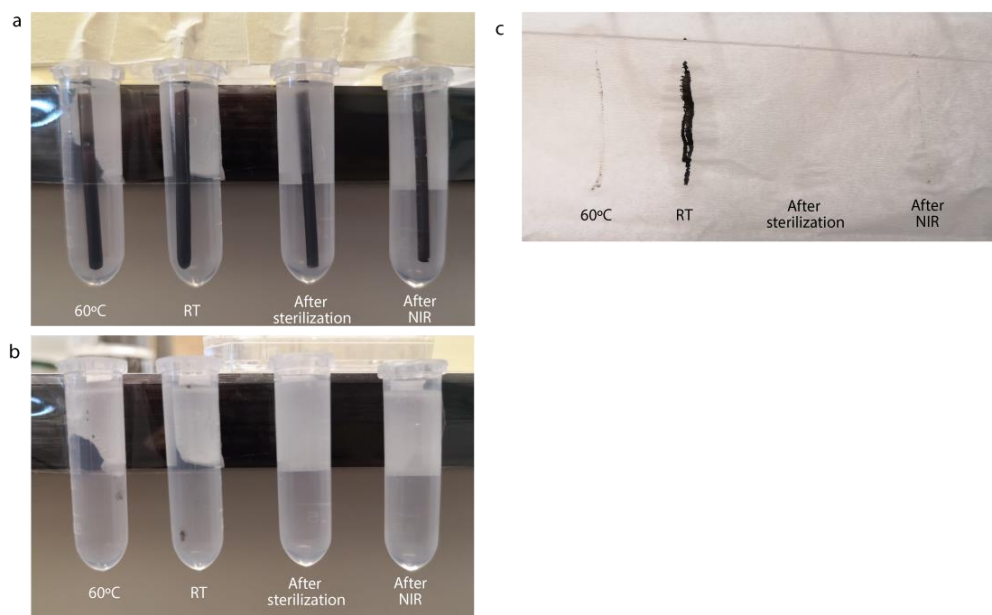


Figure 32. Photographs of the coating stability test performed to PMMA optical waveguides coated with 4 dips of the 12.5mg/mL GO suspension: dried at 60 °C and RT, after EtO sterilization and after NIR irradiation. (a) Samples in water type II after 6 h of immersion in water. (b) Water after samples removal. (c) Kimwipe after samples contact.

After 6 h of immersion in water (Figure 32a), there was no visible detachment of GO from the surface of the optical waveguides. However, the samples dried at 60 °C and RT suffered a visible swelling, more significant in the latter, compared to the other samples. The swelling of these samples can be related to their lower water contact angle and consequently higher wettability that led them to interact more with water. In those samples (60°C and RT), some graphene platelets detached from the surface of the optical waveguide after removing them from the water (Figure 32b), especially where the samples touched the walls of the eppendorf and on the surface of the water, due to the water's surface tension. On the contrary, no GO was released in the EtO sterilized and NIR irradiated samples after samples removal. These results already indicate that the stability of the coating is higher in samples subjected to sterilization and irradiation.

After pushing the samples against the kimwipe (Figure 32c), a significant amount of GO detached from the surface and adhered to the kimwipe in the sample dried at RT, whereas the sample dried at 60 °C suffered a smaller GO detachment, having therefore a more stable coating. In the sample exposed to NIR light, less GO detached from the surface, compared to the non-irradiated sample (60 °C), and in the sterilized sample no visible GO detachment

occurred, meaning that the GO coating is more stable both after EtO sterilization and NIR irradiation.

To sum up, the characterization of the GO-coated optical waveguides shows that the 60 °C used in the drying process increases the stability of the coating without affecting the physical properties of the surface. Thus, using 60 °C during the drying step not only accelerates the dip coating process without changing the physical properties of the coating, but also brings benefits regarding stability. The NIR irradiation, an unavoidable condition, seems to decrease the wettability and increase the stability, which is beneficial. Finally, the sterilization with EtO, which is a required condition for the final application of the SmartCap, also decreases the wettability of the surface and increases, more significantly than the others, the stability of the coating.

Although the preliminary coating stability tests performed herein gave important insights regarding the stability of the coating, other tests that follow international standards (as ISO 10993-13) will also be required. In fact, the outcome of these tests will determine the class of the medical device, namely if it is a class IIA if negligible release occurs, translating in negligible potential for internal exposure, or a class IIB if low release occurs, translating in low potential for internal exposure.

Overall, the dip coating process has advantages over other coating techniques, such as its simplicity and low waste of material. Besides, it is well implemented in industry, and so the transfer to a large-scale production would be relatively simple.

3 - Assembly of the SmartCap prototype

3.1 - Design and production of the plastic cap

The following figures show different caps produced throughout the work, both the CAD-designed models, in different views and with the main features and dimensions highlighted, and the printed caps. Figure 33 and Figure 34 correspond to the first cap produced.

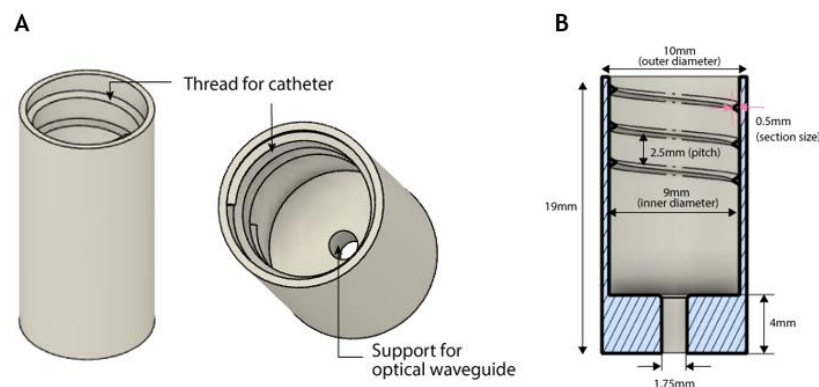


Figure 33. CAD design of the first cap: (A) 3D views and (B) section analysis.

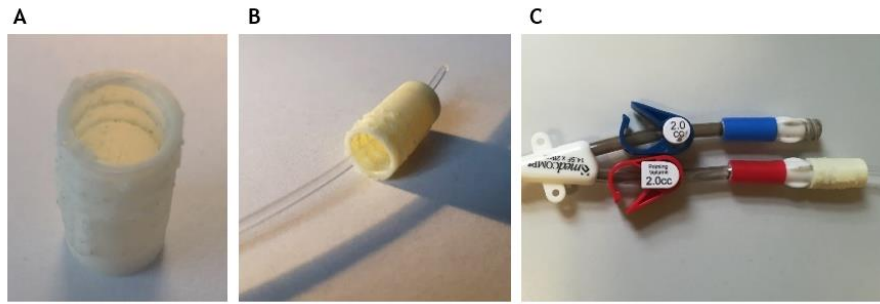


Figure 34. (A) 3D printed first cap (B) with a PMMA filament. (C) Cap inserted in a hemodialysis catheter.

The obtained plastic cap provides tight support for the optical waveguide (Figure 34B). Besides, it is compatible with the thread of a commercial catheter (Figure 34C), as desired, providing a close fit with the catheter. However, there are visible imperfections on the surface of the cap and inner thread, which are due to the defined small dimensions for the features that the resolution of the printer does not allow. For that reason, some dimensions were readjusted in subsequent prototypes.

Figure 35 shows the CAD designs and the 3D printed model of the cap prototype, after an interactive process of optimization. Figure 35 shows the model for the LED@860nm, however the model for the Laser@850nm is similar, changing the dimensions of the structure that holds the optical source.

