Optimizing a cold plasma catheter for the local treatment of biliary tract cancer

Manon SOULIER[1], Korentin GERAUD[1], Laura Fouassier[2], Marine CAMUS[2,3] and Thierry DUFOUR[1]
[1]LPP, Sorbonne Université, CNRS, Ecole Polytechnique, Paris, France
[2]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine (CRSA), Paris, France
[3]Sorbonne Université, Endoscopic Unit, Saint-Antoine Hospital, AP-HP, Paris France

ABSTRACT – This work consists in developing a cold atmospheric plasma (CAP) catheter designed to fit an endoscope and to demonstrate its safety to elicit local antitumor effects in cholangiocarcinoma, a cancer of the biliary tract. Such device relies on very constraining specifications to ensure electrical and thermal safety, both for patients and practitioners. In preclinical models mimicking human biliary tract, we demonstrate that our plasma catheter can safely operate in a confined environment ($Ø_{max}$ = 6 mm) in presence of a conductive liquid flow (bile). Exposure of a bile substitute to CAP leads to an increase in nitrite and hydrogen peroxide species (RONS). Presumably, these RONS induce an oxidative stress in cancer cells, leading to antitumor effects. These investigations constitute advances for endoscopic applications aimed at targeting cancer.

Mots-clés— cold plasma, cancer, digestive endoscopy, safety, cholangiocarcinoma

1. INTRODUCTION

Cancer therapy using cold atmospheric plasma (CAP) has been under investigation since the 2000s, demonstrating its antitumor potential [1]. This phenomenon is mostly attributed to the generation of intracellular reactive oxygen and nitrogen species (RONS) able to induce oxidative stress which subsequently triggers various cancer cell death mechanisms [2].

Experiments are typically categorized as "direct treatment" when the process involves a single step – plasma directly interacts with the cell or tumor substrate – and as "indirect treatment" when it involves two steps – a medium is first exposed to plasma and then applied to the cells or tumor. However, even in the single-step process, the interactions between cold plasma and cancer cells often occur indirectly. The commonly called *in vitro* CAP treatment corresponds to plating cells at the bottom of a culture plate, covering them with a liquid culture medium, leading CAP to interact with the liquid, not the cells [3]. Furthermore, for the major part of *in vivo* treatments, the tumor is grafted subcutaneously on murine models and CAP interacts with the mouse skin [4]. Direct treatment (*in situ*) is hypothesized to enhance the antitumor effects of CAP. Indeed, post-surgical plasma exposure was performed by Brullé *et al*. on orthotopic murine models of human pancreatic carcinoma, showing significant tumor growth regression [5]. Recently, a clinical trial demonstrated that CAP, used after surgical resection, significantly reduces local recurrence in Stage IV solid tumors by effectively targeting residual microscopic cancer cells, without adverse effects for patients [6].

Considering cholangiocarcinoma (CCA), an aggressive cancer of the biliary tract associated with poor prognosis and limited effective therapies, accessing the tumor site consists either in surgery or endoscopy [7]. Surgical resection, although effective, is available for only a minority of patients due to the presence of metastasis, risks of infection and complications such as hemorrhage and embolism. Otherwise, endoscopic procedures offer a conventional approach to reach the biliary tract, with endoscopic retrograde cholangiopancreatography (ERCP) being a standard method for both diagnosis and treatment of liver, gallbladder, pancreas, and bile duct diseases.

Our team previously demonstrated the antitumor effects of cold plasma *in vivo*, measuring a regression of CCA tumor growth in immunodeficient murine models [8]. Subsequently, our investigations were divided into two main areas: (*i*) the development of cold plasma catheters and assessment of their endoscopic safety in various biliary tract models, without tumor consideration; and (*ii*) the evaluation of CAP's biological efficacy and mechanisms involved in plasma-cell interaction, without endoscopy consideration. In area (*i*), the feasibility and safety of a transferred plasma catheter were assessed into a biliary endoscopy trainer reproducing the topography of the biliary tree and into the common bile duct of a digestive post-mortem porcine model [9]. In area (*ii*), a cold plasma generated by a dielectric barrier catheter device was studied both *in vitro* on CCA and microenvironmental cells, and *in vivo* in immunocompetent mice, showing that CAP impacts not only tumor cells directly but also modulates the microenvironment and induces antitumor immune responses [10].

