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A Novel Nanoparticle Mediated Selective Inner Retinal Photocoagulation (SIReP) for Diseases of the Inner Retina

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Abstract

A novel nanoparticle mediated methodology for laser photocoagulation of the inner retina to achieve tissue selective treatment is presented.

Methods—Transport of 527 nm, 577 nm, and 810 nm laser, heat deposition and eventual thermal damage in vitreous, retina, RPE, choroid and sclera were modelled using Bouguer-Beer-Lambert law of absorption and solved numerically using the finite volume method. Nanoparticles were designed using Mie theory of scattering. Performance of the new photocoagulation strategy using gold nanospheres and gold-silica nanoshells was compared to that of conventional methods without nanoparticles. For experimental validation, vitreous cavity of *ex vivo* porcine eyes was infused with gold nanospheres. After ~6 hours of nanoparticle diffusion, the porcine retina was irradiated with a green laser and imaged simultaneously using a spectral domain optical coherence tomography (Spectralis SD-OCT, Heidelberg Engineering).

Results—Our computational model predicted a significant spatial shift in the peak temperature from RPE to the inner retinal region when infused with nanoparticles. Arrhenius thermal damage in the mid-retinal location was achieved in ~14 ms for 527 nm laser thereby reducing the irradiation duration by ~30 ms compared to treatment without nanoparticles. In *ex vivo* porcine eyes infused with gold nanospheres, SD-OCT retinal images revealed a lower thermal damage and expansion at RPE due to laser photocoagulation.

Conclusion—Nanoparticle infused laser photocoagulation strategy provided a selective inner retinal thermal damage with significant decrease in laser power and laser exposure time.

Significance—The proposed treatment strategy shows possibilities for an efficient and highly selective inner retinal laser treatment.

Index Terms

Retinal Photocoagulation; Nanoparticles; Diabetic Retinopathy; Retinal Vein Occlusion; Neovascularization; Spectral Domain Optical Coherence Tomography; Porcine Retina

I. Introduction

Human eye is a highly evolved sensory organ of vision. Light enters the eye through cornea and pupil, and is focused on the retina by the lens through the vitreous humor. Specialized cells of the neural retina are arranged in distinct layers namely, the inner limiting membrane (ILM; the innermost layer), the nerve fibre layer (NFL), ganglion and nuclear layers, photoreceptor layer, followed by the retinal pigmented epithelium (RPE). The photoreceptor layer contains two types of light sensitive cells called the rods and cone cells that are responsible for light perception and color vision. The pigmented RPE supplies essential nutrients to the photoreceptors and prevents backscattering of light by absorbing light that are not absorbed by the photoreceptors. Choroid in the outer part of the retina is innervated with blood vessels that supply nutrients to the ocular tissue. Sclera is the outermost protective layer of the eye behind choroid that also provides mechanical support for the eye [1].

Diseases of the inner retina (the ILM, NFL and Ganglion cells) can be catastrophic when it comes to vision loss and blindness [2–5]. Some of the more frequent inner retinal diseases are *Proliferative Diabetic Retinopathy* (PDR) with leaky neovascularization in inner retina, *Diabetic Macular Edema* (DME), *Retinal Haemorrhage* in NFL, *Retinal Angiomatous Proliferation* (RAP), and *Retinal Vein Occlusion* (RVO). In advanced stages, these diseases of the inner retina may have adverse effects on vision, and in fact, the PDR, DME and RVO are few of the leading causes of vision loss and blindness [3, 5, 6].

Management of these inner retinal diseases includes drug based treatment, laser photocoagulation, and a combination of both treatment strategies. Grid (scatter) and/or focal laser photocoagulation has been in use for treatment of RVO and DME, while Panretinal (scatter) photocoagulation has been successful in treatment of PDR, especially for ischemic retinal neovascularization. The effectiveness, post treatment complications, and side effects associated with these treatments, however, are challenging. During laser photocoagulation treatment for inner retinal diseases, it may be desirable to preserve the outer retina from thermal damage, minimize patient discomfort during treatment and post treatment complications such as RPE detachment [6–8]. Some of the inner retinal diseases that may benefit from selective treatment of the inner retina while minimizing damage to the posterior retina are:

1. *Proliferative Diabetic Retinopathy* (PDR): With increase in prevalence of lifestyle related diseases such as diabetes, their associated ocular diseases are likely to affect a larger population in the coming decades. PDR is often implicated by angiogenesis led neovascularization (i.e. formation of new blood vessels) in neural retina. Retinal neovascularization may cause damage to the blood-retinal barrier and to nerve cells responsible for vision. In some instances, the newly formed weak blood vessels may leak blood into the vitreous, causing optical occlusion. Retinal neovascularization exhibits two types of vessel growth: (i) vessel sprout in the anterior part of the retina and NFL, sometimes breaking through ILM and invading the vitreous, and (ii) vessel growth in the posterior direction, through outer retina towards sub-retinal space [3, 9, 10]. Laser based

retinal photocoagulation is a clinically established non-invasive treatment procedure for diabetic retinopathy [11, 12].

2. *Brach Retinal Vein Occlusion (BRVO)* due to blockage in the small retinal veins is one of the most common vascular disorders of the retina. Treatment of BRVO for macular edema and intraretinal edema secondary to BRVO include focal and/or diffuse (i.e. grid) laser photocoagulation and in most cases has been successful but with side effects and complications. Laser treatment for intraretinal edema may not require inducing thermal damage in the outer retina and RPE, and hence a selective inner retinal photocoagulation may avoid potential post treatment complications.
3. *Retinal Angiomatous Proliferation (RAP) in Age-Related Macular Degeneration* is neovascular proliferation with origin in the retina and its severity is described as: (a) Stage 1 – Intraretinal neovascularization, (b) Stage 2 – Subretinal neovascularization, and (c) Stage 3 – Choroidal neovascularization [4, 8]. Because the stage 1 RAP primarily involves the inner retina and majority of the laser thermal energy is generally deposited in the RPE, the previously reported treatment efficiencies may be improved and complications such as RPE detachment can be minimized by selective photocoagulation of the inner retina in the selected spatial hot spots [4, 13].
4. *Retinal Hemorrhage* such as *Flame Retinal Haemorrhage* with hemorrhage located in the NFL and *microaneurysm* in inner retina is often present in RVO. Selective treatment of the retinal hemorrhage in the inner retina and microaneurysm may preserve the outer retina and RPE during laser photocoagulation.

