Light4Lungs

Inhalable Aerosol Light Source For Controlling Drug-resistant Bacterial Lung Infections

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Description of the deliverable (3-5 lines)	The document describes the required chemophysical, optical, and photophysical properties of the light-emitting particles and the aerosol, as well as the method to obtain the action spectrum for bacterial photokilling.
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DEFINITIONS & ACRONYMS

LEP – Light-emitting particle

PDT – Photodynamic therapy

ROS – Reactive Oxygen Species

PS – Photosensitiser

UVA – Ultraviolet A

ZrO2 – Zirconium oxide

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EXECUTIVE SUMMARY

This report is the second deliverable of the Light4Lungs work package WP1, Selection and development of models. The Light4Lungs project is funded by the EU's Horizon 2020 Programme under Grant Agreement number 863102. The purpose of this deliverable is to describe the aerosol required chemo- and photo-physical properties and includes the method that will be used to obtain the action spectrum for bacterial photokilling.

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1 INTRODUCTION

1.1 Project Overview

The FET Open project entitled **Inhalable Aerosol Light Source for Controlling Drug-Resistant Bacterial Lung Infections** (with acronym **Light4Lungs** and Grant agreement ID: 863102) proposes a novel approach to address the problem of antimicrobial resistance in the treatment of chronic lung infections, which are the leading cause of morbidity and mortality in patients with diseases such as cystic fibrosis and hospital-acquired lung infections. The goal is to develop a novel therapeutic scheme for the treatment of the infections, replacing antibiotics by inhalable light sources that will excite bacterial endogenous photosensitizers (e.g., iron-free porphyrins), eliminating the pathogenic bacteria by the photodynamic effect (local production of cytotoxic reactive oxygen species by the combined action of light, a photosensitiser and oxygen) irrespective of its multidrug resistance profile. The aim is to have a safe treatment for the host tissue thanks to its lack of self-photosensitising ability.

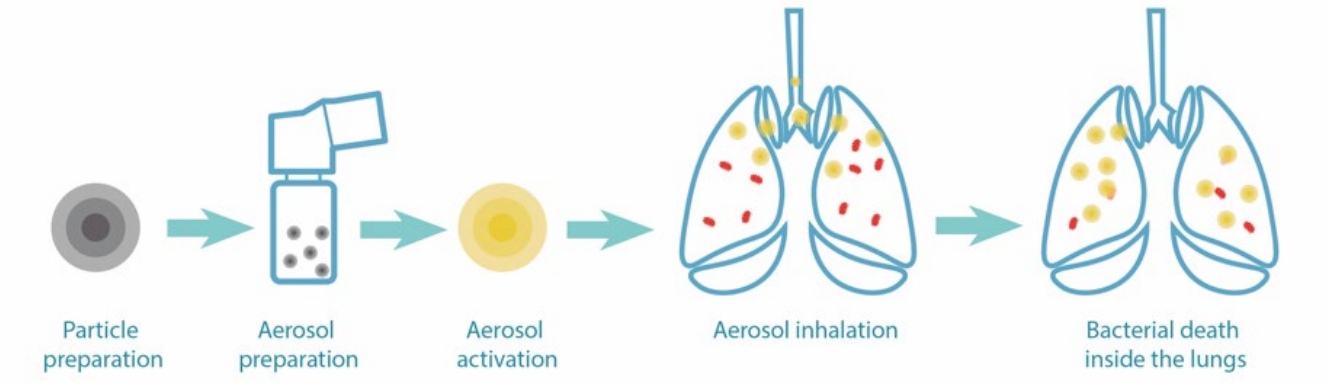

Figure 1. The Light4Lungs 5-step concept

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This project is carried out by an international consortium formed by the following institutions:

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2. UNIVERSITA DEGLI STUDI DI FIRENZE (UNIFI)
3. SORBONNE UNIVERSITE (SU)
4. FUNDACIO PRIVADA INSTITUT CATALA D'INVESTIGACIO QUIMICA (ICIQ)
5. UNIVERSITA DEGLI STUDI DI PADOVA (UNIPD)
6. CARDIFF SCINTIGRAPHICS LIMITED (CSL)
7. THE UNIVERSITY OF LIVERPOOL (UNILIV)
8. WEDO PROJECT INTELLIGENCE MADE EASY SL (WeDo)

1.2 Light-Emitting Particles and Aerosol

Development of light-emitting particles (LEPs) with persistent luminescence is the core of the project. Delivery of LEPs to the lungs is expected to achieve extensive photodynamic killing of the bacteria responsible for the infection, notably without invasive illumination devices or drugs. To achieve this goal, LEPs must meet a number of requirements concerning their optical, photophysical and chemo-physical properties, will be outlined in the following sections. Equally important is the delivery of the LEPs to the lungs, which will be achieved using aerosol technologies. The requirements for such aerosol delivery systems are also outlined in the sections below.