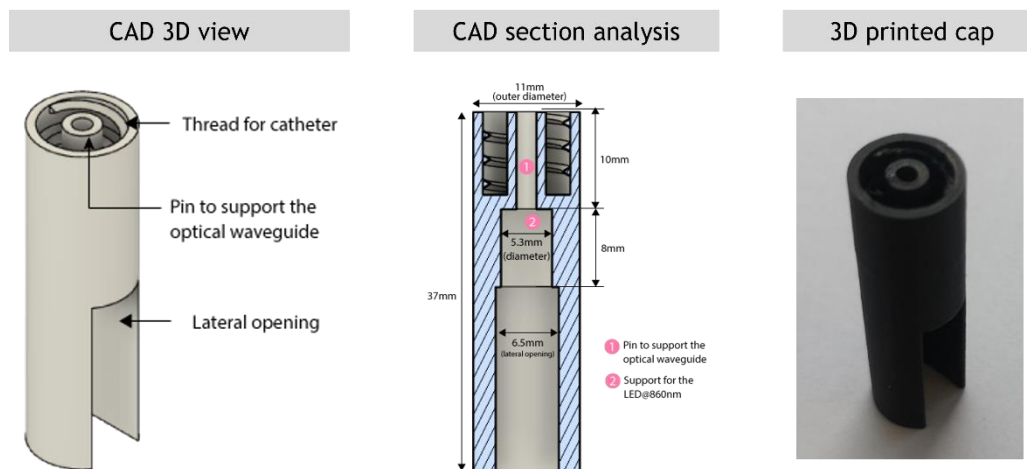


Figure 35. CAD designs (3D view and section analysis) of the final cap prototype and the respective 3D printed cap.

The upper part of the cap presented in Figure 35 is similar to the first cap (Figure 33 and Figure 34), particularly regarding the inner thread. However, the thickness of the wall of the cap was increased from 1 mm to 2 mm, by increasing the cap's outer diameter, which eliminated the defects on the surface and improved the quality and resolution of the thread. Besides, a 10 mm-length pin was designed to hold the optical waveguide, providing a more robust support than the previous 4 mm-length structure. The caps also include a hollow structure to hold the optical sources. The structure has the dimensions (diameter and length) of the respective optical source.

The lateral opening was created only for experimental purposes to be easier to fit and remove the optical sources during the experimental work.

3D printing is an additive manufacturing technique, in which parts are produced layer-by-layer, from 3D model data. Comparing to other manufacturing techniques, 3D printing has several advantages, such as freedom of design, quick manufacturing of parts, and thus efficient optimization, minimal waste and cost-effective production [131]. In this work, 3D printing allowed an inexpensive optimization and production of the cap prototypes in a short period of time, that can be easily modified.

However, 3D printing technology present some drawbacks that are important to consider, specially for transferring to large scale production, as is the case of this work. Particularly, 3D printed parts usually require a post processing to remove the printing supports and to smooth the surfaces, which adds costs and slows the production process. Besides, 3D printing has a static cost, thus production of larger volumes does not reduce the cost per unit, contrarily to other techniques, as injection molding. The plastic cap can be easily transferred to a large-scale production process by injection molding, maintaining the use of ABS as raw material.

3.2 - Assembly of the SmartCap prototype

SmartCap prototypes were obtained after assembling the optical devices, namely the optical sources and GO-coated optical waveguides, inside the plastic caps (Figure 36).

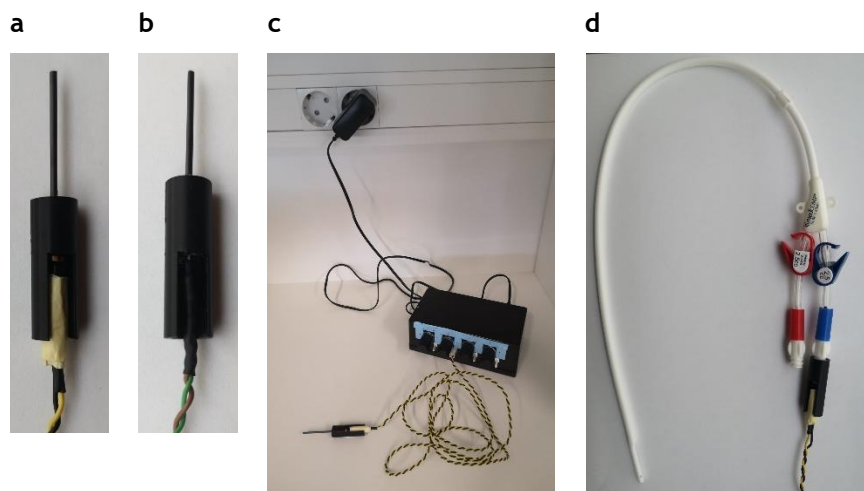


Figure 36. Assembly of the SmartCap prototype. (a, b) SmartCap prototype with Laser@850nm and LED@860nm. (c) SmartCap prototype connected to an external power source. (d) SmartCap prototype inserted in a hemodialysis catheter.

The SmartCap prototypes produced (Figure 36a and Figure 36b) provide a tight support to the optical devices (optical waveguide and optical source) and ensure their centre alignment. Besides, they are compatible with commercial catheters (Figure 36d).

The SmartCap prototypes produced allow for different experimental conditions to be tested (Figure 36b), regarding: (1) optical source - type and optical power, (2) optical waveguide - material and configuration, and (3) GO coating - GO suspension, concentration and number of layers.

3.3 - SmartCap prototype physical characterization

The results of the physical properties, including the optical and thermal properties of the prototypes, are presented together, so it is easier to relate the interaction between the NIR radiation and the GO coating to the temperature increase on the surface of the optical waveguide.

3.3.1 - Uncoated SmartCap prototypes

SmartCap prototypes with uncoated optical waveguides and using the Laser@850nm and the LED@860nm, both emitting at 45 mW, were observed with the IR viewer (Figure 37), to compare the light propagation through the different optical waveguides and the illumination ability of the optical sources. The polymeric cladding of the glass fibre was removed to decrease the light guidance and so to increase the light on the surface.

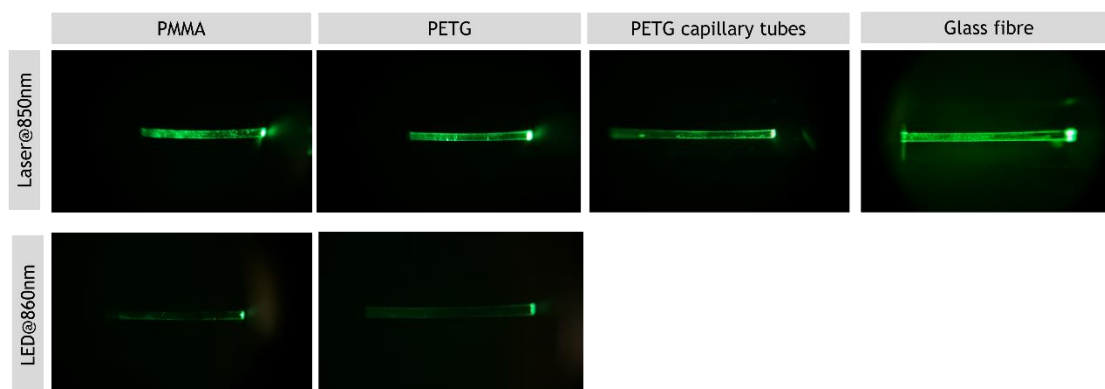


Figure 37. IR images of SmartCap prototypes with uncoated optical waveguides irradiated from the left by the Laser@850nm and the LED@860nm emitting at 45 mW.

The images in Figure 37 show that, despite emitting at the same optical power (45 mW), the Laser@850nm illuminates more the optical waveguides than the LED@860nm. This indicates that there is a significant loss of light emitted by the LED@860nm, which does not enter the optical waveguide, contrarily to Laser@850nm. These results can be explained by the differences of the numerical apertures of the beams emitted by lasers and LEDs: lasers emit collimated light beams, with a low numerical aperture, whereas LEDs emit larger beams. Thus, practically all the light emitted by the Laser@850nm enters the optical waveguides, whereas a significant amount of light emitted by the LED@860nm is immediately spread to the cap. For that reason, the Laser@850nm presents a higher efficiency than the LED@860nm, and a higher photothermal effect can already be predicted, since more light will interact with the GO on

the surface. Therefore, further experimental tests will be mainly performed using the Laser@850nm as optical source.