The laser induced thermal treatment of ocular diseases, like other forms of laser induced hyperthermia treatments [14, 15], is based on the therapeutic heat generated due to light absorption in the tissue. *Full-threshold laser photocoagulation* is a process that cause thermal damage to tissues through protein denaturation using high photo-thermal energy and is capable of producing visible lesions. The present study focuses on thermal damage in retinal layer induced by single beam laser with typical parameters of a full-threshold laser treatment procedure, referred in this article as *Retinal Photocoagulation (RPC)*.

The three important factors essential for determining the application of lasers in treating ocular diseases are [16, 17]: (a) the choice of laser wavelength based on the desired penetration depth [18], (b) duration of irradiation in case of using a *continuous wave (cw)* laser; and pulse width and pulse repetition frequency in the case of a pulsed laser, and (c) laser power density as a function of the laser output power and beam diameter. Based on the treatment requirements and the type of disease, the above factors are varied to achieve the desired treatment through targeted thermal damage. In general, the laser treatment strategies can be broadly classified into following categories [19, 20]:

1. *Retinal photocoagulation with 527 nm laser (RPC-Green)*, in which the laser heating of retina is performed using *cw* lasers. The RPC-Green procedure is mainly used for treating retinal neovascularization and other ischemic retinal

disorders. Millisecond (100 to 200 ms) pulsed laser can also be used to minimize undesired thermal damage during RPC. Typical parameters in RPC-Green are: 527 nm wavelength (green laser), 50 to 500 mW power, 50 to 500 μm beam diameter, and pulse duration of 20 to 200 ms [21]. A pulse duration of 20 ms is considered optimal in terms of achieving targeted treatment and minimizing undesired thermal damage [16].

2. *Retinal photocoagulation with 577 nm laser* (RPCYellow), in which laser photocoagulation is performed using *cw* lasers. The longer 577 nm yellow wavelength laser, with lower absorption and scattering at RPE, may reduce the thermal damage in the RPE region compared to the 527 nm laser [7, 18]. As the 577 nm wavelength is absorbed by the hemoglobin, it is a recommended treatment laser for microaneurysm and leaky vessels in retina [5]. Typical RPC-Yellow treatment parameters are: 577 nm wavelength, 50 to 500 mW power, 50 to 100 μm beam diameter, and pulse duration of 10 to 100 ms [18].
3. *Retinal photocoagulation with 810 nm laser* (RPC-NIR). With a lower laser absorption in the retina compared to the 527 nm and 577 nm lasers, near-infrared 810 nm lasers can be used for full- and sub-threshold retinal photocoagulation [22]. Typical parameters used for RPC-NIR are: 810 nm wavelength, 50 to 1000 mW power, 50 to 500 μm beam diameter, *cw* or pulsed with pulse duration of 0.1 to 1.0 ms.

Advancement in laser based photocoagulation for retinal diseases, e.g. pattern scanning laser (PASCAL), navigated laser (NAVILAS), and targeted photocoagulation (TRP), have provided spatial selectivity with good efficiency of treatment in some diseases [23–25]. However, the conventional laser based treatment still faces the following shortcomings: (i) less tissue selectivity along the path of laser (depth), especially in the inner retina as the pigmented RPE has the highest laser absorption in all therapeutic wavelengths [26], and thus maximum damage occurs invariably in or around RPE during laser induced heating, and (ii) length of the laser treatment sessions. The treatment sessions may be repeated in an eye based on the effectiveness of the laser induced thermal damage by assessing the lesions [16, 27–29]. In this study, we present a novel method of photocoagulation that can provide tissue selective, and time efficient treatment for diseases of the inner retina by improving the laser based heat transfer efficiency.

Recent advances in treating retinal and choroidal neovascularization include use of *nanoparticles* (NP) for targeted drug delivery, gene therapy and functionalized delivery of anti-angiogenic drugs [30–36]. However, the efficiency of conventional drug delivery for ocular diseases such as topical, systemic, and oral administration have challenges such as the blood-aqueous barrier and blood retinal barrier [37]. Non-conventional methods of drug delivery such as periocular and intravitreal injections provide localized drug delivery but are mildly invasive in nature. However, with the recent advancement in nanoparticle based drug delivery and nanoparticle based laser heating, it is possible to devise a new strategy to treat ocular diseases using functionalized nanoparticle mediated laser photocoagulation procedures to achieve a targeted, localized, and an efficient treatment of diseases of the inner retina [35, 38–41]. The noble metal nanoparticles mediated laser hyperthermia procedures

exhibit an increased absorption of light at a particular wavelength due to *localized surface plasmon resonance* (LSPR) [15, 39, 42–44]. Recent NP mediated laser induced hyperthermia and thermal therapies have used the LSPR phenomenon of nanoparticles in various treatment strategies such as cancer treatment and port-wine stains treatment [15].

