PDT and UVC – lung graft decontamination

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Inactivating hepatitis C virus in donor lungs using light therapies during normothermic ex vivo lung perfusion

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Availability of organs is a limiting factor for lung transplantation, leading to substantial mortality rates on the wait list. Use of organs from donors with transmissible viral infections, such as hepatitis C virus (HCV), would increase organ donation, but these organs are generally not offered for transplantation due to a high risk of transmission. Here, we develop a method for treatment of HCV-infected human donor lungs that prevents HCV transmission. Physical viral clearance in combination with germicidal light-based therapies during normothermic ex-vivo Lung Perfusion (EVLP), a method for assessment and treatment of injured donor lungs, inactivates HCV virus in a short period of time. Such treatment is shown to be safe using a large animal EVLP-to-lung transplantation model. This strategy of treating viral infection in a donor organ during preservation could significantly increase the availability of organs for transplantation and encourages further clinical development.

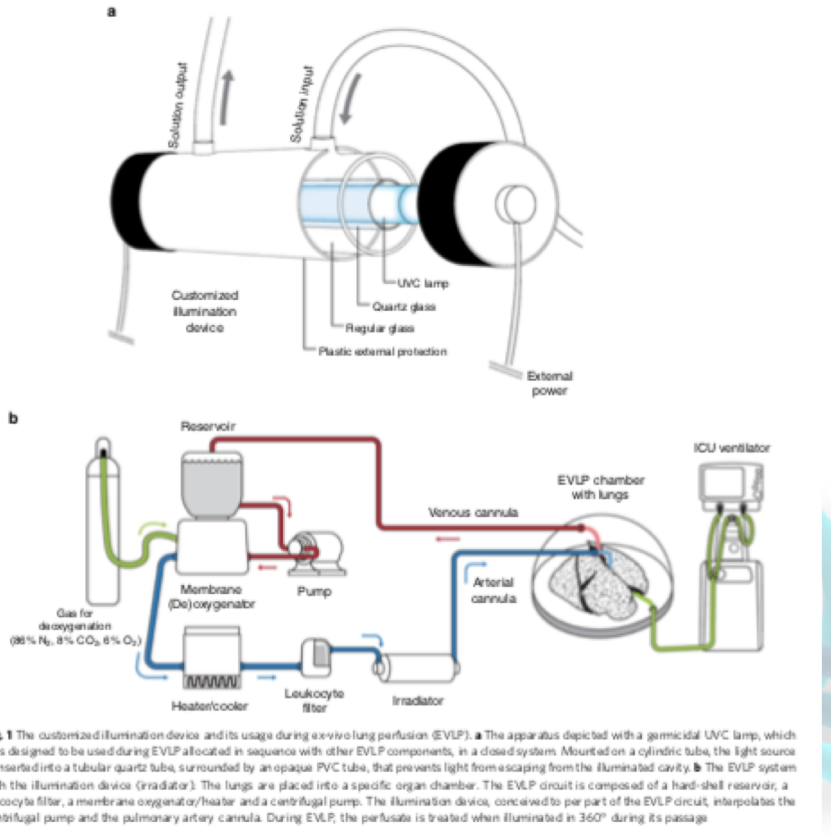
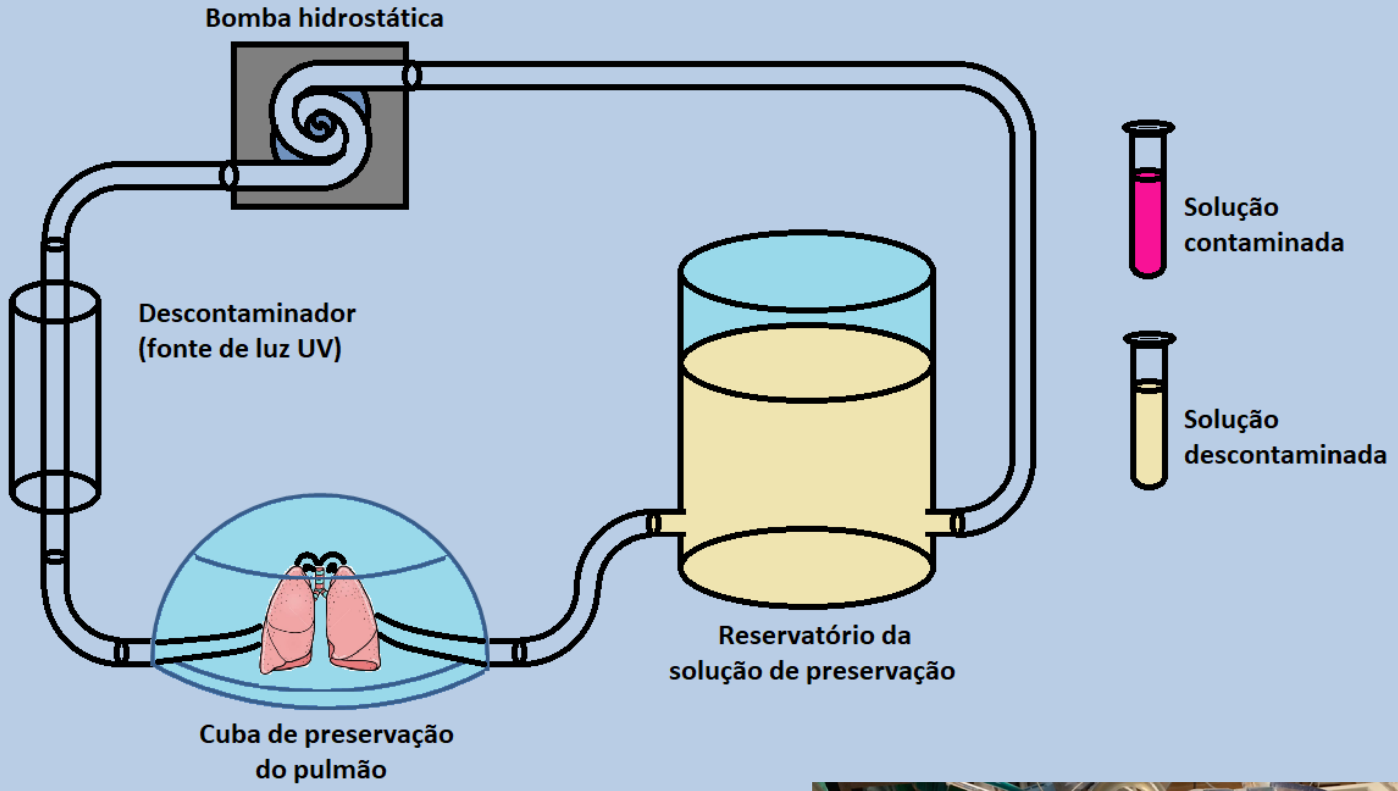


Fig. 1 The customized illumination device and its usage during ex-vivo lung perfusion (EVLP). a The apparatus depicted with a germicidal UVC lamp, which was designed to be used during EVLP allocated in sequence with other EVLP components, in a closed system. Mounted on a cylindric tube, the light source is inserted into