Comparing the optical waveguides, in all of them it is possible to see light reaching the surface, which is light that was lost in the propagation, and light exiting their end, which is light that was guided along their extension.

Then, using the Laser@850nm emitting at a high optical power of 150 mW, the thermal properties of the SmartCap prototypes with the uncoated optical waveguides were assessed to verify if there is any temperature increase of the optical waveguides caused by NIR radiation.

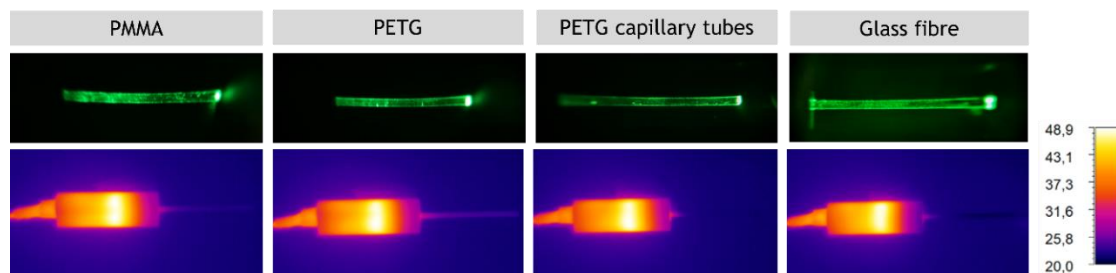


Figure 38. IR images and thermographs of SmartCap prototypes with uncoated optical waveguides irradiated from the left by the Laser@850nm. The Laser@850nm is emitting at 45 mW in the IR images and at 150 mW in the thermographs.

The thermographs in Figure 38 reveal no temperature increase in the uncoated optical waveguides, except for PETG, despite the IR images show that there is light being guided inside them and spread throughout them, meaning that light does not heat up optical waveguides, thus it is not absorbed by them. Contrarily, PETG optical waveguides absorb part of the light, generating heat. However, it is not a significant temperature rise, and therefore, in further tests, temperature rises in the GO coated optical waveguides can be attributed to the interaction between the NIR light and the GO coating.

In all thermographs, it is possible to see that the beginning of the optical waveguides slightly heats up due to the temperature of the cap in contact with it.

3.3.2 - Effect of the amount of GO on the surface of the optical waveguides

To study the effect of the amount of GO on the surface of the optical waveguide, the optical and thermal properties of SmartCap prototypes with PMMA and PETG optical waveguides coated with the two GO suspensions varying the number of dips were evaluated (Figure 39). The PETG capillary tube and glass fibre were only coated with 4 dips of the 12.5 mg/mL GO suspension, as it showed to lead to higher photothermal effects. The Laser@850nm emitting at 150 mW was used as optical source. The thermographs were acquired 15 min after turning on the optical source to allow temperature stabilization.

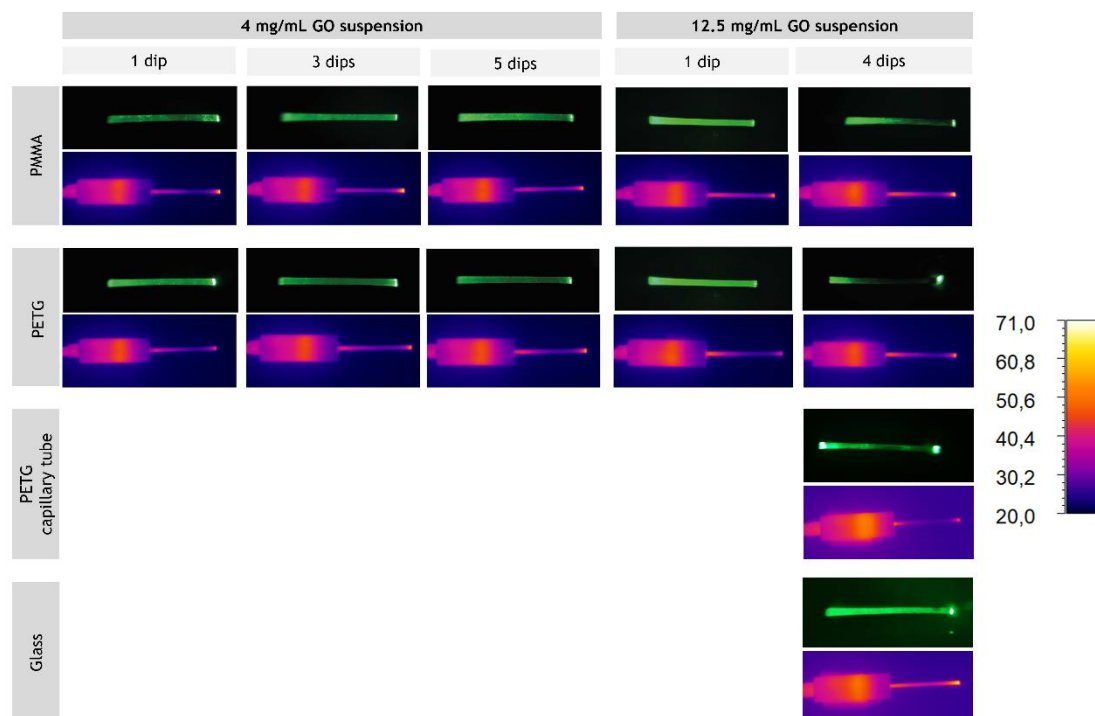


Figure 39. IR images and thermographs of SmartCap prototypes with the different optical waveguides coated with 4 mg/mL and 12.5 mg/mL GO suspensions, varying the number of dips (GO layers), irradiated from the left by the Laser@850nm emitting at 150 mW.

First, in the optical waveguides coated with the 4 mg/mL GO suspension, there is light on the surface throughout their complete (30 mm-length) GO-coated extension. However, the temperature is not uniform along the optical waveguide: there is a slight temperature increase in the beginning and a higher temperature increase in the end, being the temperature lower in the middle. For instance, in the PMMA optical waveguide coated with 5 dips of this suspension, the temperature is around 36 °C (33-38 °C) in the first 10 mm, around 31.5 °C (30-36 °C) in the following 17 mm and reaches a maximum of 57 °C (35-57 °C) in the last 3 mm. This indicates that the light interacting with GO in the middle of the optical waveguide does not promote a photothermal effect. A possible reason for that is that the light loses optical power and the optical power of the light reaching the surface at that position is not enough to cause a photothermal effect. In the optical waveguides coated with 1 dip of 12.5 mg/mL GO suspension, the same tendency was observed, with the temperature being very similar to that observed in the optical waveguides coated with 5 dips of the 4 mg/mL GO suspension.

On the contrary, in the PMMA and PETG optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension, the IR image and the thermograph are more similar to each other. Despite the temperature along the optical waveguide is similar to the others, the IR image shows less light in the middle of the waveguide. This indicates that this amount of GO absorbs all the light that is being reflected and that reaches the surface before the end of the optical waveguide. Thus, only the light being guided inside the optical waveguide reaches its end. This light either interacts with the GO deposited on the end face or is reflected backwards and

interacts with the GO deposited on the lateral surface. Comparing to the other GO-coated optical waveguides, the ones coated with 4 dips of the 12.5 mg/mL GO suspension lead to higher temperature increases both in the beginning and in the end, although a similar tendency of lower temperature in the middle is visible. For instance, in the PMMA optical waveguide, the temperature is around 43 °C (39.7-46 °C) in the first 10 mm, around 34 °C (29.5-47.3 °C) in the following 17 mm and reaches a maximum of 71 °C (48-71 °C) in the last 3 mm. The increase in the light absorption by GO and consequently in the photothermal effect with the increase in the amount of GO was also reported in literature [113], [116].