In the present study, we propose a novel NP-mediated selective retinal photocoagulation procedure in diseases of the inner retina and validate its efficacy using computational simulations and laser experiments using *ex vivo* porcine eyes. Specifically, we studied the benefits of using nanoparticles to improve the clinically standard RPC-Green, RPC-Yellow, and RPC-NIR photothermal therapies. A finite volume method based solver for transient bioheat transfer equation incorporating modified Bouguer–Beer–Lambert law of absorption [45], is developed for simulating heat transfer in laser exposed multilayer ocular tissue, with photocoagulation effects being estimated by temperature elevation and Arrhenius damage integral [11, 15, 46–50]. Enhanced optical properties of NP infused tissue are calculated using the spectral absorption and scattering in composite media with dispersed spherical nanoparticles [42, 43, 51–53]. The methodology was validated by infusing gold nanospheres in *ex vivo* porcine eyes and irradiating the retina with a green laser.

II. Methods

A. Governing Equations

Heat transfer in a living tissue is affected by several factors such as blood perfusion rate, metabolic heat generation and presence of large blood vessels in the vicinity [46]. Several models of heat transfer in biological tissues exist [14], but *Pennes bio-heat equation* (PBHE) is the most widely used bioheat model [54–56]. In the eye, computational, numerical and experimental aspects of heat transfer during laser irradiation and retinal photocoagulation have been extensively studied previously [11, 28, 47, 48, 57–65].

PBHE for a biological tissue in a 2-D axisymmetric cylindrical geometry (Fig. 1) is given as

$$\rho c_p \frac{\partial T(r, z, t)}{\partial t} = k \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial T(r, z, t)}{\partial r} \right) + \frac{\partial^2 T(r, z, t)}{\partial z^2} \right] + \eta_b \rho_b c_{pb} (T_a - T(r, z, t)) + Q_{Laser}(r, z, t) \quad (1)$$

where, ρ is the tissue density (kg/m^3); c_p is specific heat of the tissue ($J/m \cdot K$); k is thermal conductivity of the tissue ($W/m \cdot K$); ρ_b is blood density; c_{pb} is specific heat of blood perfusing the tissue; T_a is the arterial blood temperature ($^{\circ}C$); and η_b is the blood perfusion rate ($1/s$). When subjected to collimated laser irradiation, tissues generate heat by means of light absorption and scattering, which can be modelled as a source term $Q_{Laser}(r, z, t)$ in the PBHE.

The source term $Q_{Laser}(r, z, t)$, given by a modified Bouguer-Beer-Lambert law [66], is calculated as:

$$Q_{Laser}(r, z, t) = \left(\frac{I_{\max}}{\pi \cdot R_{Beam}^2} \right) \times \kappa_a(z) \times \exp(-(\kappa_a + \mu'_s) \cdot z) \quad (2)$$

where, $I_{\max}$ is the laser intensity (W/m^2), κ_a is the absorption coefficient ($1/m$) of the tissue; μ'_s is the effective scattering coefficient ($1/m$) estimated using the scattering coefficient of the tissue μ_s as:

$$\mu'_s = (1 - g) \mu_s \quad (3)$$

where g is the scattering anisotropic parameter (dimensionless quantity ranging from -1 to 1 ; a larger positive quantity represents that scattering is dominant in the same forward direction as the collimated laser beam and $g = 0$ represents isotropic scattering). Detailed description of layer wise implementation of the source term representing light induced heat deposition along the depth is given in Appendix I.

B. Nanoparticle Design

The absorption and scattering optical properties of the multilayered retinal tissues vary depending on the laser wavelength [26]. For example, laser absorbed by the retinal tissues is lower at 810 nm compared to 527 nm and 577 nm wavelengths. These spatial optical properties determine laser transport within the retinal tissue and hence determine the spatial distribution of laser photothermal response in the eye (Eq. 2). Therefore, by altering the effective absorption and scattering optical properties of the target retinal layer, it is possible to improve the laser absorption efficiency in the target retinal layer and provide targeted photothermal therapy.

Due to *surface plasmon resonance* [15, 39, 42–44], NP's such as gold nanospheres and gold-silica nanoshells exhibit enhanced light absorption and/or scattering optical properties at a specific laser wavelength [67, 68]. The resonant wavelength of a nanoparticle is dependent on its geometry. Using Mie theory of scattering, we designed three nanoparticles with resonant wavelengths at 527 nm, 577 nm, and 810 nm for use with the RPC-Green, RPC-Yellow, and RPC-NIR treatment strategies respectively. Further, at resonance, the particles were designed to have high laser absorption and very low scattering to achieve targeted heating. The absorption Q_a and scattering efficiencies Q_s of these nanoparticles were theoretically estimated using Mie theory [69]. Geometric details of the nanoparticles, and their absorption and scattering efficiencies are presented in Table IV.

Assuming that the nanoparticles are uniformly infused and distributed in the target retinal layer upon delivery (as in Table IV), the effective optical properties of the nanoparticle infused ocular tissue in Eq. 2 are updated as [42, 53],

$$\kappa_{a, NP} = \kappa_a + 0.75 f_v \frac{Q_a}{a_{ns}} \quad (4)$$

$$\mu'_{s, NP} = \mu'_s + 0.75 f_v \frac{Q_s}{a_{ns}} \quad (5)$$

where $\kappa_{a, NP}$ and $\mu'_{s, NP}$ are effective absorption and scattering dimensionless efficiency factors of absorption and scattering for a single nanoparticle (Table IV), a_{ns} is the average outer radius of a single nanoparticle and f_v is the specific volume fraction of NP. Equations (4) and (5) were developed for monodispersed approximation of spherical particles in a media after NP infusion. Q_a and Q_s are the [52]. Gold nanosphere and gold-silica nanoshell designs are provided in Table IV. Tissue-NP volume fractions ranging from $f_v = 10^{-6}$ to 10^{-4} were considered for RPC-Green, RPC-Yellow, and RPC-NIR simulations.