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2 REQUIRED OPTICAL AND PHOTOPHYSICAL PROPERTIES

The light-delivery scheme envisaged in the Light4Lungs project is based on aerosol particle light emission by phosphorescence; this corresponds to a delayed light emission consequent to particle excitation outside of the body by an appropriate external light source. This implicitly defines the presence of: (i) a "Light Emitting Particle" (LEP) to be first excited prior to its aerosolization and delivery into the relevant lung airway regions and (ii) an external excitation source for LEPs, besides the setup to produce the aerosol. We can therefore define *Light 1* as the external excitation light and *Light 2* as the emitted light by phosphorescence, which is responsible for porphyrin activation in the bacteria triggering the photodynamic effect.

To optimize the photodynamic efficacy, we have to optimize *Light 2* characteristics, which in turn depends upon optimization of *Light 1* properties.

2.1 Excitation Spectrum of the Light-Emitting Particles

According to the scheme depicted in Figure 2, the LEPs must be excited to start emitting light, and this must be done immediately prior to delivery to the lungs. The ideal excitation spectrum for LEPs is such as to maximize the aerosol emitted radiant power (*Light 2*) per unit aerosol volume. This corresponds to maximizing the emission of a single LEP, unless non negligible aerosol self-absorption is present. This effect will be reduced as much as possible by minimizing LEP self-absorption and possible optical absorption by aerosol excipients. In the general case of non-negligible aerosol self-absorption, the best LEP excitation spectrum will be defined by maximizing the product between LEPs absorption spectrum and aerosol absorbance. The expected range for the excitation spectrum of the LEPs is in the UVA-violet region, as the bacterial porphyrin absorption peak is at around 400-410nm.

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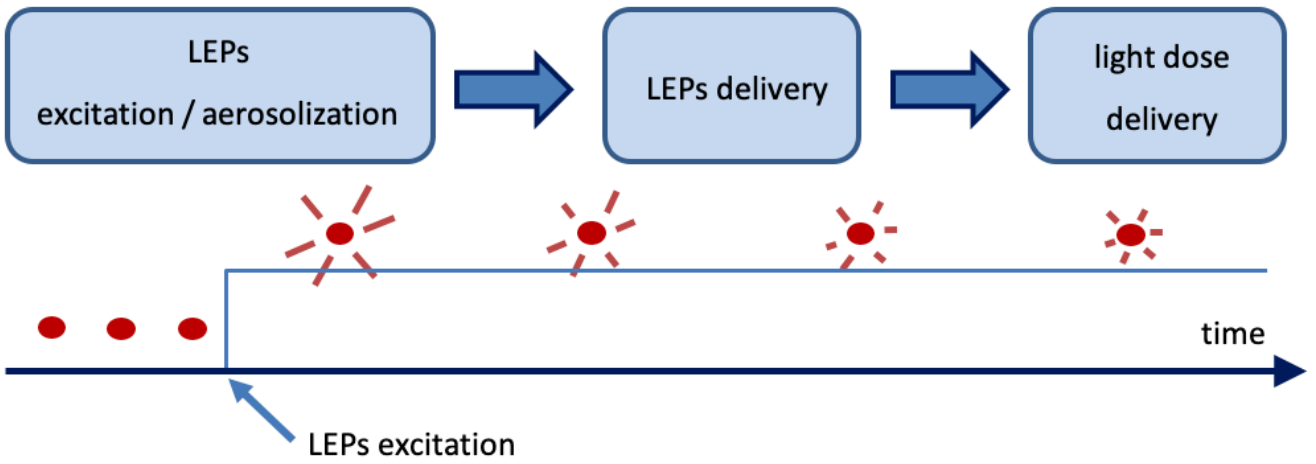

Figure 2: schematic view of the timeline leading to light delivery in the lungs

Therefore, the excitation spectrum (*Light 1* spectrum) will have to respond to the following characteristics:

- match the absorption spectrum of the LEPs, i.e. being peaked in correspondence to the absorption spectrum peak(s) of the LEPs. According to the LEPs absorption characteristics, this might result in the best choice being a monochromatic light source peaked at the LEP absorption main peak.
- be either highly focalized (e.g. laser) or diffuse (e.g. LED or lamp), depending on the final method used to excite the LEPs prior to their inhalation: the first solution (focalized) will be considered if excitation is performed on aerosolized LEPs passing through the aerosol-delivery setup and immediately before their delivery to the lungs; the second solution (LED / lamp) will be used if excitation is performed directly in the chamber containing the LEPs and prior to their aerosolization and successive delivery.
- be as much intense as possible in terms of radiant power emission (P), with the further possibility to tune P from a minimum to a maximum level. This is due to the necessity of exciting in principle all the available LEPs that will be included in the inhaled aerosol volume, taking into account: (i) their absorption efficiency (quantum yield) and (ii) the possible self-absorption by the aerosol itself during the excitation phase with *Light 1*. Generally speaking, *a priori* calculation of the needed emitted power and geometry for the excitation source is possible; nevertheless, the number of parameters upon which it depends

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undermine the real utility of this calculation, unless the chemo-physical properties of the LEPs are known and the final aerosol type and aerosolization method have been defined.

2.2 Emission Spectrum of the Light-Emitting Particles

As stated in 2.1, light - activated LEPs will in turn emit light, whose spectral characteristics need to maximize the photodynamic effect, i.e. bacteria photokilling mediated by endogenous photosensitizer absorption.

In Figure 3 the general principles of PDT are resumed. The three actors are (i) light; (ii) a photosensitizing molecule (photosensitizer: PS) and (iii) molecular oxygen. When the PS molecule is excited by light, its de-excitation in the presence of O_2 can lead to the formation of Reactive Oxygen Species (ROS) inside the target cell, leading to its death if the number or ROS produced exceeds a given threshold. In the case of the Light4Lungs project, "light" corresponds to the LEPs phosphorescent photons, while the PS is represented by the endogenous bacterial porphyrins. As the final products of the photoreactions (ROS) are proportional to the irradiating photons, → effect proportional to the dose –

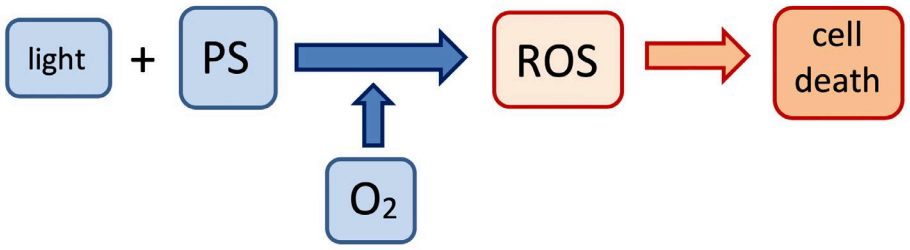

Figure 3: scheme depicting the working principle of PDT

LEPs emission spectrum can be characterized by its intensity and shape, corresponding to the normalized intensity profile. Detailed considerations about spectrum intensity are presented in section 2.5, while details about spectrum shape requirements are discussed in section 2.3. In this context, the general requirements on the LEPs emission spectrum are the following:

a) It should maximize bacterial porphyrin excitation, taking into account the effects of light scattering and absorption due to the presence of the surrounding tissue(s) and the *biological medium* (R. Giovannetti

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et al., 2008; S. T. Flock et al., 1992) where bacteria live (corresponding to biofilm and mucous layer in the case of lung infections).

b) It should minimize aerosol self-absorption, due to LEPs and/or other aerosol components' absorption

Requirements a) and b) are strictly linked to the concept of action spectrum (see 2.3) and are explained by Figure 3. The difference between the PS absorption spectrum and action spectrum is represented by the scheme in Figure 4: in case A the required emission spectrum coincides with porphyrin absorption spectrum; case B) takes into consideration the presence of e.g. a tissue ("biological medium") interacting with the light emitted by LEPs before reaching the target; case C) is the most comprehensive model, considering also the filtering effect due to the necessity of the emitted photon to exit from the aerosol itself, which acts again like a filter. The filtering action can be taken account of by dividing the porphyrin absorption by the extinction spectrum of the filter(s), accounting for absorption and scattering coupling.