Between the optical waveguides, the PMMA filaments led to a slightly higher photothermal effect than the PETG filaments, for the different coating conditions, specially in the end of the optical waveguide. Further experimental tests performed afterwards allow for a better comparison between the two optical waveguides.

As the increase in the amount of GO on the surface showed to promote the photothermal effects, the PETG capillary tubes and the coreless glass fibre (cladding removed) were only observed with this coating. Comparing all the optical waveguides, the PETG filaments and PETG capillary tubes seem to lead to the lowest photothermal effects. Thus, among the polymeric optical waveguides, PMMA showed to promote the highest photothermal effect, reaching a maximum of 71 °C in the end, although a minimum temperature of 29 °C was observed in the middle. On the other hand, the coreless glass fibre led to a more homogeneous photothermal effect along the GO-coated optical waveguide, with the temperature varying between 37 °C (minimum temperature) in the middle and 67 °C (maximum temperature) in the end.

Overall, the obtained results showed that increasing the amount of GO on the surface of the optical waveguide, both by increasing the concentration of the GO suspension and/or the number of dips, seems to lead to higher temperature rises (photothermal effect). Thus, in subsequent tests, the optical waveguides were coated with 4 dips of the 12.5 mg/mL GO suspension. Besides, the lower propagation loss of the glass fibre and PMMA optical waveguide showed to impact positively the photothermal effect, as the light interacting with GO has higher optical power. Figure 40 shows a schematic representation of the light propagation in the optical waveguides, which helps to understand the optical and thermal properties observed.

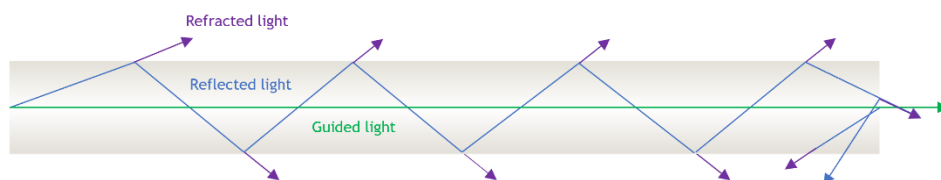


Figure 40. Light propagation in an optical waveguide.

3.3.3 - Effect of the size of the optical waveguides

To study the effect of the size of the optical waveguides in the photothermal effect, PMMA and PETG optical waveguides with different sizes were coated with 4 dips of the 12.5 mg/mL GO suspension and their optical and thermal behaviours were evaluated (Figure 41).

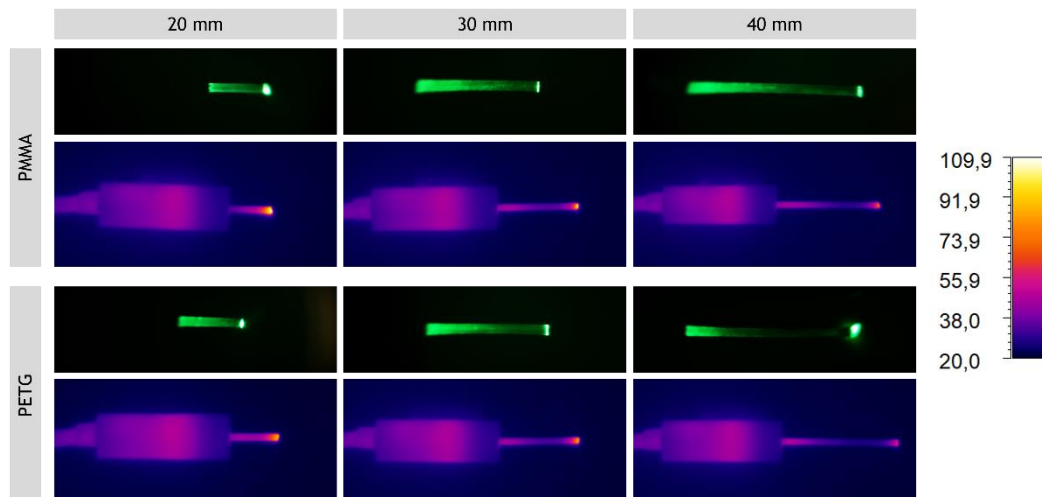


Figure 41. IR images and thermographs of SmartCap prototypes with PMMA and PETG optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension, varying the length of the optical waveguide, irradiated from the left by the Laser@850nm emitting at 150 mW.

The temperature increases more significantly in the shorter optical waveguides, particularly in their end. Those are expected results, since the light loss increases with the length and so the optical power of the light that reaches the end is lower, leading to lower photothermal effects. Particularly, in the 20 mm, 30 mm and 40 mm long PMMA optical waveguides, the temperature reaches a maximum of 109 °C, 100 °C and 71 °C in the end, respectively. Also, in the 20 mm long optical waveguides (10 mm GO-coated), there is light interacting with the GO coating through the entire optical waveguide and the temperature increases gradually along it, contrary to the 30 mm and 40 mm long optical waveguides, in which longer parts of GO do not interact with NIR light and the temperature is minimal in the middle.

Again, PMMA optical waveguides led to a higher temperature increase than the PETG optical waveguides for all lengths, both on the surface (less significant difference) and in the end (significant difference), as clear in Figure 41. This indicates that, in fact, there is higher absorption of light in the PETG optical waveguides than in the PMMA optical waveguides, since the optical power of the light reaching both the surface and the end is lower, causing lower photothermal effects.

The shorter optical waveguides have the advantage of leading to higher photothermal effects than the longer optical waveguides. However, they have lower amounts of GO exposed to the NIR light, which can eventually be disadvantageous for the photodynamic effect. It is still difficult to predict if it is better to have high power light interacting with a small amount

of GO, as in the shorter length optical waveguides, or to have lower power light interacting with a higher amount of GO, as in the longer optical waveguides in which the light is more dispersed throughout the optical waveguide. Only with further antibacterial tests using the different optical waveguides, more conclusions can be made regarding this topic. Nevertheless, an increase of the interaction of light with GO coating was attempted by increasing the light scattering along the optical waveguide through modifications. Such modifications of the optical waveguides and their effect on the physical properties of the SmartCap prototype are explored in the following subsection.

3.3.4 - Effect of the modifications of the optical waveguides

The modifications performed in the optical waveguides were firstly studied regarding their capacity of spreading the light throughout the optical waveguide. For that purpose, the optical properties of uncoated modified optical waveguides, after assembly in the cap prototypes, was evaluated. The Laser@850nm emitting at 45 mW was used as optical source. Figure 42 shows the IR images of the modified optical waveguides, comparing them to the unmodified ones.

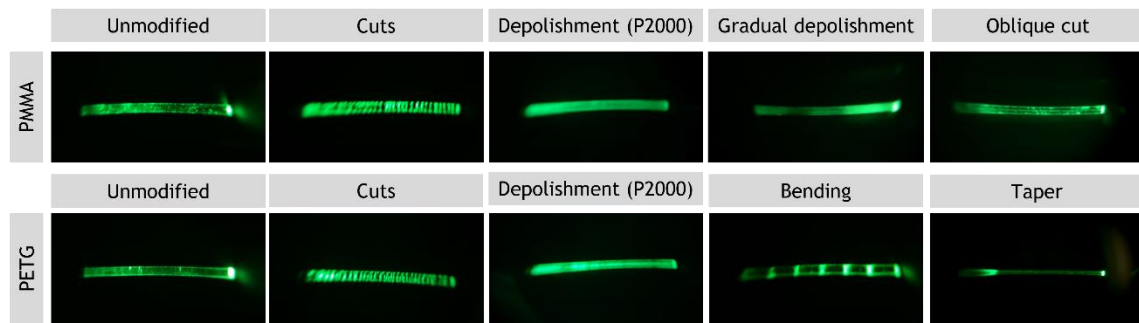


Figure 42. IR images of SmartCap prototypes with modified and uncoated PMMA and PETG optical waveguides irradiated from the left by the Laser@850nm emitting at 45 mW.