The NP design (i.e. the sphere diameter, shell diameter, core-shell ratio) was selected such that the particles exhibit LSPR at the desired wavelengths with maximum absorption and minimum scattering efficiencies (Table IV). Absorption and scattering efficiencies were estimated in Matlab using the Mie theory of scattering [52, 69]. Details of the refractive indices of NP and the tissue are presented in Appendix II.

C. Boundary Conditions

With an axisymmetric cylindrical geometry, the computational domain becomes a 2-D $r-z$ plane (Fig. 1), with the left boundary ($r = 0, z$) as the central axis of the cylinder. A no-flux boundary condition was imposed on all four boundaries. Initial temperature of 18°C was used for validating the numerical procedure as in Schule et al. [20]. The core body temperature of 37°C was used as initial temperature for all study cases. A thin layer of vitreous has been included in the geometry to model temperature elevation at the top boundary.

D. Numerical Implementation

A finite volume method (FVM) solver was implemented in C++ to solve the governing bioheat equation (Eq. 1) for the retinal temperature $T(r, z, t)$ during laser irradiation in the $r-z$ domain. The final discretised equations for all internal grid points is given by:

$$\begin{aligned}
T_{i,j}^{t+\Delta t} = & T_{i,j} + \frac{k}{r_i(\Delta r)^2 \rho c_p} \left[\left(r_i \Delta r + \frac{\Delta r}{2} \right) T_{i+1,j} - 2r_i T_{i,j} + \left(r_i \Delta r - \frac{\Delta r}{2} \right) T_{i-1,j} \right] + \frac{k}{(\Delta z)^2 \rho c_p} \\
& \times [T_{i,j+1} - 2T_{i,j} + T_{i,j-1}] + \frac{\Delta t}{\rho c_p} \\
& \times \left(\rho_{blood} C_{p_blood} \omega_b \right) [T_a - T_{i,j}] + \frac{\Delta t}{\rho c_p} [Q_{laser} \\
&]
\end{aligned}$$

(6)

where i and j are subscript for r and z direction grid points. Our bioheat FVM solver was validated using the experimental measurements, and numerical estimates presented in Schule et al [20]. With a grid size of $r \times z = 400 \times 800$, in the radial and axial directions, a uniform grid spacing of $r = 2.5 \mu\text{m}$ and $z = 1.687 \mu\text{m}$ was used. Using the explicit FVM scheme, a time step of $\Delta t = 10^{-6}$ was used in all the simulations that provided numerical stability criteria. In both directions (r, z) the *von Neumann* criteria has been imposed for numerical stability of the solver [70]. In z -direction, the stability criterion becomes:

$$\Delta t \leq \frac{\rho c_p (\Delta z)^2}{2k} \quad (7)$$

The other pertinent details of our FVM procedure such as discretization steps, grid independence, and solution procedure has been skipped for brevity and can be found in dedicated texts [70].

E. Tissue Damage Estimation

Using the FVM estimated tissue temperature distribution over time $T(r, z, t)$ in Eq. 1, the ocular tissue thermal damage due to laser irradiation was estimated using the Arrhenius equation,

$$\omega(r, z, t = \tau) = A \int_0^\tau \exp \left(\frac{-E_a}{RT(r, z, t)} \right) dt \quad (8)$$

where, ω is the Arrhenius damage integral, A is the pre-exponential frequency factor ($1/s$), τ is the total heating time (s), E_a is the activation energy barrier (J/mole), and R is the universal gas constant ($8.3143 J/\text{mole/K}$). In the present study, the following parameter values were used for estimating the Arrhenius thermal damage [11, 47]:

$$\left. \begin{array}{l} A = 0.0 \\ E_a = 340000 \\ A = 1.6 \times 10^{54} \end{array} \right\} \begin{array}{l} \text{for } T \leq 43^\circ\text{C} \\ \\ \text{for } T > 43^\circ\text{C} \end{array}$$

F. Ex vivo Laser Photocoagulation and OCT Imaging Setup

We experimentally validated the computational model of RPC-green photocoagulation described earlier using *ex vivo* porcine eyes. Fig. 2 shows a schematic and experimental arrangement of our laser photocoagulation setup with a modified optical coherence tomography (OCT) arrangement for simultaneous laser irradiation and real-time imaging of *ex vivo* retinal tissues [71]. A 532 nm wavelength green laser (LRS-0532 DPSS Laser System, Laserglow Technologies, West Toronto, Canada) was collimated and delivered to the porcine retina through a spectral beamsplitter/dichroic long-pass filter with a cut-on wavelength of 650 nm (Edmund optics, # 69-891). Spectralis SD-OCT device (Heidelberg Engineering, GmbH) with operating wavelengths of 820 nm (for reflectance imaging) and 870 nm (for OCT) was arranged as shown in Fig. 2b to image the porcine retina through the spectral beamsplitter during laser irradiation using its built-in *b-scan movie* mode.

III. Results

The posterior segment of the porcine eye was modelled as a multi-layered tissue using a 2-D axisymmetric cylindrical geometry of radius 1 mm and depth of 1.35 mm (Fig. 1). The model comprised of the vitreous (thickness 200 μm), retina (190 μm), RPE (10 μm), choroid (250 μm), and sclera (750 μm). The top retinal surface was irradiated with laser to induce thermal damage in the posterior segment of the eye for three types of laser treatment strategies namely RPC-Green, RPC-Yellow, and RPC-NIR. Laser parameters for all treatment strategies are presented in Table I. Thermophysical properties of the porcine retinal layers are presented in Table II [62, 72, 73]. Optical properties of the porcine ocular tissue layers at 527, 577, and 810 nm are presented in Table III [26]. We assumed the blood perfusion level in choroid to be 50% [26]. The anisotropic parameter g was kept constant for each tissue layer at $g_{\text{Retina}} = 0.97$, $g_{\text{RPE}} = 0.84$, $g_{\text{Choroid}} = 0.90$, and $g_{\text{Sclera}} = 0.90$ [26] independent of the irradiation wavelength. The vitreous was assumed to be transparent to all the wavelength, and included in the geometry for thermophysical effects.