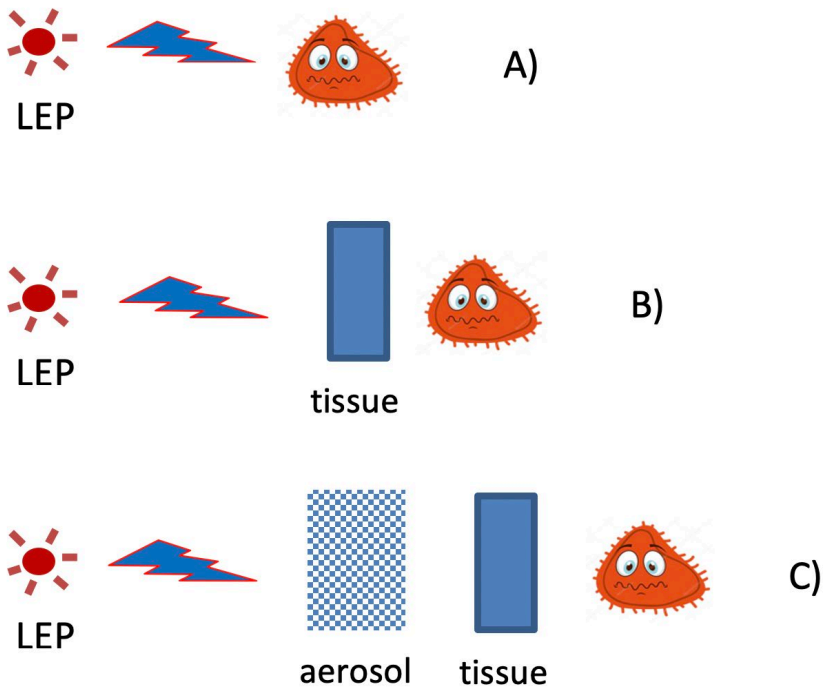

Figure 4: modelling of the contribution of the aerosol and relevant biological media to the definition of the best LEPs emission spectrum characteristics.

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c) Finally, i should minimize possible photodamage to the healthy tissue. This is linked to the possible presence of a residual UVA light component in the LEPs emission.

2.3 Method to Obtain the Photokilling Action Spectrum

The experimental action spectrum for *in vivo* bacterial photokilling will be represented by a normalized curve depicting the relative efficacy of the various visible wavelengths to perform antibacterial photokilling (Figure 5). As such, the action spectrum is intrinsically dependent on the specific conditions to which it refers, i.e. *in vitro* (planktonic bacterial cultures / biofilm) or *in vivo*. The main interest of the Consortium is to define and measure the photokilling action spectrum in *in vivo* conditions, in accordance to the final model developed and studied in the Light4Lungs project.

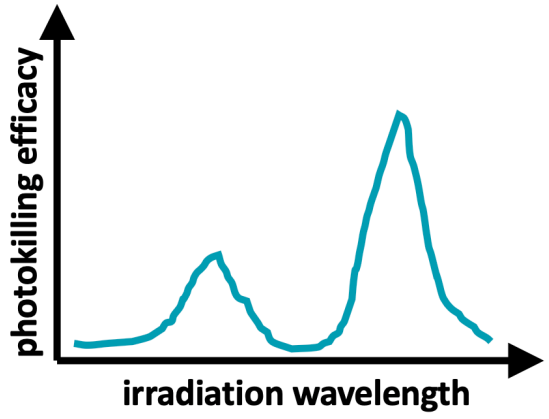

Figure 5. Picture depicting the action spectrum curve

The procedure to obtain the *in vivo* action spectrum S (λ) can be divided into 2 steps leading to its increasingly accurate determination:

1) semi-theoretical determination of a first estimate of the *in vivo* photokilling action spectrum

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This will follow the methodology present in other studies performed by the UNIFI partner (Gnerucci 2020) and will be composed by the following steps:

- Identification of the affected lung districts and analysis of the pathological parameters

- Geometric and optical modelling of the interaction between light and the infected lung districts, by considering literature experimental data for the relevant biological media optical data (i.e. mucous, biofilm, the relevant tissues of the lung airways, bacteria themselves).

- reduction of the problem to a 1-dimension only model and relative Monte Carlo simulations to obtain T (λ) = transmittance of the relevant *biological media*

- expected action spectrum: $S_1(\lambda) = T(\lambda)\, \epsilon(\lambda)\, \phi(\lambda)$

where $\epsilon(\lambda)$= bacterial porphyrin extinction coefficient and $\phi(\lambda)$ = porphyrin quantum yield for ROS production

2) final experimental determination of the *in vivo* photokilling action spectrum from *in vitro* experiments

To perform this step, results in 1) will guide and inform the choice of the best wavelengths to be used in *in vitro* experiments, as depicted in the following:

- perform *in vitro* photokilling experiments, first with LED sources then with the aerosol, to obtain the *in vitro* photokilling efficacy vs wavelength curves in different experimental conditions i.e. (i) with the chosen strains of *P. aeruginosa* / *S. aureus*; (ii) in the presence of planktonic culture / biofilm; (iii) with at least 4 different visible wavelengths, to be chosen according to the results obtained in step 1) and including both more effective and less effectives wavelengths.