This study is integrated in area (*i*), focusing on the feasibility of cold plasma endotherapy (Fig. 1a), specifically plasma-assisted ERCP (PA-ERCP). It involves designing a suitable cold plasma catheter capable of reaching the biliary tract safely, generating a plasma in this confined environment, addressing electrical and thermal safety concerns for both patients and practitioners and managing fluids and overpressure inside a living organism, either due to bile or helium flows. Additionally, the study aims to refine preclinical models of the biliary tract for clinical application.

2. MATERIAL AND METHODS

2.1. Cold plasma catheter

The cold plasma catheter is basically a dielectric barrier plasma jet device, featuring an elongated flexible capillary (3 m-

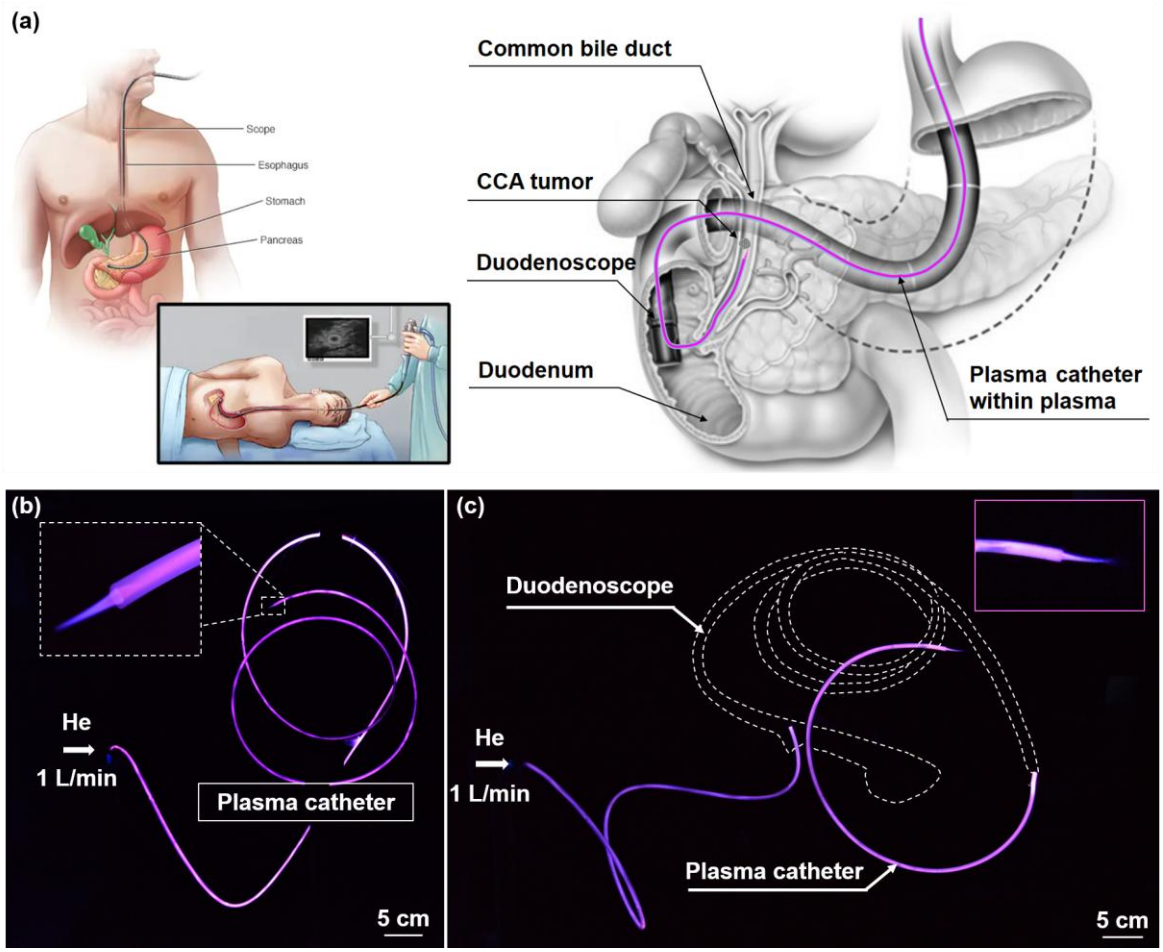

Fig. 1. (a) Schematic description of the digestive system and a PA-ERCP, intrabody plasma propagation. Plasma propagation all along the catheter, in ambiant atmosphere: (b) without duodenoscope and (c) within the duodenoscope.

length) and a reduced diameter within a high-voltage electrode embedded in a dielectric material. This device is supplied in helium (≤ 1 slm) and powered by a nanopulse generator device (Nanogen 1 – RLC Electronic) which controls the high-voltage delivered by a DC power supply (SLM 10 kV 1200 W – Spellman). This configuration allows plasma propagation all along the catheter to generate a plasma plume at its distal extremity, as depicted in Fig. 1b. All experiments are conducted with the catheter inserted inside a duodenoscope, the endoscopic device used to bring the catheter from the mouth to the biliary tract (Fig. 1a). Although the inner walls of the operating channel can present conductive parts, plasma propagation is conserved all along the catheter and a plume emerges at its distal extremity (Fig. 1c).

2.2. Preclinical models of the biliary tract

Assessments are conducted upgrading the models of the biliary tract from a physical one to a living organism in order to answer the different issues related to the generation of plasma in a patient biliary tract:

- **Artificial bile duct (ABD) model**: a copper tube associated to a resistance-capacity parallel circuit is selected to reproduce the biliary tract impedance based on RLC measurements realized on PDP model (R = 300 Ω – C = 400 pF). Voltage-frequency couples are tested in this configuration to ensure safety parameters: bile duct leakage current (I_{RMS} < 100 µA) and lateral wall temperature (T ≤ 40°C). In addition, a bile substitute (H_2O + NaCl) with the bile conductivity (σ = 10 mS/cm) can be inserted in the tube. Nitrite (NO_2^-) and oxygen peroxide (H_2O_2) content are assessed in this liquid to evaluate the antioxidant ability of plasma under realistic and penalizing scenarios. While the realistic scenario corresponds to a catheter's outlet not immerged in the bile substitute, leaving plasma to interact with ambient gas before reaching the liquid, the penalizing scenario concerns catheter's outlet totally submerged in the bile substitute.
- **Post-mortem digestive porcine (PDP) model**: a human-like digestive system used to check the catheter insertion, the previously defined safety parameters and to deal with unexpected issues such as electromagnetic interferences. Experiments are performed on 3 PDP models with the guidance of a professional endoscopist.
- **Live porcine (LP) model**: a living organism in which plasma delivery is provided directly into its common bile duct thanks to a PA-ERCP. Experiments are conducted on 4 LP models, 2 with a direct autopsy 20 min after PA-ERCP and 2 with waking-up, monitored during 40 days before autopsy at The Fondation Carpentier, Hôpital Européen Georges Pompidou (HEGP, Paris) with the guidance of professional endoscopist and veterinarian (Ethics number: APAFIS #44177-2023041311592688 v4). Vital constants such as cardiac frequency and oxygen saturation are recorded all along PA-ERCP. Blood samples of the waking-up LP are analyzed at days 0, 1, 7 and 40 of the monitoring.

2.3. *Diagnostics and analyses*

The leakage current is calculated from the voltage measured across ABD model with a probe (Teledyne LeCroy PP020 10:1) and monitored using an analog oscilloscope (Rohde & Schwarz HMO 3004). An infrared-camera (JENOPTIK & INFRATEC VarioCAM HD head 680/30) is used to determine the copper wall temperature in the ABD model and the biliary epithelium temperature in PDP models.