A. Model Validation

Validity of our geometrical model of the eye and the numerical accuracy of our FVM implementation was assessed using experimental retinal temperature measurements as well as using numerical temperature estimates from *in vitro* laser irradiation of a porcine eye using a micro-pulsed laser presented in Schule et al. [20]. Laser parameters used for validation are presented in Table I. Data from Fig. 5 of Schule et al. [20] were manually extracted using the Plot Digitizer software (ver. 2.6.8). Pearson coefficient between present and calculated temperature of Schule et al. [20] is $r = 0.9774$, which shows a good agreement between the two.

B. Retinal Photocoagulation with 527 nm (RPC-Green)

The retinal surface was irradiated for 100 ms with a continuous wave laser beam of radius $100\ \mu\text{m}$ at 50 and 100 mW power levels [74]. Figure 4 shows axial temperature distribution (z-axis) along the laser axis after 10 ms of laser irradiation without any nanoparticle infusion (i.e. conventional RPC-Green treatment) and with 10^{-4} volume fraction of gold nanospheres (Table IV) infused in the retina. With the conventional RPC-Green treatment, the highest temperature increase was observed at RPE due to the lower light absorption characteristics of the neural retina and the highest light absorption by the pigmented RPE [26]. With NP infusion, the highest temperature increase was observed in the inner retina without any significant temperature increase in RPE. Further, the desired retinal coagulation temperature of $\sim 55^\circ\text{C}$ was achieved using a significantly lower laser power and laser irradiation time.

At 50 mW laser power, the temperature of NP infused retinal tissue layer reached $\sim 55^\circ\text{C}$ (at the mid-retina) in 10 ms while the tissue without NP infusion reached a maximum of $\sim 39^\circ\text{C}$ in 10 ms at the same location. A similar laser thermal irradiation response was observed at 100 mW power level (Fig. 4). Fig. 5 shows the effects of laser power and NP infusion on the spatial temperature distribution (r-z plane) in the posterior segment of the eye. With NP infusion, peak of the heat deposition shifted from the RPE layer to the mid-retinal layer.

Figure 6 shows thermal damage at a location along the laser axis as a function of laser irradiation duration at the center of the retinal layer and at the top of the RPE, estimated using the Arrhenius damage integral (Eq. 8). With a 50 mW laser, the desired thermal damage threshold ($= 1$) in the retinal layer was achieved after ~ 41 ms of irradiation without any nanoparticles and in less than 15 ms with nanoparticles infused in the inner retina. Simultaneously, the time to thermal damage of the RPE increased from 4 ms without nanoparticles to 33 ms with nanoparticle infusion.

Therefore, it is evident that, for the same laser parameters, the proposed treatment strategy using nanoparticles is selectively enhancing thermal damage in the inner retina without causing thermal damage to the RPE and the photoreceptor layers.

C. Retinal Photocoagulation with 577 nm (RPC-Yellow)

The retinal surface was irradiated with a 577 nm yellow laser of $100\ \mu\text{m}$ beam radius at 50 mW and 100 mW power levels. For infusion in the retina, gold-silica nanoshells were designed ($R_{IN} = 15\ \text{nm}$, $R_{OUT} = 25\ \text{nm}$, Table IV) to provide peak laser absorption at 577 nm.

Fig. 7 shows the axial temperature distribution along the laser axis and Fig. 8 shows the spatial temperature distribution after 10 ms of laser irradiation. Fig. 9 shows the Arrhenius retinal thermal damage along the laser axis as a function of laser irradiation duration. With nanoparticle infusion in the retina, the 577 nm yellow laser provided selective thermal damage to the inner retina without damaging the RPE and the photoreceptor layers similar to the 527 nm green laser. The yellow laser improved the selectivity in treating the inner retina (axial temperature distribution/spread in Fig. 4 vs. Fig. 7) and did not cause RPE damage when compared to the green laser (tissue damage threshold in Fig. 6 vs. Fig. 9).

D. Retinal Photocoagulation with 810 nm (RPC-NIR)

Because the laser absorption and scattering properties of the retina, RPE and choroid are significantly lower for infrared lasers (Table III), the 810 nm laser is generally used for choroidal photocoagulation as it provides more depth of penetration compared to 527 nm and 577 nm lasers. Therefore, to eliminate RPE and photoreceptor damage and to provide highly selective inner retinal thermal damage, we irradiated the retinal surface with 810 nm lasers with and without nanoparticle infusion in the retinal layer. We designed gold-silica nanoshells to achieve peak laser absorption at 810 nm ($R_{IN}=23\text{ nm}$, $R_{OUT}=26\text{ nm}$, Table IV).

Figure 10 shows the axial temperature distribution during RPC-NIR along the laser axis with and without nanoparticle infusion in the retinal layer. It is evident that the 810 nm laser with tuned gold-silica nanoshells infused in the retina, a highly selective inner retinal treatment can be achieved without any damage to the RPE and photoreceptors. Further, with the infrared lasers, the selective inner retinal treatment can be achieved at reduced laser power and laser exposure duration. Figure 11 shows the Arrhenius thermal damage plot for RPC-NIR.

In general, we observed a significant increase in the thermal damage as well as a significant reduction in the irradiation time during RPC treatment by infusing nanoparticles in the retina. In case of RPC-Green, RPC-Yellow, and RPC-NIR treatment, infusion of nanoparticles in the retina provided a significant increase in the thermal damage in the target layers. Observations in NP mediated RPC-Green, RPC-Yellow, and RPC-NIR simulations were promising and displayed a *Selective Inner Retinal Photocoagulation* (SIReP).