- merging previous data with experimental data regarding the optical transmittance of the relevant biological media, i.e. mucous/biofilm layer and lung airway tissue. Data will be obtained both from the literature (where applicable) and also experimentally.

This step will determine S_2 (λ) as:

final action spectrum = S_2 (λ) = (*in vitro* photokilling efficacy) x (experimental transmittance data)

This result will guide the final choice of the best aerosol emission spectrum.

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2.4 Modelling of Therapeutic Photon Delivery

Given an initial number N_0 of excited LEPs (with $N_0 >> 1$), the number of emitted phosphorescence photons at time t is assumed to be represented by the formula:

$N(t) = N_0 (1 – \exp(-t/\tau))$,

where τ is the phosphorescence decay time constant, i.e., in every timespan τ, the number of excited LEPs decreases of a factor "e". In the general perspective of delivering the aerosol (i.e. light) through successive inhalations, be it V the aerosol volume inhaled in one single breath, so that the above-described model can be applied to the excited LEPs contained in the single-breath volume V.

In this hypothesis, we can develop a photon-delivery model in the lungs, which can inform us about the best decay time for LEPs phosphorescence, once all the other variables have been defined. Even more, the same model can be used to understand the numerical dependence of the delivered number of photons on the "problem variables", as illustrated below.

Even if this model can be implemented with additional parameters, nevertheless it gives a first estimate that will have to be refined only once experimental data will be available from the Consortium. Thus, the model for photon delivery is as follows:

a) At time t=0 a volume V containing a number N_0 of LEPs is excited out of the body. with negligible duration for excitation.

b) The aerosol begins to emit light immediately. The emission follows a monoexponential decay law (see above) with time constant τ, which depends on the LEPs chemo- and photophysical characteristics.

c) At time $t=t_i$, the excited aerosol volume V reaches the desired lung districts, where it remains for a timespan Δt, during which it delivers a number N* of therapeutic photons.

d) Light emitted before time t_i corresponds to a lost photon dose. The t_i parameter depends on the method for both LPEs excitation and aerosolization. We will endeavour to minimize it to ensure the fullest possible use of the LEPs illumination potential.

e) At time $t= t_i + \Delta t$, all the inhaled aerosol volume V is exhaled, so that no more photons are delivered to the lungs after this time. For the purpose of modelling, this includes the case $\Delta t = +\infty$, corresponding to the LEPs remaining inside the lung airways (no LEPs in the actual exhaled volume).

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f) Reasonable *a priori* values of the model parameters, based on previous knowledge of the aerosolization/excitation/delivery processes and literature data, are: τ = 2-100s; t_i = 0.5-2s; Δt = 5-15s (10s is a reasonable value in real clinical cases).

Using these hypotheses, we can calculate the number of therapeutic photons N* delivered during the lungs residence time Δt. Starting from the equation for N(t) we have:

$N^* = N(t_i + \Delta t) - N(t_i)$,

which gives $N^*(t_i, \Delta t, \tau) = N_0 \exp(-t_i / \tau)(1 - \exp(-\Delta t / \tau))$.

The results of the simulations are shown in Figure 6.

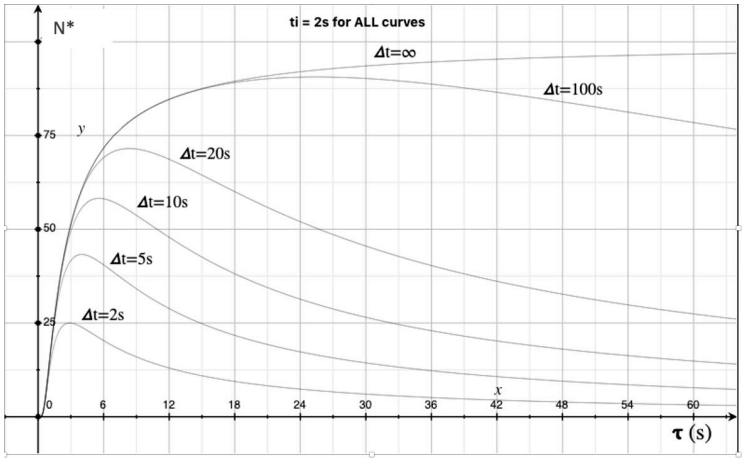

Figure 6: N* as a function of τ, considering Δt as a parameter (ti = 2s as an exemplary case).

