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Photothermal lesions in soft tissue induced by optical fiber microheaters

Reinher Pimentel-Domínguez,[1,2,*] Paola Moreno-Álvarez,[3] Mathieu Hautefeuille,[4] Anahí Chavarría,[5] and Juan Hernández-Cordero[1]

[1]*Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de Mexico, Circuito Exterior, Ciudad Universitaria, Coyoacán D.F. 04510, Mexico*
[2]*Posgrado de Ciencias Biológicas, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán D.F. 04510, Mexico*
[3]*Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán D.F. 04510, Mexico*
[4]*Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán D.F. 04510, Mexico*
[5]*Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán D.F. 04510, Mexico*
[*]*postdata.reinher@gmail.com*

Abstract: Photothermal therapy has shown to be a promising technique for local treatment of tumors. However, the main challenge for this technique is the availability of localized heat sources to minimize thermal damage in the surrounding healthy tissue. In this work, we demonstrate the use of optical fiber microheaters for inducing thermal lesions in soft tissue. The proposed devices incorporate carbon nanotubes or gold nanolayers on the tips of optical fibers for enhanced photothermal effects and heating of *ex vivo* biological tissues. We report preliminary results of small size photothermal lesions induced on mice liver tissues. The morphology of the resulting lesions shows that optical fiber microheaters may render useful for delivering highly localized heat for photothermal therapy.

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1. Introduction

In recent years, the use of therapeutic heat has become a promising means to treat malignant tissues. Experimental evidence demonstrates that thermal therapy by increasing temperature above normal body temperature has different biological consequences such as protein denaturation, irreversible damage to subcellular structures, blood coagulation, inducing apoptotic signaling pathways or tissue charring [1–4]. All these effects cause the death of cancer cells or destruction of malignant tissue.

Different devices have been developed for heating locally malignant tissue; these include radiofrequency generators, microwave antennas and highly focused ultrasound probes [5]. However, the use of light to heat biological tissue offers certain advantages over these devices such as a precise space-control, or the possibility to provide in situ administration of light through optical fibers. Furthermore, some wavelengths of light allow for penetration depths reaching deep tissues (mm-cm), providing attractive capabilities for non-invasive or minimally invasive therapy. Photothermal therapy, i.e., thermal therapy with light, allows for the selective destruction of cancer cells achieving the reduction or complete destruction of the tumor, minimizing the damage in the surroundings of the malignant tissue [6].

Several studies have explored the effects of photothermal therapy in different tumor models, both in animals and humans. As an example, laser light has been used to reduce and destroy liver cancer in mice [7]. Also, photothermal therapy with lasers has been reported in the destruction of a human malignant liver tumor, achieving complete necrosis of the targeted tissue in 95% of cases, with collateral damage of 5mm in healthy tissue [6]. Light has also been used in treatments for different types of brain tumors by interstitial photothermal therapy using optical fibers [8, 9]. The main limitation of this therapy is the availability of appropriate wavelengths (e.g., infrared light) to generate the desired photothermal effects in different tumors avoiding damage in the surrounding healthy tissues. Moreover, the low absorption of some tissues requires using high power lasers (several Watts of optical power) and/or prolonged therapy time (> 10 minutes) [6,8]. Large doses of energy can also cause unwanted effects such as tissue breakdown or cell death due to cavitation effects that may occur during

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laser light delivery [10, 11]. Hence, new tools to provide more effective means for photothermal therapy are required.

Nanotechnology has allowed for the fabrication of nanoparticles with high efficiency for converting light into heat. This attractive feature offers new possibilities for developing highly efficient nanoheaters capable of heating smaller and highly delimited spatial regions. Numerous studies have demonstrated the effectiveness of photothermal therapy with different types of nanoparticles [12,13]; in particular, carbon nanotubes and gold nanoparticles showing enhanced photothermal effect have yielded very promising results [14–17]. However, in all of these studies the nanoparticles had to be incorporated into the tumor by systemic or intratumoral injections [14,17]. Furthermore, the biotoxicity of nanoparticles remains a controversial topic [18,19]; their biodistribution in the body of the patient and their final destination are still under scrutiny and nanoparticle tracking is always required after therapy.