Almost all the modifications performed to the surface of the optical waveguides seem to increase the spreading of light along them, although in different ways. The depolishment with the P2000 grid sandpaper is a more uniform modification of the surface and thus allows for a continuous and more homogeneous light scattering. The gradual depolishment, on the contrary, leads to a gradual increase of the light on the surface. The scattering of light in the optical waveguides modified with cuts and bending occurs periodically where the defects are placed. The oblique cut of the initial end of the optical waveguide does not seem to have much impact on the light scattering. Finally, in the taper it is also not visible an increase in the light traveling on the surface.

All of these optical waveguides were afterwards coated with 4 dips of the 12.5 mg/mL GO suspension and their optical and thermal properties were evaluated (Figure 43).

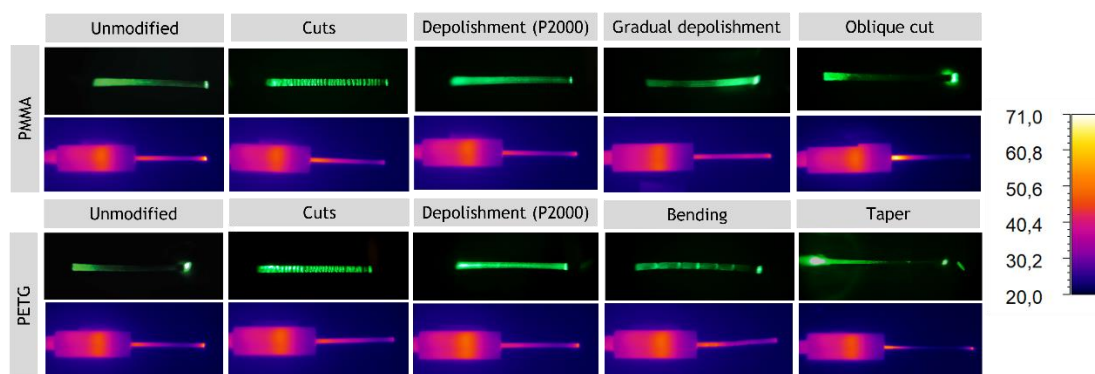


Figure 43. IR images and thermographs of SmartCap prototypes with modified PMMA and PETG optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension, irradiated from the left by the Laser@850nm emitting at 150 mW.

Overall, in the modified optical waveguides, the temperature in the end of the waveguide is lower than in the respective unmodified optical waveguide, indicating that part of the light that was being guided inside the optical waveguide is now being scattered along it, leading to a lower photothermal effect in the end.

Despite there is light reaching the surface along the whole extension of the P2000 depolished and cut optical waveguides, the thermal profile does not differ much from the unmodified ones, with a mild temperature in the beginning and a lower temperature increase in the middle. However, in the cut optical waveguides, the temperature of the coating in the beginning is slightly higher than that of the unmodified waveguides (average of 47 °C vs average of 43 °C for cut PMMA in the first 10 mm). Similarly, in the bent PETG optical waveguide, the photothermal effect is mild in the beginning but low in the rest of the length. Contrary, the gradually depolished PMMA optical waveguide presents the most homogeneous thermal profile, although with a mild temperature increase. Along this optical waveguide, the temperature ranges between 34 °C (in the middle) and 46 °C (in the beginning and in the end). In the obliquely cut PMMA optical waveguide, the light enters the optical waveguide with multiple directions and therefore all the light reaches the surface in the beginning, causing a high temperature increase there, and no light is left to interact with the rest of the GO coating. In the taper, there is also a high temperature increase in the beginning (before the diameter is reduced) and in the end. In these two latter cases (obliquely cut and taper), a longer length of the optical waveguide suffers no temperature increase at all, meaning that almost all light is absorbed in the beginning.

Overall, it seems that only the gradual depolishment of the PMMA optical waveguide contributes to the uniformity of the temperature increase along the waveguide, although the temperature is lower than that of the unmodified PMMA optical waveguide.

An experimental SmartCap prototype capable of guiding NIR light, emitted by an optical source, through a GO-coated optical waveguide was therefore developed. The interaction

between the light and the GO causes a photothermal effect, desired for the bactericidal action of the prototype. Therefore, the developed prototypes were evaluated regarding their antibacterial performance (presented and discussed next subsection).

As an additional observation, the thermographs show that the plastic cap heats up to around 49 °C, which is higher than the critical temperature tolerated by the skin (43 °C, [132]). This is caused by heating of the optical source and resistor coupled to it, which depends on the optical power of the optical source. Therefore, in the final prototype, after the conditions are optimized, particularly the optical source and its optical power, the temperature of the plastic cap must be measured and, if needed, a thermal insulation material must be added or the cap must be redesigned, so that the optical source does not contact directly with the plastic, so the external surface of the cap does not reach 43 °C.

As already mentioned, the goal of this thesis was to develop an experimental prototype that can be optimized, so it includes an optical source that connects to an external power supply. However, the final prototype will include, besides the GO-coated optical waveguide and the optical source, an integrated circuit and a battery. As it is not the focus of this thesis, more information regarding this prototype, as well as a schematic representation of it, can be found in Annex C.

4 - Antibacterial effect of the SmartCap prototype

Several antibacterial tests were performed using the SmartCap prototypes that were thought to have a potentially greater antibacterial effect. Despite being described in the text, the conditions of the antibacterial assays and the respective SmartCap prototypes are summarized in Table 6. The first six assays in the table were performed using the first setup described in Chapter 3, section 4.4, in which the GO-coated optical waveguide is immersed in an Eppendorf with the bacterial suspension, whereas the seventh was performed in the second setup, with the GO-coated optical waveguide immersed laterally in a well of a 48-well plate containing the bacterial suspension. In each assay, there are three different groups: a control of bacteria growth, consisting of a bacterial suspension left untouched, a control of GO, where the SmartCap prototype without NIR irradiation is used, and the experimental group, where the SmartCap prototype is used.

Table 6. Conditions of the antibacterial assays and the respective SmartCap prototypes.

Assay	Figure	Assay conditions		SmartCap prototypes		
		Bacterial suspension	Assay conditions	Optical source	Optical waveguide	GO coating
1	44	[2.65x10 ⁷ CFU/mL] Volume: 2 mL	Time: 6 h Temperature: 37 °C	Laser@850nm at 110 mW	PMMA unmodified 40 mm, non-sterile	4 mg/mL GO suspension
2					PETG unmodified 40 mm, non-sterile	5 dips 30 mm-coated
3	45	[0.5x10 ⁴ CFU/mL] Volume: 2 mL	Time: 2 h Temperature: 37 °C	Laser@850nm at 150 mW	PMMA unmodified 40 mm, non-sterile	12.5 mg/mL GO suspension
4					PMMA gradually depolished 40 mm, non-sterile	4 dips 30 mm-coated
5	46	[0.5x10 ⁴ CFU/mL] Volume: 1.75 mL	Time: 2 h Temperature: 37 °C	Laser@850nm at 150 mW	PMMA unmodified 20 mm, non-sterile	12.5 mg/mL GO suspension
6					PMMA unmodified 20 mm, sterile	4 dips 10 mm-coated
7	47	[0.5x10 ⁴ CFU/mL] Volume: 300 µL	Time: 2 h, 4 h Temperature: 37 °C	Laser@850nm at 150 mW	PMMA unmodified 17 mm, sterile	12.5 mg/mL GO suspension 4 dips 7 mm-coated

In the first two antibacterial tests, SmartCap prototypes with PETG and PMMA optical waveguides (unmodified and 40 mm-length) coated with 5 dips of the 4 mg/mL GO suspension were evaluated. These SmartCap prototypes, as previously discussed, presented a continuous and uniform light scattering along the optical waveguide, thus having light interacting with the GO in their 30 mm-length GO-coated portion (Figure 39). Figure 44 shows the results of these antibacterial assays.