Maximizing N* with respect to τ, we find the condition:

$\tau^* = \Delta t / \ln(1 + \Delta t / t_i)$,

corresponding to the best estimate for the requested decay lifetime for LEPs.

Albeit with limitations due to its simplicity, this model informs that, once t_i and Δt are fixed, there is an optimal value for τ that maximizes the number of delivered therapeutic photons.

This perspective, then, starts by defining the protocols for both LEPs excitation, aerosolization and delivery (fixing t_i) and the LEPs residence time in the lungs for the single inhalation, being it understood that repetition of multiple inhalations is always possible.

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Interestingly, this approach is not unique, being it possible to start from fixing t_i and τ (to reasonable values, in the range 1-4s and 2-30s respectively) while maximizing N respect to Δt. This alternative approach suggests that, if t_i and τ have been already defined/measured, the best strategy for LEPs residence in the lungs corresponds to $\Delta t = +\infty$, which in real terms means to minimize LEPs exhaled fraction with possible consequences on the side of LEPs accumulation. This possible choice will be evaluated in the light of other considerations, such as those about biocompatibility/toxicity.

Out of this model, two possible scenarios can be considered in particular; the final choice between them will be driven by experimental results coming from LEPs synthesis, the excitation and aerosolization protocols and the biocompatibility/toxicity studies.

First scenario: "short τ and small LEPs": Shorter lifetime is compatible with a strategy where LEPs are designed to be inhaled and fully exhaled afterwards. This strategy will request smaller LEPs dimensions (~ a fraction of μm). See also 3.3 and 4.

Second scenario: "long τ and big LEPs": Longer lifetime is compatible with a strategy where LEPs are designed to remain inside the lung airways and eventually cleared, but not exhaled, delivering the light payload during longer time respect to the previous scenario. This strategy will request bigger LEPs dimensions (~ some μm). See also 3.3 and 4.

2.5 Emission Intensity of the Light-Emitting Particles

In bacterial photokilling experiments, the positive correlation between the photokilling efficacy and the impinging light dose D (J/cm^2) is very well known since decades. This informs us that maximization of the delivered photon number N is paramount, assuming a proportionality relationship between N and D to be precisely determined during the project.

In general, the dose can be delivered in one or more successive irradiations ("steps"), which in our case correspond to e.g. multiple inhalations. By restricting our considerations to the single dose (inhalation) release, the main request is about the maximization of the aerosol emitted radiant power per unit aerosol volume over the whole solid angle (dP/dV), being it understood that the emitted spectrum should comply to the requirements stated in 2.2 and 2.3. Of course, dP/dV can be transformed into a requirement about the emitted photon number per unit aerosol volume dN/dV, to be formally intended per further unit wavelength (thinking of a continuous

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spectrum). This corresponds to obtain as much photons as possible emitted by the unit volume of aerosol. It is important to remember that the above-described quantities are not per unit time but refer to a single inhalation or "dose step". As such, they correspond to the time integration of the emitted photons, following the exponentially decreasing behaviour described in 2.4. If aerosol scattering and absorption phenomena are neglected, this corresponds to have as much photons as possible emitted by the unit volume of LEP, irrespective of their size and shape. Only once LEPs are defined in all their chemo-physical characteristics (including size), this also means a maximization of the emitted photons per LEP.

In case of non-negligible scattering/absorption by the aerosol itself, specific measurements should be performed to assess whether a change in the required emitted spectrum is necessary (see 2.2).

3 REQUIRED CHEMICAL AND PHYSICAL PROPERTIES

3.1 Core Material

The core material of the LEPs is a key element for the success of the Light4Lungs project since it is responsible for providing the therapeutic light. In most luminescent materials, the decay of the light emission lasts no longer than a few milliseconds after the end of the excitation. On the contrary, persistent phosphors can continue emitting light for minutes or hours. In general, these materials can be divided into four different groups: silicates, non-silicates oxides, non-oxides and glasses; and they are activated by using different cations. By now, over 200 combinations of host materials and activating ions have been described. It is quite common to use oxide hosts to obtain blue and green afterglow (K. van den Eeckhout et al. 2010).