In this work, we present the fabrication and characterization of optical fiber microheaters (OFMH) potentially useful for delivering highly localized heat for photothermal therapy. The proposed devices incorporate carbon nanoparticles or gold nanostructures on the tips of conventional optical fibers to enhance photothermal effects in ex vivo biological tissues. Upon combining these nanostructures with the laser light guided by the optical fiber, we expect to obtain improved heat generation owing to the enhanced optical absorption of these nanomaterials. We report promising preliminary results in mice liver tissue, obtaining small-sized photothermal lesions when exposed to the OFMH at different optical powers. Since the tissue minimally absorbs the light used with these devices, local heating of the nanoparticles yields little or no damage to the surrounding healthy tissue, with potential applications in selective cancer cells destruction.

Based on standard fiber optic technology, the proposed OFMHs offer an interesting alternative to other techniques involving nanoparticles for photothermal therapy. In particular, confinement of the nanoparticles within the tip of the fiber requires low optical power to generate adequate heat in highly localized areas. Furthermore, nanoparticle confinement avoids the need for injecting them into the body, thereby reducing potential problems associated with nanostructure toxicity. The aim of these devices is at implementing a technique offering a more controlled and less aggressive solution to induce small-sized, highly localized lesions, hence minimizing collateral damage in biological tissues.

2. Materials and methods

2.1. Fabrication of optical fiber microheaters

We present two types of OFMHs based on nanostructures with high optical absorption. The first type is fabricated with carbon nanotubes and the second one uses gold nanolayers. Both were chosen due to their enhanced photothermal effects; furthermore, they have also been used for photothermal therapy [14–17]. The nanostructures are incorporated onto standard single-mode optical fibers; the light launched into the optical fiber is absorbed at the output end by the nanostructures and heat is generated and dissipated in the vicinity of the tip. Similar OFMHs have been previously used to heat liquids up to their evaporation point [20,21]. An attractive feature of these microheaters is their ease of fabrication with the use of off the shelf elements. Hence, we show comparative results for two types of OFMH when used as highly-localized heat sources for inducing lesions in tissue.

2.1.1. Optical fiber microheaters with carbon nanotubes (OFMH-CNT)

This microheater uses a thin layer of carbon nanotubes to generate the required photothermal effects. For these experiments, we used multiwalled carbon nanotubes (CNTs) (Sigma-Aldrich, 724769) due to their ability to generate heat through light absorption [14,20]. We used a standard single-mode optical fiber (SMF-28e, NA 0.11, 8 micron core) serving as the laser waveguide and as the deposition target. The optical fiber is cleaned and cleaved to

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obtain a flat end-face. CNTs are subsequently deposited onto the optical fiber end face using an optically driven deposition technique [20]. This is achieved using a CW fiber-coupled laser diode (Thorlabs, 975nm) as the light source, with an output power of 137mW and a deposition time of 240s to guarantee an adequate layer. Finally, the optical fiber end face with the CNTs is coated with a thin gold layer (~10nm) deposited by sputtering (Cressington Sputter Coater, 108auto) using a 0.1mbar chamber pressure and a current of 40mA; Fig. 1(A) schematically depicts the fabrication process for this microheater.

2.1.2. Optical fiber microheaters with gold nanolayers (OFMH-Au)

This type of microheater incorporates a layer of gold nanostructures onto the optical fiber end face. The fabrication procedure is simpler than the previous one since after cleaning and cleaving, the optical fiber is simply coated by sputtering with a non-homogeneous thin gold layer (~10nm). This procedure is schematically shown in Fig. 1(B).