E. Photocoagulation using 532 nm (RPC-Green) Laser in Ex Vivo Porcine Eyes

Porcine eyes were obtained from a local USDA inspected meat processing facility within 4 hours of harvesting and used within 24 hours. For optical clarity, cornea was trephined (8 mm) and the refractive power of the cornea was replaced with a 50 D lens. 1 ml of gold NP solution made of 50 nm diameter gold nanospheres with a peak absorption at 531 nm (50 nm PEG Nanoxact Gold, nanoComposix, San Diego, CA, USA) was injected at the limbus at approximately 45° toward the posterior pole. The nanoparticles were allowed to diffuse into the retina for ~6 hours before irradiation. Laser photocoagulation was performed using 532 nm laser in one control eye without NP and in one study eye with NP infusion. During laser irradiation, real-time infrared fundus images and OCT b-scans at the location of irradiation were acquired at a frequency of 1 Hz for ~45 secs.

In the control eye without NP infusion, thermal damage at the RPE level, and thermal expansion were primarily observed in the anterior direction (Fig. 12). The retinal thermal expansion observed in the control eye was similar to previously reported observations [75]. However, the RPE damage and thermal expansion in NP infused eye was significantly less than the control eye (Figs. 12(a) – (e)). OCT imaging at ~1 minute post laser irradiation revealed a lower thermal damage at the RPE level in NP infused tissue (Fig. 13(a)). Lesion due to irradiation (Fig. 13(b)) appeared larger in the NP infused eye in comparison with that

of the control eye. This observation may be due to higher inner retinal thermal damage in the NP infused tissue when compared with the control eye.

The fact that RPE has the highest absorption, and our experimental observations of delayed and lower thermal damage at the RPE, reduced thermal expansion (Fig. 12d, e) and larger lesion in the NP infused eye (Fig. 13) likely indicate the ability of our nanoparticle mediated photocoagulation procedure to achieve a selective inner retinal treatment while minimizing damage to the RPE and photoreceptors. Future histological evaluations will document the extent of cellular damages in the neural retina and RPE in these study eyes.

IV. Discussion

In this study, we have presented a novel nanoparticle based photocoagulation laser procedure to treat ocular diseases affecting the inner retina that we call as *Selective Inner Retinal Photocoagulation* (SIReP). The proposed method provides a highly selective, efficient and targeted treatment of the inner retina without damaging the photoreceptor layer and the RPE. The efficacy of the new procedure was assessed using a computational model of the posterior segment of the porcine eye. Validity and performance of the proposed SIReP in *in-vitro* and *in-vivo* studies of porcine eyes will be presented in future.

We studied the efficiency of the SIReP procedure with various volume fractions of gold and gold-silica nanoparticles in the target location. In this study, we assumed that the nanoparticles are uniformly infused in the retina, however for scenario of a non-uniform NP accumulation is possible. At present, however, there is no clear quantitative reports in literature regarding the actual levels of nanoparticle uptake, nanoparticle retention and concentration available at the target location, and their rate of systemic removal from the ocular tissues. Further, limited *in vivo* studies report possible toxic effects of nanoparticles to the retinal cells [34, 76]. Therefore, we chose a conservatively lower volume fraction of nanoparticles for the SIReP procedure that is likely to be safe based on the previous studies [42, 53]. More studies on the nanoparticle uptake, systemic removal and retention as well their toxic effects on retinal cells are necessary to understand their longer-term effects in living tissues and to assess patient safety in clinics.

In general, nanoparticles can be functionalized and delivered to the target location through the systemic route. Because the retina can be directly accessed (via the vitreous), the nanoparticles can be delivered to the target location through non-systemic route. The NP delivery to the selected tissue layer is possible with advancement in drug delivery procedures in ocular tissues. Functionalization of NP based drug delivery has been reported in recent literature. Intravitreal injections and topical NP delivery in retina is a possibility and further investigation on the subject matter is required [77, 78]. The NP functionalization, and combination of metallic NP and anti-angiogenesis drugs, may provide a new possibility of laser assisted drug based treatment of ocular diseases affecting the inner retina. The present study is based on the confidence of recent advancements in the field of functionalized NP drug delivery in ocular tissue. Specific disease targeting based on the surface chemistry of retinal tissues has been recently reported.

The feasibility of gold-silica nanoshell design used for LSPR in the case of RPC-NIR ($R_{IN} = 23$ nm, $R_{OUT} = 26$ nm) may be limited by the required ultrathin gold coating on silica core. The current commercially available nanoparticle fabrication technology limits gold coating on spherical particles to ~ 10 nm. Nanospheres (for 527 nm wavelength) and nanorods (for 810 nm wavelength) with high absorption efficiencies are commercially available with biocompatibility coatings. Studying the possibility of nanorods may provide a more feasible design than the ultrathin 3 nm coating (815 nm wavelength, Table IV) for LSPR at 810 nm wavelength [79].

In general, Ganglion cells at the sites of retinal photo-coagulation are destroyed due to thermal damage. Because Ganglion cells are neuronal cells, it is highly unlikely to preserve or restore visual function at these locations after retinal photocoagulation. In SIREP, retinal thermal damage can be highly depth selective with a narrow depth extent as small as ~ 40 microns using a low energy laser irradiation (Figs. 3, 6, and 9). With a narrow depth extent of thermal damage, depending on the retinal location treated, we may be able to also minimize Ganglion cell death at the sites of retinal photocoagulation. In addition, by preventing deeper laser penetration, SIREP may also minimize patient discomfort during treatment, post treatment complications and side effects associated with conventional panretinal photocoagulation such as RPE detachment etc. [6, 8].

In this study, we experimentally validated the computational model of photocoagulation using green laser (RPC-green) in *ex vivo* porcine eyes. In the *ex vivo* eyes, we characterized the protective nature of the nanoparticle based targeted therapy by irradiating the retina for a significantly longer duration (15 sec) in contrast to our theoretical model (100 ms). Because the method shows protective evidence and targeted thermal damage for longer duration of irradiation, such targeted therapeutic outcome is also naturally expected for shorter duration of irradiation.