Among the different core phosphors reported in the literature with persistent luminescence properties, zirconia (ZrO_2) is a very good candidate without much concern of biocompatibility and long-term toxicity. Moreover, it presents high chemical stability, good transparency, and highly efficient luminescence properties (Z. Zhao and Y. Wang, 2012). In comparison to more complex afterglow materials, zirconia can be synthesized by a simple sol-gel method and calcined at relatively low temperatures without the necessity to use a reductive conditions atmosphere (T.M.A. Ellateif and S. Mitra, 2017). In addition to the size distribution, the sol-gel method also provides an opportunity to control the morphology of as-prepared materials by easily varying the synthesis

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conditions. All these advantages, and the high availability of the reagents required for its preparation, will lead us to obtain the LEPs with low economical costs, very good yield performance and facilitates its scalability to the market demands. Furthermore, zirconia particles can be easily doped with different cations to provide persistent luminescent at any convenient emission wavelength. Finally, due to the presence of hydroxyl groups on the surfaces of most oxides, the LEPs surfaces can be easily coated and functionalised with appropriate ligands.

3.2 Shape

For practical applications, the LEPs with spherical shape are highly preferred over other morphologies because they present the highest surface-to-volume ratio, which we deem essential to obtain the highest luminescence. Besides spheres minimize the light scattering effects. Moreover, most of the inhalation studies are focused on spherical particles, since they are more suitable for aerosolization over elongated particles due to their lower attractive forces (Y. Zhang et al., 2014; M.S. Hassan and R.W.M. Lau, 2009).

3.3 Size

Particle size of the LEPs is a critical factor in determining the luminescence quantum efficiency. In general, the efficiency increases with decreasing particle size. Moreover, the size also plays an important role in defining if and where the aerosol particles will deposit in the human lung (J. Hayder, 2004). Numerous experimental and theoretical studies have demonstrated that particles of mean aerodynamic diameter of 1-3 µm deposit minimally in the mouth and throat and maximally in the lung's parenchymal region. Tracheobronchial deposition, generally not desired for an inhalation therapy, is maximized for aerodynamic diameter between 8 and 10 µm. Particles possessing an aerodynamic diameter smaller than 1 µm are mostly exhaled and therefore will remain in the lungs for the air residence time (which can be extended by holding the breath). Finally, and particles larger than 10 µm have little chance of reaching beyond the mouth and throat D.A. Edwards et al., 1998).

Therefore, a compromise between the maximum persistent luminescence efficiency and the optimum residence time of the particles in the human lungs will be found in the Light4Lungs project.

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3.4 Surface coating

Surface coating of the LEPs by different stabilizing agents such as alcohols, polyols, polyethers, etc. has been widely used to enhance the functional activity of nanomaterials and increase their biocompatibility (M. Kahn et al., 2020). Coated LEPs are generally more stable and have better dispersibility/solubility for the proper interaction between bacteria and LEPs. This functionality will also allow us to tailor the surface in accordance with the LEPs delivery media, stabilize the LEPs in a suitable solvent, and increase its adhesion to bacteria or biofilms, should that be beneficial.

3.5 Surface charge and functionalization

A definition of the required surface charge of the particles needs to bring several aspects into consideration. Gram positive *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa* are the bacteria Light4Lungs wants to treat by light irradiation in the human lungs. They show strong differences in their cell envelope, yet both possess a negatively-charge surface. Binding of the LEPs to the bacteria surface could thus be facilitated by endowing them with positive surface charge to take advantage of the electrostatic attraction, as observed for cationic molecules (J. Ghorbani et al., 2018). However, the most likely state of the bacteria in the lungs is forming biofilms, in which bacteria are embedded in a scaffold, the extracellular polymeric substance (EPS) matrix, formed by polysaccharides, proteins, lipids and nucleic acids. Targeting the biofilms EPS matrix has been demonstrated using antibodies and peptides (H. Koo et al., 2017). Of specific interest for Light4Lungs, an antibody targeting PSI, a polysaccharide widely present in clinical isolates of *P. aeruginosa*, has shown efficacy against *P. aeruginosa* infections (A. DiGiandomenico et al., 2012). Similar approaches have proven successful in MRSA lung infection models (A. Estelles et al., 2016). Thus, biofilm targeting, rather than free bacteria targeting, seems a more desirable approach to attach the LEPs to the site of the infection.

On the other hand, the Light4Lungs project considers the use of LEPs that match the lung residence time to the duration of the luminescence. Since once their light emission is exhausted LEPs cannot be “recharged”, at least not inside the lungs, it may be desirable to avoid any binding at all, to prevent long-term toxicity effects derived from LEP accumulation in the lungs. In this case, a neutral or even a negative surface charge may be desirable.

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At this stage of the project, we opt for the use of close-to-neutral LEPs. Based on experimental results, we will — if necessary —reconsider the question of the surface charge.