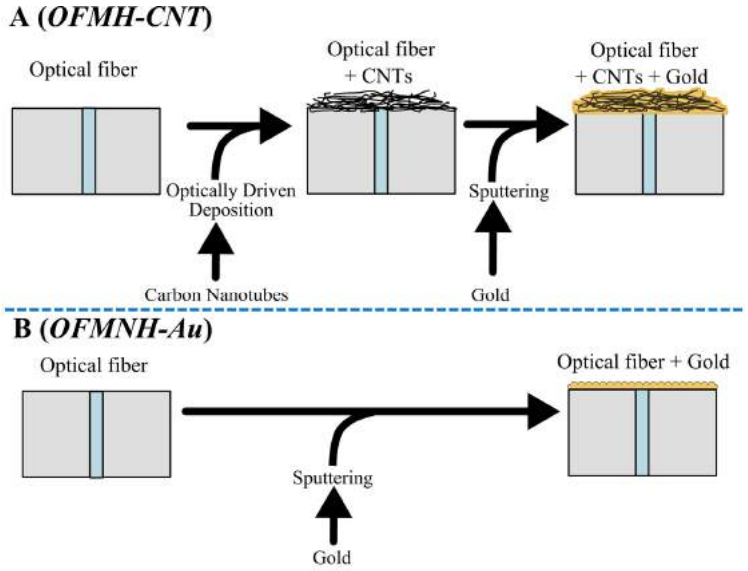

Fig. 1. Schematic of the fabrication process of the OFMH. The illustrations show the procedure followed for coating the optical fibers end faces with different nanostructures: (A) carbon nanotubes with a gold layer, and (B) gold nanolayers created by sputtering.

2.2. Evaluation of photothermal effects

Several experiments were performed to test the potential impact of the proposed microheaters. The microheaters were used to provoke light-induced localized lesions at the surface of livers extracted from laboratory mice to demonstrate the proof of concept of this technique.

Five male mice of a CD1 strain, aged 8-10 weeks and weighing from 30 to 35g were used for these experiments. The mice were sacrificed by cervical dislocation and their livers were subsequently removed from the peritoneal cavity. The hepatic lobes were sliced and placed in a Petri dish with a phosphate buffered saline solution (PBS). The samples were kept at 4°C with ice during the experiments. Animal handling was carried out according to the guidelines of the Ethics Committee of the School of Medicine of the UNAM.

The OFHM was placed directly on the liver surface to induce localized thermal lesions. A zirconia ferrule for optical fibers was used to fix the OFMH to a linear translational stage intended to draw near the device to the liver surface (see Fig. 2). The opposite ends of the OFHM were connected to a CW fiber-coupled laser diode (Thorlabs, 975nm), which was turned on for a prescribed time for each test and then turned off. Finally, the OFMH was removed from the liver, and the samples were inspected either by optical microscopy

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(DinoLite, AM7115MZT) or scanning electron microscopy (SEM). The size and other features of the lesions were subsequently measured using ImageJ software. Both types of OFMHs were tested for various laser diode powers (70-270mW) and for different irradiation times (10-240s). Table 1 summarizes the experimental conditions used for the tests performed in our experiments.

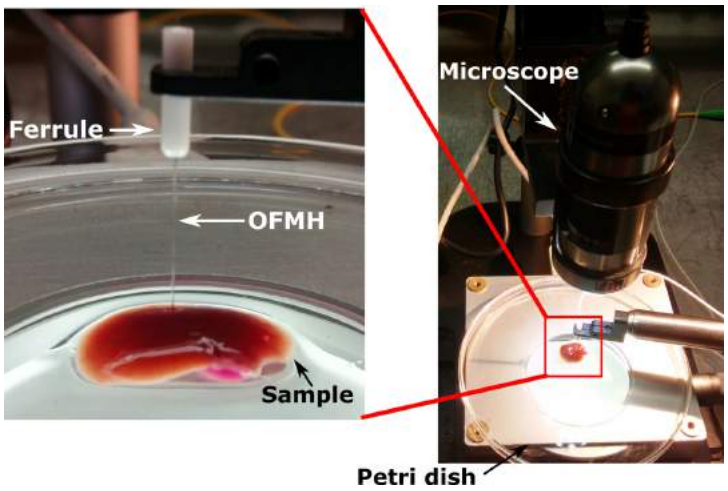

Fig. 2. Experimental setup for generating photothermal lesions in mouse liver with the OFMH.

Table 1. Experimental parameters used to obtain photothermal lesions

Mouse number	Device	Laser Power (mW)	Irradiation Time (s)
1	OFMH-CNT	70-270	10
		70-270	60
2		165.5	10-240
3	OFMH-Au	70-270	10
		70-270	60
4		165.5	10-240
5	Optical fiber (pristine)	70-270	60
		269	10-240

2.3. Biological analysis

After performing several photothermal lesions on the hepatic surface at different power levels and irradiation times, we assessed the damage to the tissue by histological analysis. Surface observations by electron microscopy were also carried out.