In conventional panretinal photocoagulation for treating diabetic retinopathy, the target treatment pathway is to induce anti-VEGF activity and reduce oxygen consumption of the retina by destroying few photoreceptors and RPE [7, 80]. In such treatment targets, the proposed methodology is not applicable.

V. Conclusion

We have presented a novel nanoparticle based photo-thermal strategy known as Selective Inner Retinal Photocoagulation (SIREP) for efficient treatment of diseases affecting the inner retina such as proliferative diabetic retinopathy, diabetic macular edema, retinal haemorrhage in NFL, retinal angiomatous proliferation, and retinal vein occlusion. Compared to the existing laser therapies for retinal photocoagulation, the proposed strategy, evaluated using computational simulation, shows promise for achieving highly selective treatment of inner retinal layer, minimizing damage to healthy tissues and cellular layers, and reducing laser power requirement and irradiation duration. The proposed strategy can also be used for treating neovascularization in the choroid during age related macular degeneration.

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Appendix I

A. The Heat Deposition in Different Tissue Layers

The source term in Eq. 1, which is a function of space and time (Eq. 2), attenuates exponentially with depth [81]. For the five tissue layers considered in the eye model (Fig. 1) Q_{Laser} is calculated as following:

$$\begin{aligned}
Q_{\text{Laser_Vitreous}}(r, z, t) &= 0 \\
Q_{\text{Laser_Retina}}(r, z, t) &= \left(\frac{I_{\max}}{\pi \cdot R_{\text{Beam}}^2} \right) \times \kappa_{\text{a_Retina}}(z) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Retina}} \cdot z_{\text{Total}} \right) \\
Q_{\text{Laser_RPE}}(r, z, t) &= \left(\frac{I_{\max}}{\pi \cdot R_{\text{Beam}}^2} \right) \times \kappa_{\text{a_RPE}}(z) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Retina}} \cdot z_{\text{Retina}} \right) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{RPE}} \cdot (z_{\text{Total}} - z_{\text{Retina}}) \right) \\
Q_{\text{Laser_Choroid}}(r, z, t) &= \left(\frac{I_{\max}}{\pi \cdot R_{\text{Beam}}^2} \right) \times \kappa_{\text{a_Choroid}}(z) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Retina}} \cdot z_{\text{Retina}} \right) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{RPE}} \cdot z_{\text{RPE}} \right) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Choroid}} \cdot (z_{\text{Total}} - (z_{\text{Retina}} + z_{\text{RPE}})) \right) \\
Q_{\text{Laser_Sclera}}(r, z, t) &= \left(\frac{I_{\max}}{\pi \cdot R_{\text{Beam}}^2} \right) \times \kappa_{\text{a_Sclera}}(z) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Retina}} \cdot z_{\text{Retina}} \right) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{RPE}} \cdot z_{\text{RPE}} \right) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Choroid}} \cdot (z_{\text{Choroid}}) \right) \\
&\times \exp \left(-(\kappa_a + \mu'_s)_{\text{Sclera}} \cdot (z_{\text{Total}} - (z_{\text{Retina}} + z_{\text{RPE}} + z_{\text{Choroid}})) \right)
\end{aligned}$$

where the z_{Total} represents the total depth of tissue geometry and z_{Retina} , z_{RPE} , z_{Choroid} , and z_{Sclera} , represents the corresponding tissue thickness as described in results section.

Appendix II

Refractive Index of NP and Tissue Medium

The retinal layers in the posterior segment of the eye are reported to have differing refractive indices. The refractive index of retina and RPE-choroid of bovine eye vary from $n = 1.34$ to $n = 1.38$ [82]. By considering the melanin concentration, the refractive index of bovine RPE

has been reported to vary with wavelength [83]. Refractive index of RPE has also been reported to vary with temperature changes [84, 85]. The retinal layer shows birefringence (change in n), due to its polarization properties. Refractive index of tissue used by most commercial optical imaging devices is constant, $n = 1.35$ [86, 87]. The refractive index for the porcine neural retina is reported as $n = 1.35733 \pm 0.00167$ [88]. At the cellular level, retinal cell layers have differing refractive indices. In rats, the refractive index of individual retinal layers varied between $n = 1.358$ and 1.369 [89]. These reported variations in the refractive index are assumed to make minimal change in the calculated efficiencies of the nanoparticles. Therefore, in this study, we assumed that the refractive index of retina to be constant ($n = 1.35$).

The refractive indices of gold and silica were calculated based on the relation given in [42, 52, 53].

Fig. 1. Schematic representation of laser irradiation of the multilayer ocular tissue modeled geometrically as an axisymmetric cylindrical domain (i.e. 2-D r - z domain) under laser irradiation. Laser parameters for various treatment strategies given in Table I. Thermo-physical properties and optical properties of ocular tissues given in Table II and Table III respectively.

Fig. 2.
 (a) Schematic and (b) laboratory laser photocoagulation and OCT experimental setup.

Fig. 3.

Validation of our geometric model and numerical finite volume solution using experimental temperature measurements and numerical temperature estimates from the RPE of a laser irradiated porcine eye in Schule et al., 2004 [20].

Fig. 4.

Temperature distribution during retinal photocoagulation (RPC-Green) at various retinal layers (vitreous, retina, RPE, choroid, and sclera) along the laser axis when irradiated with a 527 nm continuous wave laser at 50 and 100 mW power levels with and without nanoparticle (NP) infusion in the retina.

Fig. 5. Spatial temperature distribution in the posterior segment of the eye during retinal photocoagulation (RPC-Green) after 10 ms of laser irradiation at the top of retina (a) at 50 mW laser power without NP infusion, (b) at 50 mW laser power with NP infused in the retinal layer.