3.6 Biocompatibility

LEPs must be non-toxic to normal tissue surrounding the infection. Measures taken to ensure maximum biocompatibility include the use of biocompatible materials to prepare them. The core material, zirconium dioxide, is an excellent choice in this regard due to its intrinsically low toxicity and biological inertness. It shows good cyto- and hemocompatible properties and has been used as implant and dentistry materials. It may become necessary to coat the particles with a layer of silica, which may induce inflammatory lung reactions. A recent report shows that the toxicity can be traced back to nearly free surface silanols and can be modulated by thermal treatments (C. Pavan et al., 2020). Additional surface coatings such as polyalcohols, polyethers and peptides have been largely used to increase the biocompatibility of nanomaterials and, given the added benefit of increasing their dispersibility and solubility in biological media, we will use them in the Light4Lungs project. On the other hand, we will minimise the residence time and persistence of the particles inside the lungs to further decrease the potential induction of toxicity.

4 AEROSOL MODEL

4.1 Aerosol Type

As discussed in Section 2.4, two scenarios are considered for the aerosol. The first is where the LEPs are presented as an aerosol particles in the nanoparticulate size of less than 1 μm; the second where the LEPs are presented as aggregates in aerosol particles in the low micron size, ideally in the range of 1-3 μm. The sub-micron aerosol type is most suited for LEPs which have short phosphorescence decay times, since these particles will be inhaled, deliver the light to the airways during their short residence time in the lung and then be exhaled. The low micron aerosol type is most suited for LEPs which have longer phosphorescence decay times, since these particles will need to have longer residence times in the lungs to deliver their light energy. The low micron aerosols will be

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inhaled, deposit in the small airways of the lung, deliver their emitted light over a longer period of time, before being removed by the lungs defence mechanisms - primarily, mucociliary clearance and phagocytosis.

We are currently modelling the residence time of the sub-micron aerosol type in the human lung using data on file for Kr-99m gas. This gas is commonly used for obtaining "ventilation images" in clinical diagnosis. Kr-99m has a radioactive decay half-life of 13s, which is similar, but not identical to, some of the LEP phosphorescence decay half-lives.

The high surface area and surface energy of the LEPs results in a high potential for the nanoparticles to aggregate. This aggregation needs to be controlled, for both the sub-micron and low micron aerosol types and methods to achieve this goal will be discussed in Section 4.2.

4.2 Vehicle, Carrier, and Excipients

Aerosols used for the delivery of drugs to the lungs can be classified as either wet or dry aerosols. Typically, wet aerosols are delivered via nebulisers whereas dry aerosols are delivered using dry powder inhalers. The use of a pressurised metered dose inhaler is not appropriate for this project.

For the sub-micron aerosols, a type of nebuliser is most likely to be effective for delivery since the energy involved to generate sub-micron aerosols will be large. For the low micron aerosols either a nebuliser or a dry powder device could be used for human studies. The LEPs are insoluble in water and biological media, which suggests that a dry powder aerosol is the most appropriate formulation. To achieve controlled aggregation of the LEPs an aerosol particle size in a dry powder formulation would entail using a "carrier system" where another particle such as lactose is introduced to improve flow properties and reproducibly generate a high quality aerosol cloud. This is certainly an option worthy of investigation for human studies, however for the in vitro and pre-clinical studies in this project, a type of nebuliser device is deemed to be the best choice. In addition, certain nebulisers offer greater potential for controlling the aerosol particle size.

To achieve a good dispersion of the LEPs in an aqueous medium for use in a nebuliser formulation the use of some type of dispersing agent will likely be necessary. Certain surfactants are naturally occurring in lung tissue and these are administered in certain clinical conditions, e.g. respiratory distress syndrome. These surfactants will be investigated for their potential to give reproducible dispersions of the LEPs as a priority over other dispersing agents which regulatory approval in other nebuliser formulations.

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4.3 Aerosol Activation

The relatively short decay times of the LEPs indicate that the most efficient delivery of light to the lungs will occur if activation occurs immediately before lung exposure. Therefore, we will investigate methods of activating the aerosol particles after they have been generated from the formulation inside the nebuliser chamber. This will be achieved by using a pulsed light source around a modified elongated nebuliser mouthpiece. Some key parameters for activation which will be investigated include controlling the particle size and density of the aerosol cloud, the pulse intensity and the duration of exposure, in order to optimise the aerosol to produce the highest possible number of light emissions. The time lapse between aerosol excitation and delivery will be also investigated and be fine-tuned to the different types of experiments in the in vitro and pre-clinical studies in this project.

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