2.3.1. Histological assessment

For histological analysis, liver injured immediately following the application of the photothermal lesion was isolated and fixed in 4% (w/v) formaldehyde and 30% sucrose. Afterwards, the liver was sectioned in 5µm thick samples using a cryostat (Leica, CM1860 UV) and the samples were mounted onto glass slides. The tissue slides were stained with hematoxylin and eosin (H&E).

2.3.2. Scanning electron microscopy

The surface of the lesions was also observed with SEM. The samples were fixed in 0.5% glutaraldehyde in PBS buffer for 6 h and subsequently dehydrated in a series of ethanol concentrations (30-100%). Afterwards, the samples were dried in a desiccator for 4 h. Finally, the liver slices were observed with a SEM (JEOL, 7600) and digital images were captured at various magnifications for inspection.

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3. Results

Figure 3 shows an example of a typical OFMH. The microheaters are very small compared to other devices used for thermal therapies such as radiofrequency or microwave probes. The proposed devices have a diameter of 125 μm, close to the human hair diameter (70-100 μm). Previous work has reported the possibility to coat thoroughly the fiber tips with CNTs [20]. For these experiments, sputtering was also employed to deposit a final coating on the tips with thin gold nanolayers. Sputtering is a simple technique, with controlled conditions hence providing repeatable results. Gold coating provides a more biocompatible interface and further improves the adhesion of the CNTs to the optical fiber. Gold is also a good heat conductor.

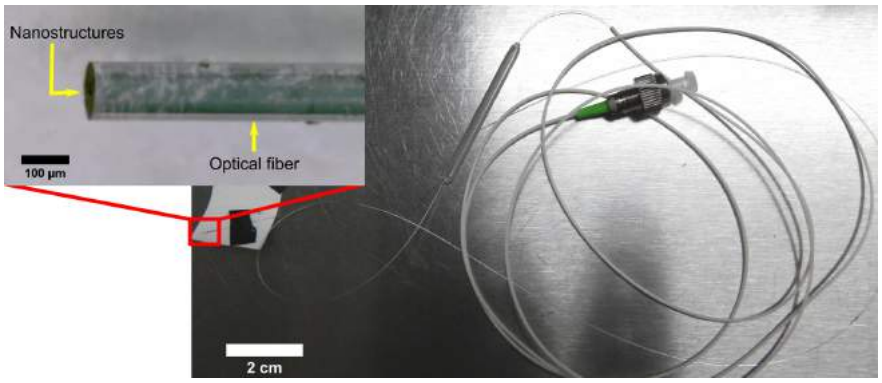

Fig. 3. Images of typical OFMH; the diameter of the microheater is 125μm.

Figure 4 shows the SEM micrographs of two types of fabricated OFMHs; for comparison, an optical fiber without nanostructures is also included in the figure. The first row shows a comparison of the surfaces obtained with the different coatings and a pristine optical fiber. The red circles in the micrographs identify the area of the optical fibers, and the green circles define the location of the optical fiber core. In the second row, we present a magnification of the surface of the OFMHs.

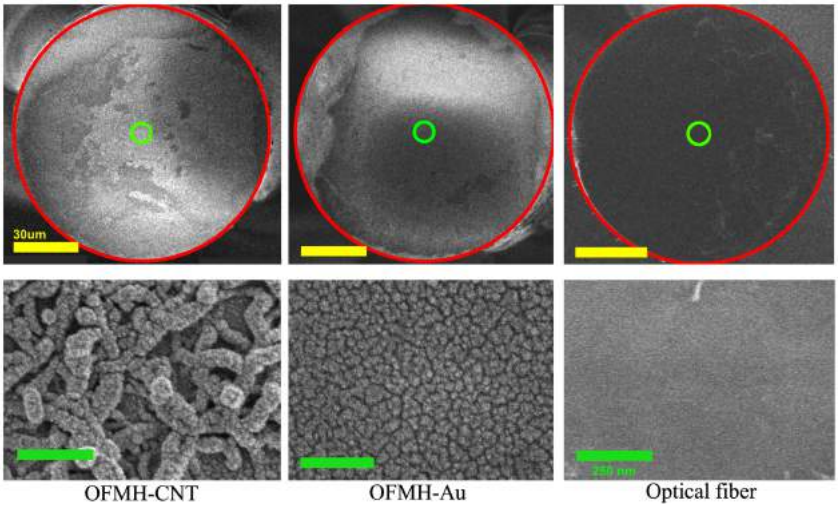

Fig. 4. Micrographs of the surface of the OFMH-CNT and OFMH-Au, compared to a pristine optical fiber.