Nitrite and hydrogen peroxide levels are quantified using colorimetric assays with Griess reagent and Titanium Oxysulfate IV (Sigma-Aldrich, Saint-Quentin Fallavier), respectively, to detect reactive oxygen and nitrogen species (RONS) in the bile substitute. Samples (200 µL) are mixed with 50 µL of reagent and analyzed after 10 minutes using a Biotek Cytation 3 spectrophotometer, measuring absorbance at 548 nm for NO_2^- and 409 nm for H_2O_2.

Cardiac frequency and oxygen saturation are measured using a patient monitor (Philips Intellivue X2). LP serum is analyzed by a chemistry analyzer (Beckman Coulter AU 480).

3. Results and Discussion

3.1. *Defining safety and plasma efficiency parameters*

First measurements concern a mapping of the leakage current (I_{RMS} – eq. 1) transmitted to the ABD model, varying voltage-frequency couples of the pulse generator for a constant duty cycle of 10 %.

$$I_{RMS} = \sqrt{\frac{1}{T}\int_{t_0}^{t_0+T}\left(\frac{V_{ABD}(t)}{R}\right)^2 dt} \qquad (1)$$

The leakage current corresponding to the 40°C-thermal threshold depends on voltage-frequency couples. Whatever the frequency, incrementing voltage induces a rise of the leakage current (data not shown), so that the augmentation of the voltage-frequency couple increases this current too, up to drastic values ($I_{RMS} \geq 100$ µA) which is forbidden here. As depicted in Fig. 2, the leakage current at the 40°C isotherm stays below 35°C, indicating that the temperature is the more restrictive parameter to ensure the safety, *i.e.* without cell thermal damages.

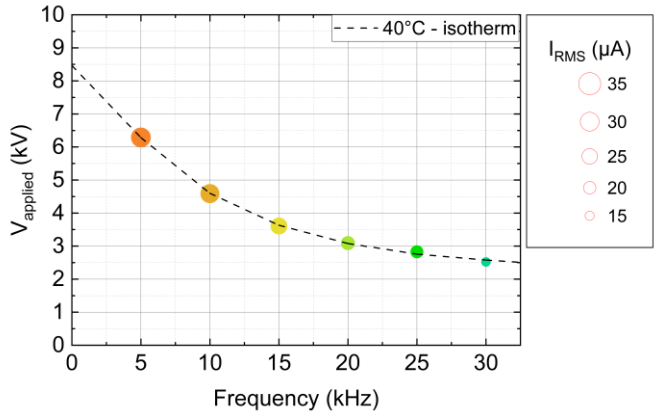

Fig. 2. Leakage current as a function of applied voltage-frequency couples from 0 to 10 kV and 0 to 30 kHz for the 40°C isotherm.

The RONS content in the bile substitute of the ABD model is quantified by the titration of nitrites (NO_2^-) and hydrogen peroxide (H_2O_2) species considering two configurations: a realistic one where a gap separates plasma and bile substitute and a penalizing one where the catheter's outlet is submerged in bile substitute. As shown in Table 1, whatever the scenario, plasma induces an increase of RONS content. As expected, in the penalizing configuration, nitrites are not produced because plasma is not interacting with air molecules. Furthermore, a rise of frequency to 10 kHz, increases as well the RONS content in the liquid (data not shown).

Table 1. Nitrite and hydrogen peroxide species in bile substitute, not exposed (CTRL) or exposed to plasma (CAP: 6 kV – 5 kHz – 10 %) during 10 min.

Configurations	$[NO_2^-]$ (µmol/L)		$[H_2O_2]$ (µmol/L)	
	CTRL	CAP	CTRL	CAP
catheter-bile substitute gap (realistic)	0.00 ± 0.00	1.9 ± 0.5	2 ± 4	19.4 ± 5.9
Catheter submerged in bile substitute (penalizing)	0.00 ± 0.02	0.00 ± 0.05	1.380 ± 0.002	8.3 ± 3.9

These investigations conduct to a selection of safety values (5 to 10 kHz and 5 to 7 kV) that can also be efficient to produce RONS, agents for cancer therapy.