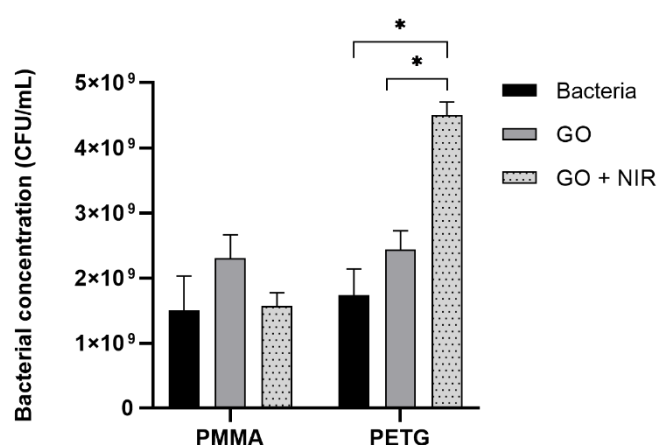


Figure 44. Antibacterial action of SmartCap prototypes composed of Laser@850nm at 110 mW and non-sterile PMMA and PETG optical waveguides coated with 5 dips of the 4 mg/mL GO suspension (30 of 40 mm length). * Statistical significance, $p < 0.05$: unpaired t-test with Welch's correction.

The results in Figure 44 indicate that the SmartCap prototypes tested have no bactericidal effect, as no decrease in the bacterial concentration was observed in the experimental group (GO + NIR) compared to the bacteria control (Bacteria). On the contrary, an increase in the bacterial concentration occurred in the SmartCap prototype with the PETG optical waveguide. This indicates that the photothermal effect of the SmartCap prototypes is not enough to heat the suspension up to a critical temperature for bacteria. Similarly, the photodynamic effect, if exists, is also not enough to damage bacteria by oxidative stress.

In the next antibacterial assays (Figure 45), the concentration of the bacterial suspension was reduced to 0.5×10^4 CFU/mL, maintaining the same volume, and the time of the assay was reduced to 2 h. Both these changes avoid an excessive growth of bacteria that could be preventing the inhibitory or bactericidal effect of the prototype from being seen. The two prototypes tested differ in the optical waveguide, as one uses an unmodified PMMA optical waveguide and the other uses a gradually depolished PMMA optical waveguide. Both optical waveguides were coated with 4 dips of the 12.5 mg/mL GO suspension, since it revealed to promote the photothermal effect. The Laser@850nm emitting at 150 mW was used as optical source. The unmodified PMMA optical waveguide showed to have a higher photothermal effect, with a high temperature increase in its end (Figure 39), on the other hand, the gradually depolished PMMA optical waveguide showed a more homogeneous temperature rise (Figure 43), having NIR light interacting with the GO coating along the 30 mm length. The results of these assays are shown in Figure 45.

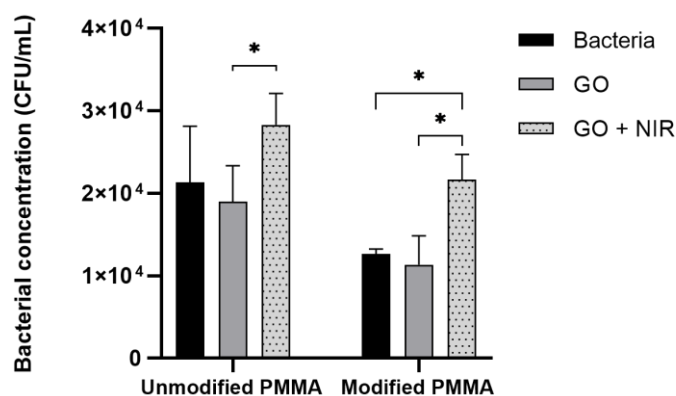


Figure 45. Antibacterial action of SmartCap prototypes composed of Laser@850nm at 150 mW and non-sterile PMMA unmodified and gradually depolished optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension (30 of 40 mm length). * Statistical significance, $p < 0.05$: unpaired t-test with Welch's correction.

Similarly to the previous assays, no decrease in bacterial concentration was observed, indicating that none of the SmartCap prototypes had bactericidal action. Again, in one of the assays, namely with the SmartCap prototype with the gradually depolished PMMA (Modified PMMA) optical waveguide, the bacterial growth appears to have been promoted, as there was a significant increase in the bacterial concentration compared to the bacteria control.

Subsequently, prototypes with 20 mm-length PMMA optical waveguides were tested as they showed to have the highest photothermal effect, reaching temperatures above 100 °C in the end of the optical waveguide (Figure 41). The GO coating and the optical source were maintained as the previous assay. In one of the assays, the GO-coated optical waveguide was previously sterilized with EtO.

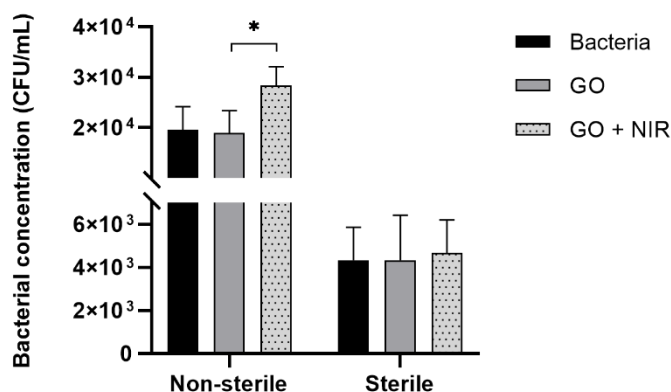


Figure 46. Antibacterial action of SmartCap prototypes composed of Laser@850nm at 150 mW and non-sterile and sterile PMMA unmodified optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension (10 of 20 mm length). * Statistical significance, $p < 0.05$: unpaired t-test with Welch's correction.

As before, the tested prototypes showed no antibacterial action, as no differences were observed between the bacteria control and the SmartCap group in both the assays. Even these prototypes that reach 100 °C, in dry conditions, are insufficient to heat the bacterial suspension around them to temperatures that cause bacteria damage or death. The photothermal effect of the SmartCap prototype used in this assay on the temperature of the supernatant was evaluated in a separate assay. The setup was mounted, as in the bacterial assay, and 1.75 mL of TSB (without bacteria) were added to the eppendorf. The temperature of the TSB was measured using a thermocouple immersed in the TSB. During this test the system was kept in the oven at 37 °C, to replicate the conditions of the antibacterial assay. No temperature increase was observed, and the temperature of the supernatant remained around 36 °C over time, up to 30 min, which is not enough to cause damage in bacteria.

Since none of the most promising prototypes was showing bactericidal action, the experimental setup of the antibacterial assay was changed to replicate the antibacterial assays performed with the graphene films by P. Henriques et al. [118] where high antibacterial effect was observed. The aim was to understand if the lack of bactericidal effect of the SmartCap prototypes was exclusively related to the prototypes themselves, or if it was related to the assay's conditions, namely the volume of bacterial suspension in which the GO-coated optical waveguide was immersed. The results of this assay are presented in Figure 47.

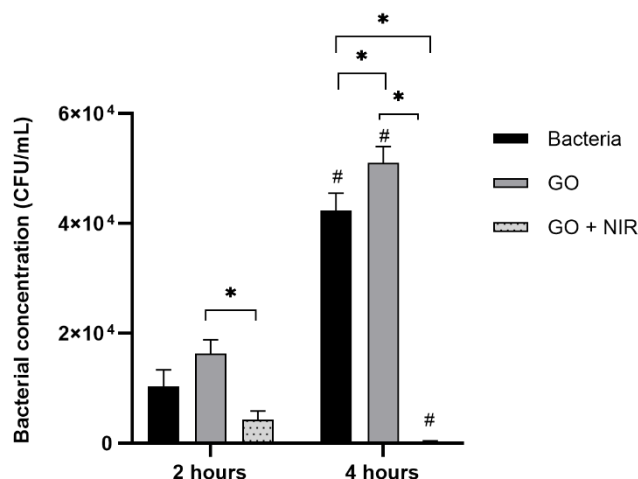


Figure 47. Antibacterial action after 2 h and 4h of SmartCap composed of Laser@850nm at 150 mW and PMMA optical waveguide coated with 4 dips of the 12.5 mg/mL GO suspension (7 of 17 mm). *, # Statistical significance, $p < 0.05$: unpaired t-test with Welch's correction to compare (*) conditions within the same timepoint and (#) same condition in different time points.