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The qualitative effectiveness of the microheaters was measured as a function of the diameter of the lesions obtained in the liver tissues after photothermal exposure. In particular, the influence of the laser parameters (power density and exposure time) on the resulting lesion diameter was observed. Figure 5 shows an optical microscopy image with typical examples of the lesions obtained in a mouse liver with an OFMH-CNT for different optical powers while maintaining a constant exposure time (10s). The resulting lesions are circular and a strong dependency of the lesion diameter with the laser power and exposure time was observed. Similar results were obtained using the OFMH-Au tip and pristine fiber. Under some exposure conditions we were able to observe highly localized tissue carbonization (see inset in Fig. 5).

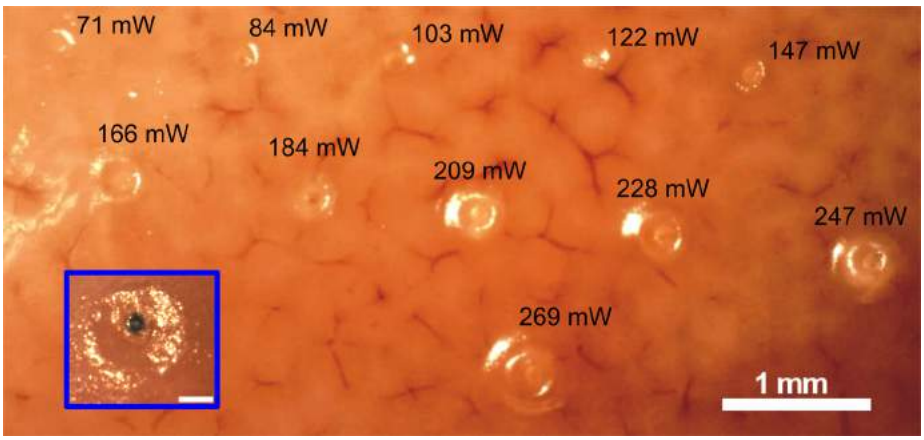

Fig. 5. Lesions induced in mouse liver by an OFMH-CNT. The exposure time was 10s. Inset: carbonization obtained with 166mW and 30s for an OFMH-CNT (scale bar: 100μm).

The resulting lesions diameters obtained on liver tissue are plotted in Fig. 6. These were obtained for both types of microheaters, using different power levels and exposure times as well as for different coating materials (see Table 1). The characteristic dimension chosen to evaluate the performance of the microheaters was the outer circle of the lesion, and this was measured using image-processing software for all the micrographs captured after treatment. As shown in the figure, the resulting experimental data for the lesion diameter as a function of laser power fits to a linear curve; in contrast, data for the lesion diameter a function of exposure time was fitted is to a logarithmic curve.