3.2. *Ensuring the safety on a biological model*

These parameters have been verified in the PDP model before investigating living organisms. The feasibility of cold plasma endoscopy is validated by generating plasma within a healthy common bile duct. The PDP model is depicted in Fig. 3, the digestive system can be observed on the left panel, and plasma emission inside its common bile duct is highlighted on the right panel, visible through the epithelial tissue. The orange hue is not the actual plasma color, which is more purple-magenta as seen in Fig. 1, but results from viewing through epithelial tissue and the camera's integration time.

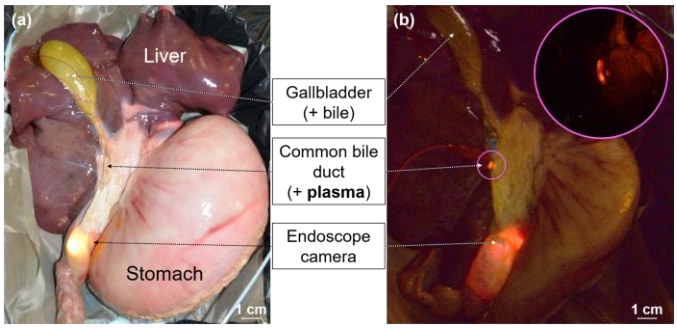

Fig. 3. Photography of the PDP model and plasma generation in its biliary duct: (a) at ambient light and (b) in dark to see plasma through the biliary epithelium.

The PDP model simulates a confined environment with bile flow, allowing to assess electrical and thermal safety for both patients and practitioners, while also managing fluid dynamics. As mentioned in Fig. 2 for the ABD model, the temperature is the more restrictive property to ensure the safety. Comparing ABD and PDP models for a plasma exposure time of 10 min, ABD model begins at ambient temperature, reaching a threshold temperature of 38°C after a 10 min-plasma exposure. Unfortunately, PDP models are conserved in freezers and cannot reach ambient temperature when the experiments are performed. The PDP models begin at 13°C and the epithelium reaches 20°C after 10 min of plasma exposure. In Fig. 4, the variations of temperature based on the temperature at plasma ignition are compared on ABD and PDP models. This representation suggests that this plasma exposure condition is safe because in the biological organism, the temperature increase is less drastic than in the physical and highly conductive model.

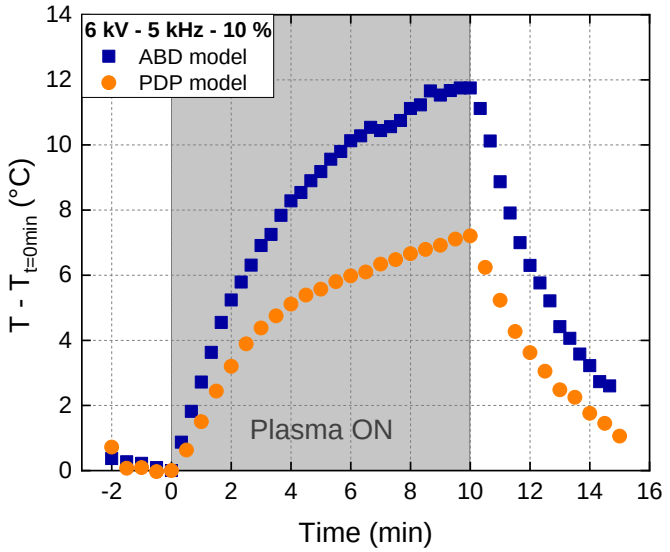

Fig. 4. Temperature variations comparing ABD and PDP models for a plasma generated by 6 kV, 5 kHz, 10 %; the thermal reference is the temperature measured at t = 0 min.