The SmartCap prototype tested showed to have an antibacterial action against the bacteria in the supernatant, which increased with the time of irradiation. Compared to the control of bacteria of each time point, the SmartCap prototype promoted a reduction in bacterial concentration of 58.1% and 99.4% after 2 h and 4 h, respectively, despite with no statistical significance in the first. On the contrary, bacteria in contact with non-irradiated GO grew when compared with the control of bacteria, which means that the reduction of bacteria is attributed exclusively to the combination of GO with NIR light.

The obtained results suggest that in the first antibacterial setup the volume of the bacterial suspension was too high for the prototypes to heat it up to a damaging temperature for bacteria. To confirm this, the photothermal effect of the SmartCap prototype used in this assay on the temperature of the supernatant was evaluated in a separate assay, similarly to before. The setup was mounted, as in the antibacterial assay, and 300 μ L of TSB (without bacteria) were added to the well. Thermographs were acquired over time, namely 0, 15 and 30 min after turning the Laser@850nm on. During this test, the system was kept in the oven at 37 $^{\circ}$ C, to replicate the conditions of the antibacterial assay, and was only taken out for thermograph acquisition. Figure 48 shows the acquired thermographs with a range of temperatures of 33-40 $^{\circ}$ C. After 15 min, the maximum temperature of 40 $^{\circ}$ C was reached, near the end of the GO-coated optical waveguide, and it did not increase with time. However, with time the temperature gets more uniform, as it increases in the rest of the TSB. After 15 min, the temperature ranges between 34-40 $^{\circ}$ C and after 30 min between 35-40 $^{\circ}$ C. Compared to the first setup used in the antibacterial assays, in this one there is a mild temperature increase in the supernatant, enough to increase the membrane permeability of bacteria.

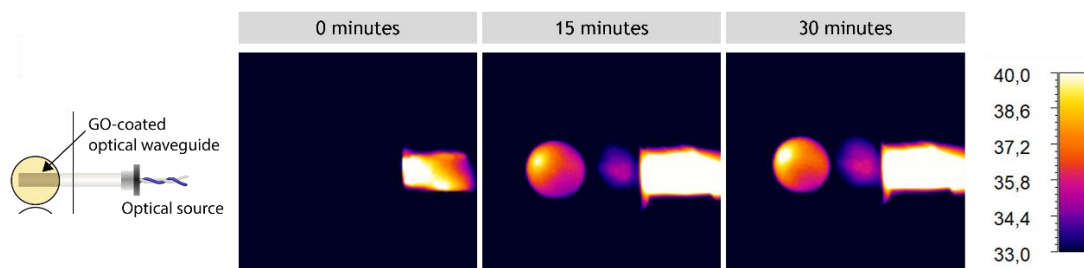


Figure 48. Thermographs of the well where the GO-coated optical waveguide is immersed in 300µL of TSB. The optical waveguide is irradiated from the right by the Laser@850nm emitting at 150mW. Schematic representation on the left.

In addition to the photothermal effect, a photodynamic effect is expected to be occurring, allowing for a synergistic effect, as shown by P. Henriques et al. [118]. Therefore, in future tests, it is important to quantify the oxidative stress in bacteria, measured by the glutathione levels and reactive-oxygen species generation, to estimate the contribution of the photodynamic effect to bacteria elimination.

Chapter 5

Conclusions and Future Work

1 - Conclusions

In this work, SmartCap prototypes were developed and their physical and antibacterial properties were evaluated.

Several optical devices, namely optical sources and optical waveguides, were explored. As optical sources, a laser diode (Laser@850nm) and a LED (LED@860nm) were characterized. The Laser@850nm emits monochromatic light at 851 nm, whereas the LED@860nm emits polychromatic light with a peak wavelength of 861 nm and a full width at half maximum of 30 nm. The optical power of the Laser@850nm is approximately the double of that of the LED@860nm (160 mW and 80 mW, respectively, for an applied voltage of 3.32 V). As optical waveguides, PMMA and PETG solid filaments, PETG capillary tubes and glass fibre were explored. Among the polymeric optical waveguides, PMMA filaments presents lower propagation losses (0.18 dB/cm), followed by PETG filaments (0.42 dB/cm) and PETG capillary tubes (0.96 dB/cm), which are significantly higher than the losses of the glass fibre (12 dB/km).

The optical waveguides were coated by the dip coating method, and two GO suspensions (4 mg/mL and 12.5 mg/mL) were used. A homogeneous coating of $\sim 1.95 \pm 0.12$ mg was achieved with 4 dips of the 12 mg/mL GO suspension. The morphology of this GO coating, assessed by SEM, showed a wrinkled surface, typical of GO films. The GO-coated optical waveguides reveal to be hydrophilic (contact angle of $\sim 51^\circ$), although the contact angle increases after EtO sterilization ($\sim 78^\circ$) and NIR irradiation ($\sim 68^\circ$). Preliminary tests showed that coating stability increases when the coating is dried at 60 °C and after EtO sterilization and NIR irradiation.

Plastic cap prototypes, with a thread compatible with commercial hemodialysis catheters, were designed by CAD software and produced by 3D printing. The cap couples the optical source to the optical waveguide, which is irradiated. The interaction of NIR light with the GO coated

on the surface of the optical waveguide promotes a photothermal effect, which increases with the amount of GO and optical power, being the latter achieved with shorter waveguides.

A SmartCap prototype consisting of the Laser@850nm emitting at 150 mW and a 17 mm long PMMA optical waveguide coated (7 mm) with 4 dips of the 12.5 mg/mL GO suspension showed to have bactericidal action against *S. epidermidis*, killing 99.4% of the bacteria in the supernatant after 4 h.

Overall, the results of this thesis show that it is possible to transfer the technology that refers GO as a photosensitizer for phototherapy into a medical device for antibacterial purposes. Besides, the work herein reported show that SmartCap has a scalable production process.

2 - Future work

Despite the promising results achieved with the experimental SmartCap prototypes developed in this thesis, some important work still needs to be done so a more complete and fully functioning prototype can be developed. Future work includes:

- i) Exploring optical sources, preferably LEDs, with lower wavelengths aiming to decrease the optical power needed. LEDs are preferred over lasers because they are low cost and more robust devices. The optical power of the optical source will determine the durability of the battery. Higher optical powers require higher currents and decrease the durability of the battery. Therefore, the reduction of the optical power needed is critical for the final application in SmartCap.
- ii) Exploring different geometries for the optical waveguides to increase the light travelling on the surface and improve the prototype's thermal properties, using a lower optical power. As explained in the previous point, reducing the optical power required to have sufficient thermal and antibacterial effects increases the durability of the battery, and consequently the durability of the SmartCap.
- iii) Validating the coating stability under international standards (e.g., ISO 10993-13). Despite the preliminary test performed in this work gives an idea regarding the coating stability and the conditions that increases it, further tests are required to quantify the release of GO from the surface. Particularly, ISO 10993-13 allows to identify and quantify degradation products from polymeric medical devices. It consists in a first accelerated degradation test in which the device is immersed in a solution, similar to the intended use, for a certain period of time (>60 days) under a certain temperature (>37 °C), and a second real time test, similar to the first, but

with lower temperature. The degradation products are quantified by the mass, after separation from the solution.

- iv) Performing antibacterial assays in hemodialysis catheters and validating the antibacterial action of the SmartCap prototype.