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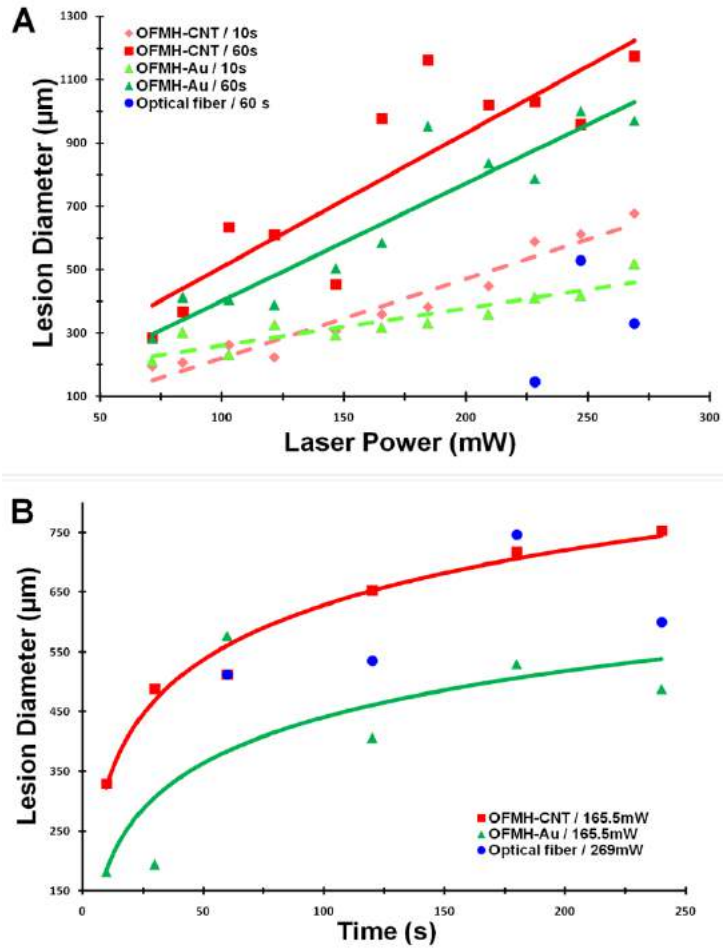

Fig. 6. Size of mouse liver lesion induced with the OFMH as a function of: (a) the optical power, and (b) exposure time. The curves corresponding to OFM-CNT are shown in red color, the curves for OFMH-Au are shown in green, blue dots corresponds to the lesions induced with pristine optical fiber. The R^2 values for the linear fit of (a, solid lines) are 0.943 (OFMH-CNT/10s), 0.762 (OFMH-CNT/60s), 0.834 (OFMH-Au/10s) and 0.868 (OFMH-Au/60s). For (b, solid lines), the resulting fitting is logarithmic with with R^2 values of 0.977 (OFMH-CNT/165 mW) and 0.834 (OFMH-Au/165 mW).

Figure 7 shows typical SEM pictures of the central region of the lesions obtained with the two types of microheaters and a pristine fiber. In the micrographs, the differences between the lesions induced with both OFMHs are evident. Further comparison of these effects can be made with Fig. 8, showing a representative image of histological sample.

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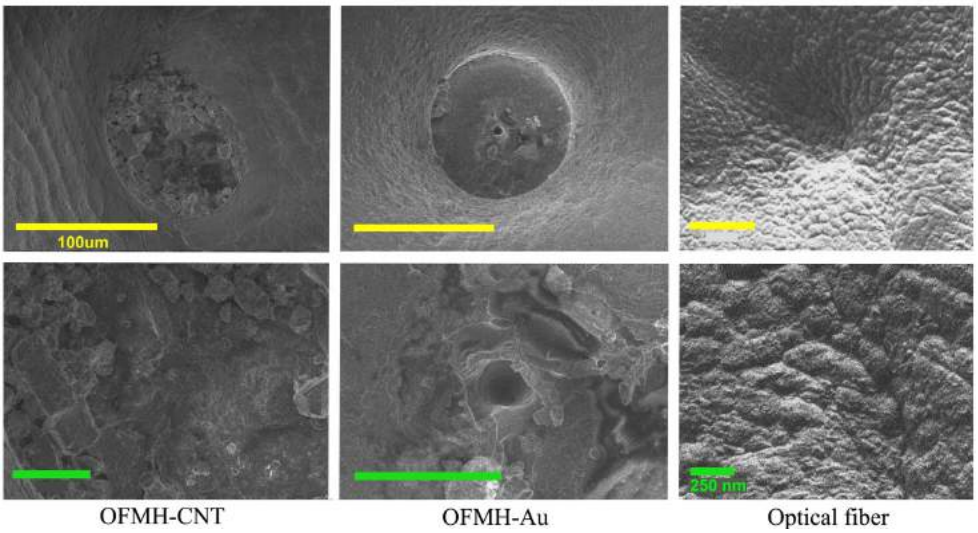

Fig. 7. SEM micrographs of lesions caused by OFMH-CNT (184mW and 60s), OFMH-Au (146mW and 60s) and a pristine optical fiber (269mW and 240s).