3.3. *Performing a PA-ERCP on a living organism*

In the LP model, the endoscopy practitioner achieves a PA-ERCP with a precise plasma delivery. The safety of the model is ensured during and after the procedure, as shown in Fig. 5. Cardiac frequency as well as oxygen saturation remain constant from 10 min before plasma exposure to 15 min after it. At the end of ERCP, cholangiograms of the biliary tract, *i.e.* X-ray scan of an injected contrast agent, demonstrate no perforation of the biliary epithelium.

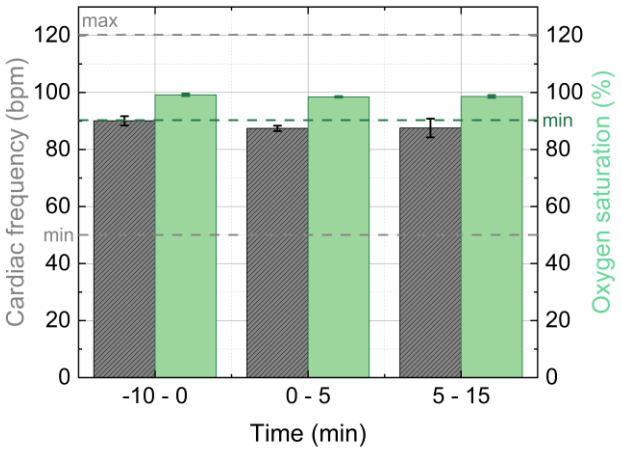

Fig. 5. Vital constants monitored during PA-ERCP for 4 PDP models: cardiac frequency and oxygen saturation.

After PA-ERCP, 2 LP models are directly autopsied while the other 2 are waking-up as in conventional procedures, without complication. All autopsies, realized directly or 40 days after PA-ERCP, reveal no macroscopic damage, as illustrated in Fig. 6.

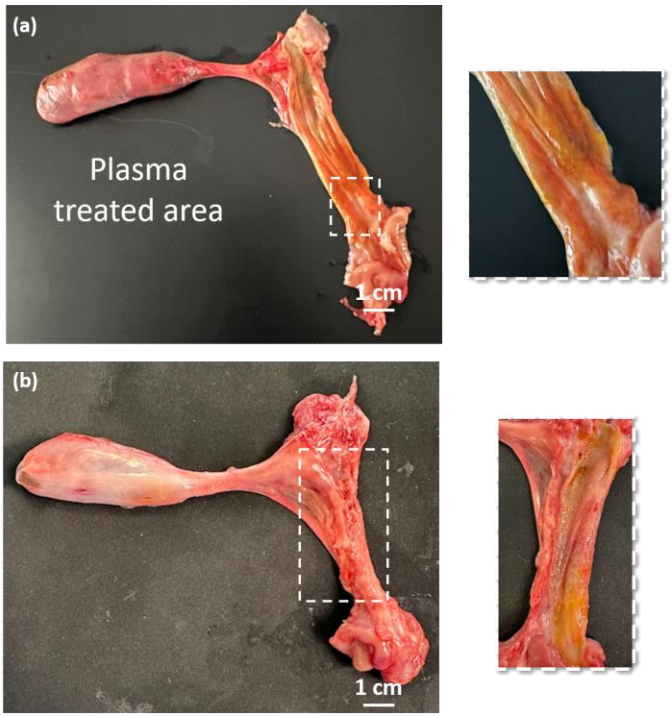

Fig. 6. Macroscopic observation of the biliary tract: (a) directly after PA-ERCP and (b) 40 days later.

Blood samples of the waking-up LP models collected 4 times during the 40 days of monitoring reveal no anomaly.

In the two biological models, electromagnetic interferences appeared on the duodenoscope camera and monitoring screens due to plasma generator.

4. Conclusions

This study shows that incorporating CAP technology into endoscopic devices is both feasible and safe. Addressing significant issues in preclinical models of biliary tract, the results comfort CAP's potential as an innovative *in situ* treatment approach for cholangiocarcinoma. Future research aims to refine the device electromagnetic compatibility and launch clinical trials, in order to translate these promising findings into practical medical applications.

5. Acknowledgments

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