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Annexes

A - 3D printed supports

In the experimental setup for the IR images and photographs acquisition of the SmartCap prototype, two plastic supports, produced by 3D printing, were used. The following Figure 49 and Figure 50 show those supports.

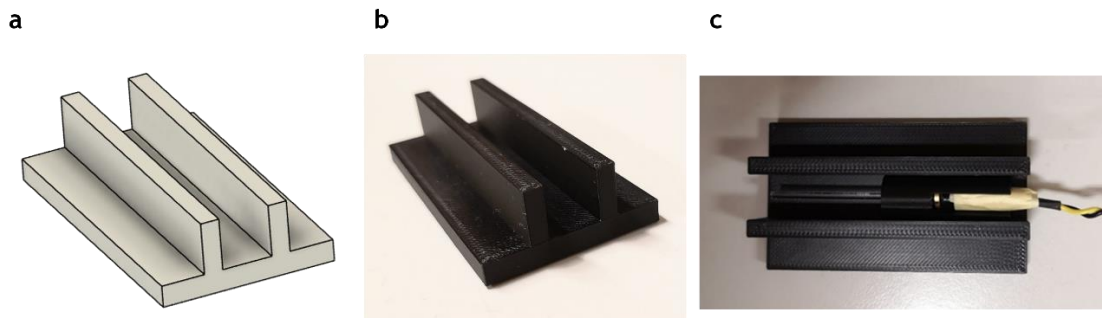


Figure 49. Support for the SmartCap prototype. (a) CAD design. (b) 3D printed support. (c) SmartCap prototype on the support.

To ensure a static position of the SmartCap prototype during the IR images acquisition, the support represented in Figure 49 was used. This support is a simple piece consisting of a rectangular platform with two rectangular elevations 11.5 mm apart to ensure a tight fit of the prototype (the plastic cap has an outer diameter of 11 mm) between them. The dimensions of the support are 70 mm in length, 40 mm in width and 17 mm in height.

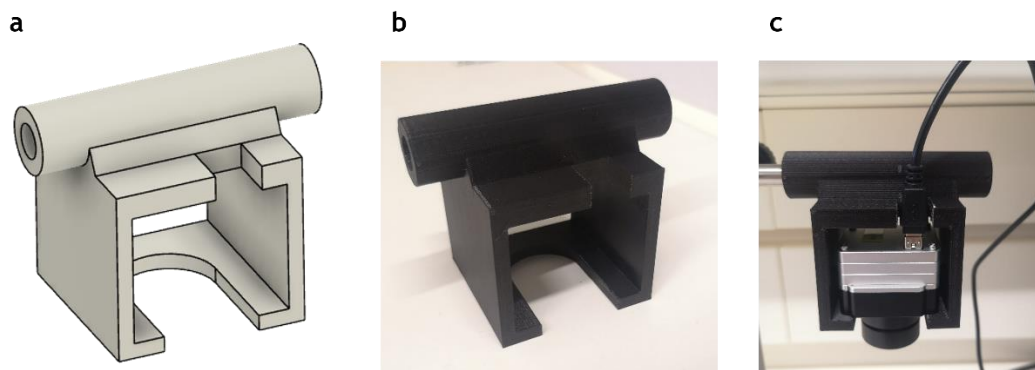


Figure 50. Support for the thermal camera. (a) CAD design. (b) 3D printed support. (c) Thermal camera on the support.

To hold the thermal camera in a static position above the SmartCap prototype during the thermal images acquisition, the support represented in Figure 50 was used, in combination with a general laboratory metalware support where it is placed. Despite being a single piece, the support consists of two main parts: one to fit into the vertical support and the other to hold the thermal camera. The first part is a cylindric tube to fit the other support. Its dimensions are 10mm and 20 mm in inner and outer diameter, respectively, and 90 mm in lateral length. The second part is a hollow cube with a curve opening in the bottom face where the camera is placed with the lens facing down. This part has a small cut on the top face to allow the thermal camera cable to fit the support. The dimensions of the cubic part are 50 mm in length, 60 mm in width and 55 mm in height. The diameter of the bottom opening is 30.5 mm.

B - SEM images of GO-coated optical waveguides after sterilization and irradiation

SEM images of the GO-coated optical waveguides after EtO sterilization and NIR irradiation were acquired in different positions of their extension: first 10 mm (beginning), second 10 mm (middle) and last 10 mm (end). Figure 51 shows the SEM images with magnifications of 500x, 2000x and 5000x.

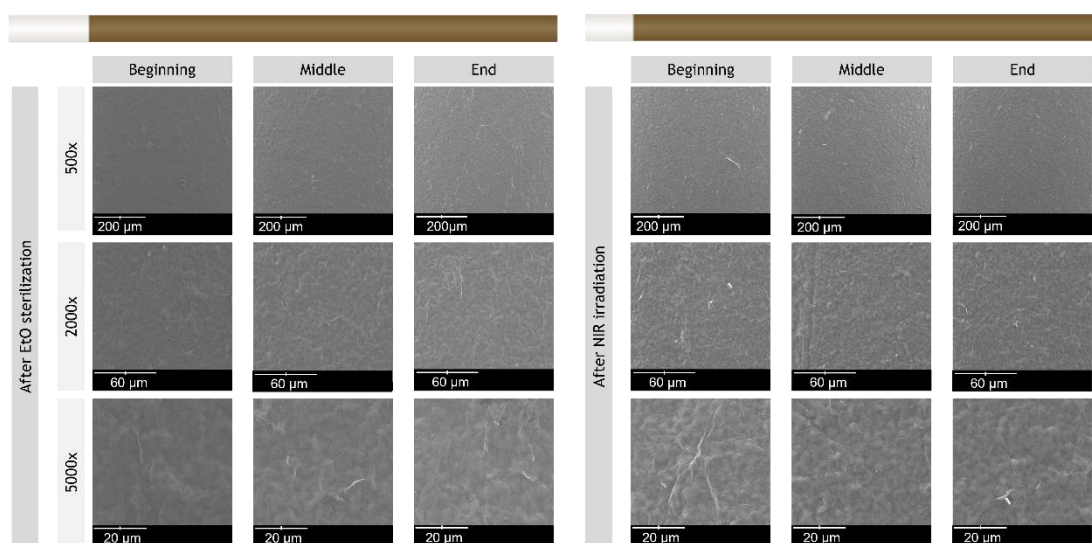


Figure 51. SEM images of optical waveguides coated with 4 dips of the 12.5 mg/mL GO suspension after EtO sterilization and NIR irradiation, in the beginning, middle and end of the optical waveguides, with magnifications of 500x, 2000x and 5000x.

The morphology of GO coating does not change along the optical waveguide in any of the conditions (after EtO sterilization and after NIR irradiation). Therefore, the heterogeneity of the optical waveguide cannot be related to the surface morphology, as discussed in Chapter 4, subsection 2.3.1.

C - SmartCap prototype

The SmartCap is a graphene-based light-activated cap for hemodialysis catheter hub sterilization. Therefore, it consists of a plastic cap, compatible with commercial hemodialysis catheter, in which an optical source is coupled to a GO-coated optical waveguide that extends into catheter hub, a chip with an integrated circuit and other electronic components and a battery. Additionally, it has a signaling LED and a switch or button. Figure 1 (Chapter 1, section 1) shows a schematic representation of all components of the SmartCap, which will be included inside the plastic cap.

The integrated circuit chip includes a microcontroller Tiny85 20-Pu Dip-8 Atmel Original, with a processor of 8 bits and 20MHz of frequency, that will be programmed to control the time of action of the system. As the other components, the battery is still being studied and optimized, however it should fill some requirements, including to provide enough current to the optical source and have a high capacity to last for a satisfactory amount of time for the application.

Finally, the switch or button will be used to turn on the optical source that illuminates the GO-coated optical waveguide, and the signaling LED indicates when the optical source is turned on, so when the SmartCap is operating.