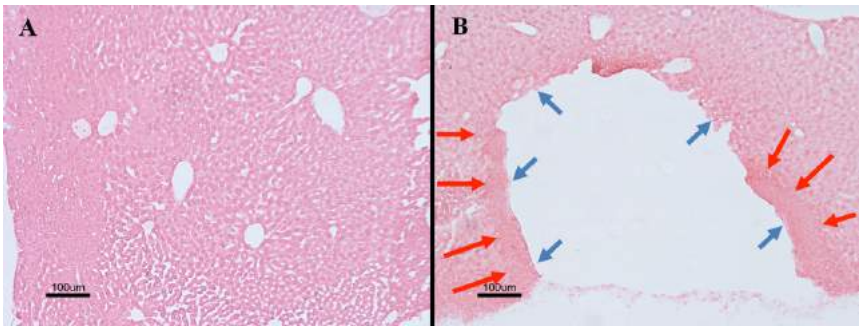

Fig. 8. A representative image of histological lesion induced by a microheater in mouse liver. A) Effect of the physical placement microheater without light, showing liver tissue integrity. B) Photothermal effects on the tissue induced by the microheater: normal histology of the liver tissue is interrupted by a lesion (the blue arrows point to the inner edge of the lesion) and a necrotic border is also observed (red arrows delimited the necrotic border). The lesion was induced with OFM-CNT (269mW and 30s).

4. Discussion

It is clear from the micrographs (Fig. 4) that the presence of CNTs greatly influences the geometry, dimensions and density of the superficial nanostructures coating the tip of the fiber. This plays an important role in the absorption process of the infrared light guided and exiting the fiber; as discussed elsewhere [20], this feature has an impact on the heat generated at the tissue-fiber interface. Gold nanolayers may be deposited by sputtering on the tip of the fibers either with or without CNTs. We found experimentally that gold adheres well to both, the surface of the fiber tip and the deposited CNTs layer when deposited by sputtering. Furthermore, we also noticed that the gold layers would not easily stick to the biological tissue after treatment. This is an attractive and desired feature to avoid potential contamination by cell uptake. Finally, we observed the formation of gold nanolayers in the OFMH-Au tips.

Figure 5 shows a typical example of the lesions. It is also interesting to note that several concentric circles were obtained inside the lesions. While the inner circle corresponds to the diameter of OFMH (see Fig. 7), the two outer circumferences correspond to a ring of a crater

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that is formed around the lesion (damage zone). Analogous concentric features are commonly observed in tissue ablation experiments, using high power lasers, with short pulses and long exposure times. Typically, the ablation zone is located at the center of the lesion, and this is surrounded by a coagulation and a carbonization zone at the limit of the ablated tissue [7, 22]. Notice also that there is no apparent extension of the lesion outside the region delimited by the diameter of the fiber: the lesion extends up to a millimeter around the OFMH (see Figs. 5 and 6).

Clearly, the observed effects arise from a temperature gradient generated by the OFMH. As seen in the plots, for both types of OFMHs the diameter of the induced lesions is proportional to the optical power launched into the optical fiber. Thus, the photothermal process that takes place at the fiber tip (i.e., within the layers of nanostructures) involving light absorption and scattering, along with heat generation and dissipation, seems to sustain a linear relation with the optical power. Concerning the size of the induced lesions, a higher optical power results in a larger diameter lesion, and a larger amount of heat leads to higher temperatures thereby producing greater tissue damage (see Fig. 6(a)).

Regarding the exposure time, Fig. 6(b) shows that the dimension of the lesion increases as well with this parameter for all the coatings used in the microheaters. Longer exposure times favors heat diffusion and dissipation within the tissue thus leading to extended damage. Although this feature is observed for both types of devices, the OFMH-CNT and the OFMH-Au, the resulting lesion diameters differ slightly owing to the differences in the photothermal characteristics of the materials. The OFMH-CNT produces lesions with larger diameters, which means that more heat is generated by these fiber tips owing to the higher photothermal efficiency of CNTs compared to that of gold nanolayers. Previous reports have shown that CNTs have a higher photothermal efficiency compared to gold nanoparticles [23] and this seems to hold for gold nanolayers as well. It is interesting to note that the use of OFMH allows for inducing lesions over a wide range of diameters. While the largest diameter achieved was around 1.2mm (OFMH-CNT, 269mW and 60s), the smallest lesion was measured to be within 200 microns (OFMH-CNT, 71mW and 10s).