Fig. 6.

Thermal damage during retinal photocoagulation (RPC-527 nm) estimated using the Arrhenius integral at the middle of the retinal layer and at the top of RPE, both along the laser axis for a total irradiation time of 100 ms and laser power of 50 mW.

Fig. 7.

Temperature distribution during retinal photocoagulation (RPC-Yellow) at various retinal layers (vitreous, retina, RPE, choroid, and sclera) along the laser axis when irradiated with a 577 nm continuous wave laser at 50 and 100 mW power levels with and without nanoparticle (NP) infusion in the retina.

Fig. 8. Spatial temperature distribution in the posterior segment of the eye during retinal photocoagulation (RPC-Yellow) after 10 ms of laser irradiation (a) at 50 mW laser power without NP infusion, (b) at 50 mW laser power with NP infused in the retina.

Fig. 9.

Thermal damage during retinal photocoagulation (RPC-Yellow) estimated using the Arrhenius integral at the middle of the retinal layer and at the top of RPE, both along the laser axis for a total irradiation time of 100 ms and laser power of 50 mW.

Fig. 10.

Temperature distribution during retinal photocoagulation (RPC-NIR) at various retinal layers (vitreous, retina, RPE, choroid, and sclera) along the laser axis when irradiated with a 810 nm continuous wave laser at 50 and 100 mW power levels with and without nanoparticle (NP) infusion in the retinal layer.

Fig. 11.

Thermal damage during retinal photocoagulation (RPC-NIR) estimated using the Arrhenius integral at the middle of the retinal layer and at the top of RPE, both along the laser axis for a total irradiation time of 100 ms and laser power of 50 mW.

Fig. 12.

Thermal expansion and damage during retinal photocoagulation (RPC-Green) as seen in real-time OCT images. (a) – (e) OCT of retinal tissue at 0 s, 1 s, 5 s, 10 s, and 15 s of laser irradiation respectively. Laser beam of 100 μm radius with 200 mW power was used in the experiments.

Fig. 13.

OCT images of thermal expansion and RPE damage during retinal photocoagulation (RPC-Green). (a) Retinal b-scan after one minutes of laser OFF period, and (b) Fundus image after one minute of laser OFF period.

TABLE I

Laser parameters for the validation case and three study cases

1. Validation case	
Laser wavelength - 527 nm	
Mode - micro-pulsed laser	
Energy per Pulse - 32 μ j	
Pulse Width - 1.7 μ s	
Beam radius - 80 μ m	
Total No. of Pulses - 100	
Repetition Frequency - 500 Hz	
2. Retinal photocoagulation green (RPC Green)	
Laser wavelength - 527 nm	
Mode - continuous wave (cw) laser	
Laser Power - 50 mW and 100 mW	
Beam radius - 100 μ m	
Exposure time - 100 ms	
3. Retinal photocoagulation yellow (RPC Yellow)	
Laser wavelength - 577 nm	
Mode - continuous wave (cw) laser	
Laser Power - 50 mW and 100 mW	
Beam radius - 100 μ m	
Exposure time - 100 ms	
4. Retinal photocoagulation NIR (RPC-NIR)	
Laser wavelength - 810 nm	
Mode - continuous wave (cw) laser	
Laser Power - 50 mW and 100 mW	
Beam radius - 100 μ m	
Exposure time - 100 ms	

TABLE II

Thermophysical properties of ocular layers in the posterior segment of the eye [11, 47, 71, 80]

Tissue	Thermal conductivity k (W/m/K)	Specific heat capacity c_p (J/Kg/K)	Density ρ (Kg/m ³)	Blood perfusion rate η_b (l/s)
<i>Vitreous</i>	0.603	4178.0	1000.0	0.0
<i>Retina</i>	0.628	4190.0	1000.0	0.005
<i>RPE</i>	0.628	4190.0	1000.0	0.005
<i>Choroid</i>	0.628	4190.0	1000.0	0.3
<i>Sclera</i>	0.58	4178.0	1050.0	0.00046
<i>Blood</i>	N/A	3720.0	1060.0	N/A

TABLE III

Optical properties of ocular tissues [26]. For vitreous, coefficients κ_a and μ_s were assumed to be zero.

Tissue	Absorption Coefficient $\kappa_a(1/m)$	Scattering Coefficient $\mu_s(1/m)$	Extinction Coefficient $\kappa_a + (1 - g)\mu_s$
Wavelength = 527 nm (Green Laser)			
<i>Retina</i>	421.0	38188.0	1446.6
<i>RPE</i>	110243.0	114637.0	128584.9
<i>Choroid</i>	15939.0	73587.0	25505.3
<i>Sclera</i>	411.0	89827.0	9393.7
Wavelength = 577 nm (Yellow Laser)			
<i>Retina</i>	390.0	29000.0	1260.0
<i>RPE</i>	97000.0	12000.0	116200.0
<i>Choroid</i>	14000.0	68000.0	20800.0
<i>Sclera</i>	390.0	89000.0	9290.0
Wavelength = 810 nm (NIR Laser)			
<i>Retina</i>	69.0	692	761.0
<i>RPE</i>	43986.0	25614.0	69600.0
<i>Choroid</i>	4577.0	5126.0	9703.0
<i>Sclera</i>	47.0	5810.0	5857.0

TABLE IV

Nanoparticle design

Treatment	NP	R_{IN} (nm)	R_{OUT} (nm)	SPR λ (nm)	Q_d	Q_s
<i>RPC-Green</i>	Gold Sphere	0	25	525	3.527	0.4628
<i>RPC-Yellow</i>	Gold-Silica Nanoshell	15	25	575	5.249	1.0005
<i>RPC-NIR</i>	Gold-Silica Nanoshell	23	26	815	11.33	2.4362