In general, data scattering of the resulting diameters of the lesions shown in Fig. 6 are due to various parameters such as contact between the OFMH and liver and CNTs deposits. Also, physical contact between the microheater and the tissue can vary in depth and strength. In addition, the OFMH sometimes adheres to the tissue and the lesion geometry may change during fiber removal. Evidently, further work is needed in order to fully assess the capabilities of the OFMH for photothermal therapy. Nonetheless, our results show that the highly localized heat generated with these devices can readily induce thermal lesion in tissue.

The proposed devices do not rely on tissue absorption and heat is effectively generated at the tip of the OFMH device. Experimental data obtained with a pristine optical fiber is shown in Fig. 6 for comparison. In this case, lesions are due to the optical absorption of the tissue; chromophores of the liver (e.g., blood hemoglobin) absorb light and convert it into heat. The OFMH generates a tissue lesion through the photothermal effect due to the optical absorption of the nanostructures. The lesions induced with the pristine optical fiber are smaller than those induced with the OFMHs, and this approach clearly offers less control over the dimensions of the photoinduced lesions. Furthermore and more importantly, larger laser powers are necessary to obtain noticeable results. Hence the proposed approach does not require high-power laser systems at a predetermined wavelength.

Figure 7 shows the center of the lesions obtained for two types of OFMHs and a pristine fiber. Clearly, the damage caused by the microheaters is more defined and locally aggressive, as traces of tissue debris are not visible within the exposed region of the tissue. In contrast, irradiation with the pristine optical fiber seems to maintain the integrity in cell morphology even inside the lesion. This is consistent with a large temperature increase expected with the microheaters due to enhanced optical absorption. The lesion induced with the OFMH-Au has a cleaner appearance within the lesion. This may indicate that despite not producing as much

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heat as the OFMH-CNT, the fiber tip with gold nanolayers yields more concentrated heat owing to reduced scattering because of the absence of nanotubes. In addition, the lesion is localized within a more confined area, making this microheater a better candidate for photothermal therapy applications.

A histological test shows that by simply placing the microheater on the sample without turning the laser on preserves normal liver histology (Fig. 8(A)). Notice that hepatic parenchyma, central veins, and hepatic sinusoids are easily identified. In contrast, Fig. 8(B) clearly shows the thermal effect of the microheater, causing a lesion with the loss of tissue, and an eosinophilic border surging a necrotic border. The limits between the necrotic border and the normal hepatic tissue are well defined, suggesting a more focal necrotic process; similar results to the histological findings have been reported previously using a focal hyperthermia technique [7]. Similar results were obtained for both types of microheaters and analogous necrotic borders in the lesions were observed. The OFMHs have also been tested in other mouse tissues such as skin, muscle and brain, showing similar macroscopical results.

Our results show that the OFMHs offer an interesting alternative to induce thermal lesions with light. Among their main advantages are their size, which makes them ideal for local treatment of malignant tissue, and the use of nanostructures to enhance the photothermal effect, avoiding the use of high power lasers and reducing the time required to generate tissue damage. Other advantages include their ease of fabrication and their compatibility with laser diodes to generate lesions, requiring low power laser devices to generate hyperthermia or thermal ablation. Finally, confinement of the nanoparticles within the tip of the fiber avoids the need for injecting the nanoparticles into the body, thereby reducing potential problems associated with nanostructure toxicity. Hence, the OFMHs may render useful for hyperthermia applications or for triggering thermally triggered local biological responses in cells. Future work will consider histological assessment, lesion depth studies and cell viability tests.

5. Conclusion

The proposed optical fiber microheaters may provide a powerful tool for photothermal therapy. Our results demonstrate the ability of the OFMH to induce lesions with very limited photothermal damage in ex vivo soft tissue. The use of micron-size optical fiber microheaters coated with carbon nanotubes or gold nanolayers allow inducing thermal damage in tissue samples in direct contact with the devices. This thermal effect does not rely on the optical absorption of the tissue, and hence avoids the need of a predetermined laser wavelength, providing a versatile device to generate and deliver heat in a highly localized manner. These features may be attractive for developing hyperthermia devices that could find direct application for tumor treatments.

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