a tubular quartz tube, surrounded by an opaque PVC tube, that prevents light from escaping from the illuminated cavity. b The EVLP system with the illumination device (irradiator). The lungs are placed into a specific organ chamber. The EVLP circuit is composed of a hard-shell reservoir, a leucocyte filter, a membrane oxygenator/heater and a centrifugal pump. The illumination device, conceived to be part of the EVLP circuit, interpolates the centrifugal pump and the pulmonary artery cannula. During EVLP, the perfusate is treated when illuminated in 360° during its passage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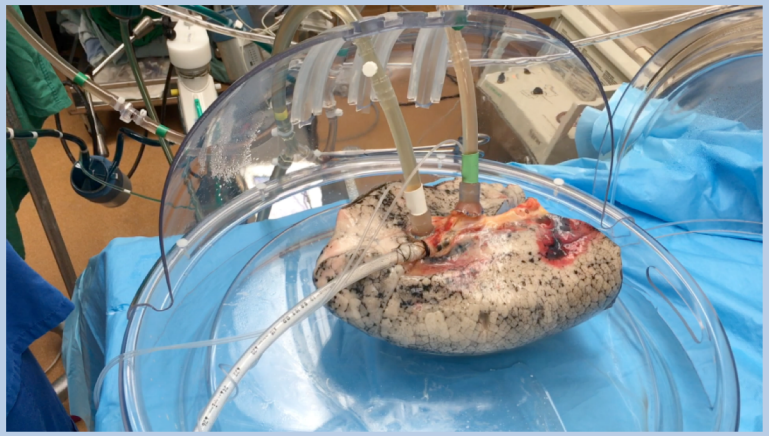


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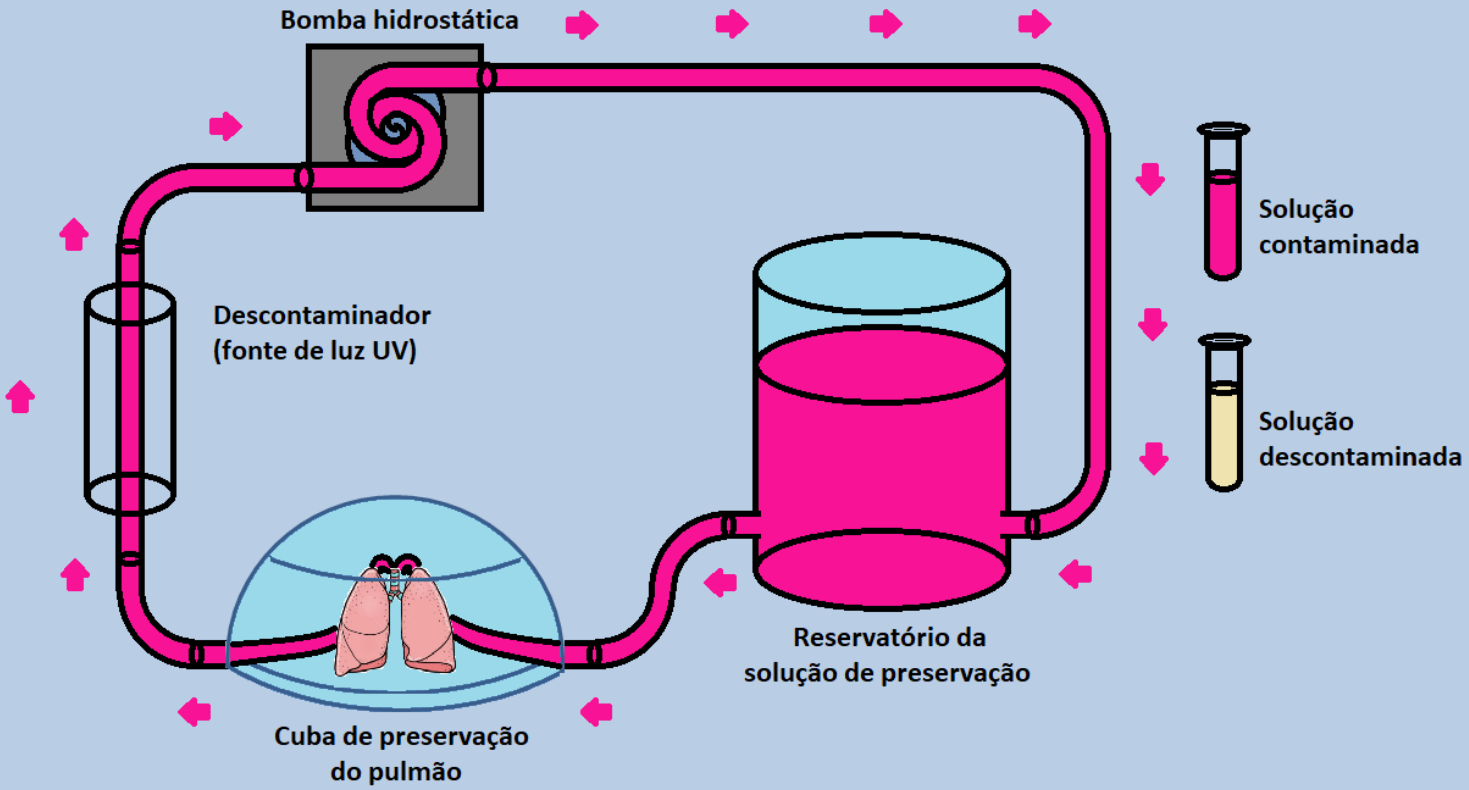
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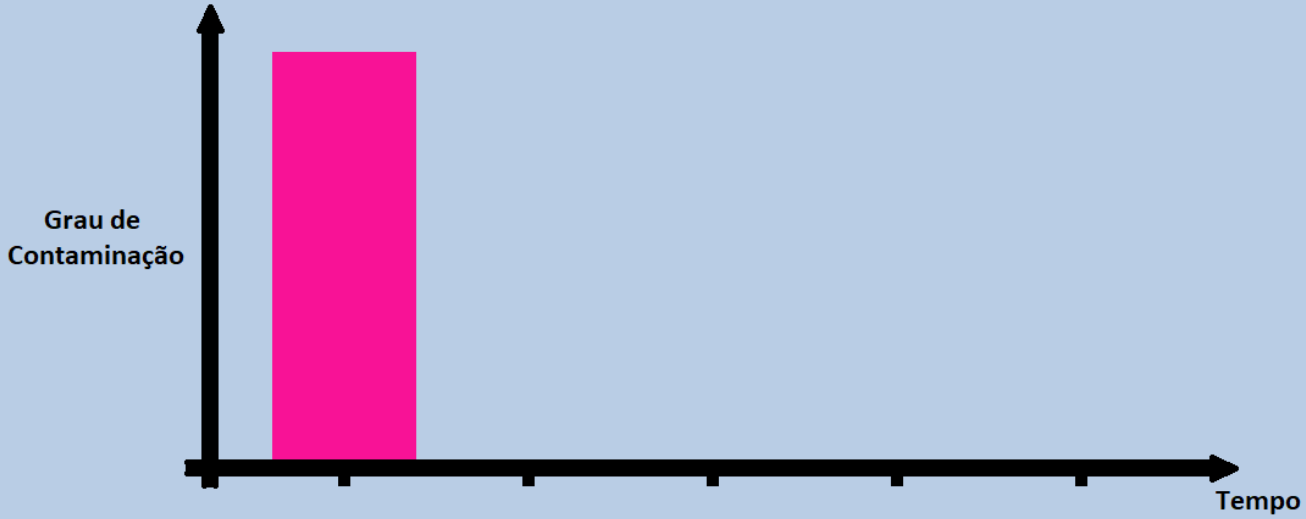
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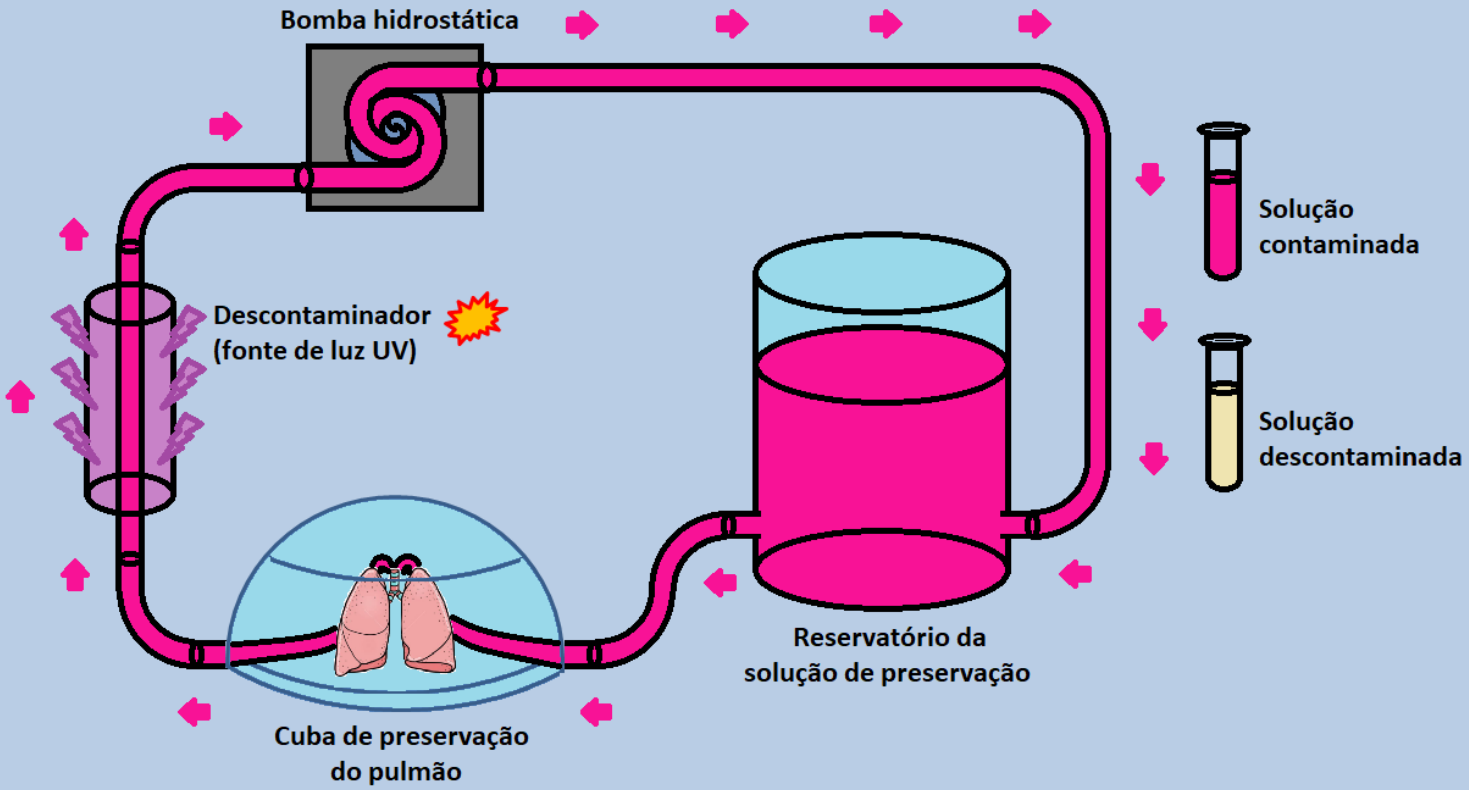
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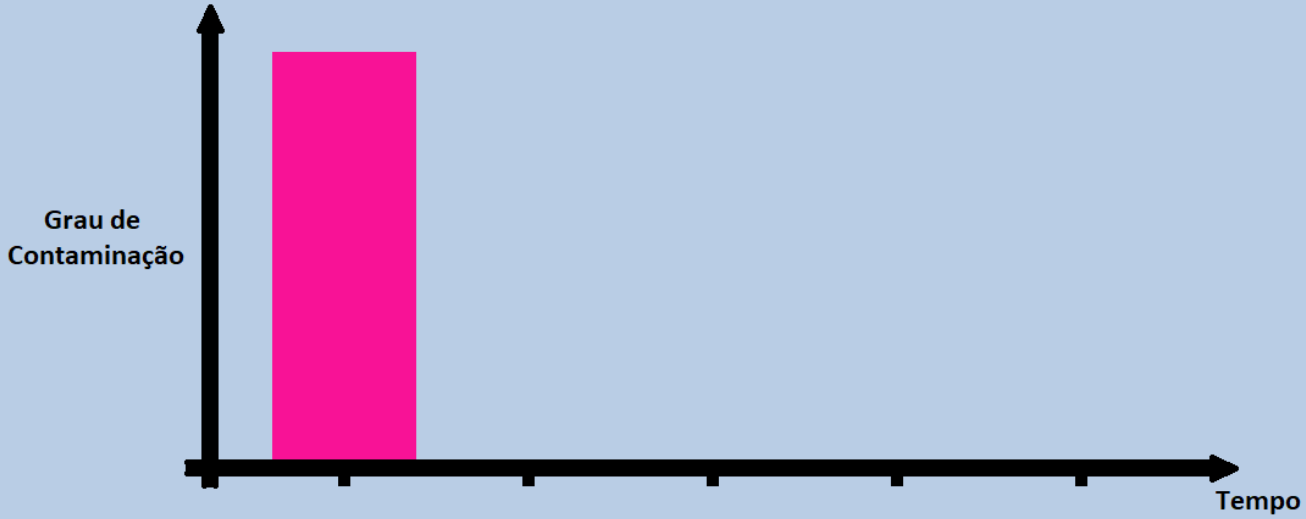
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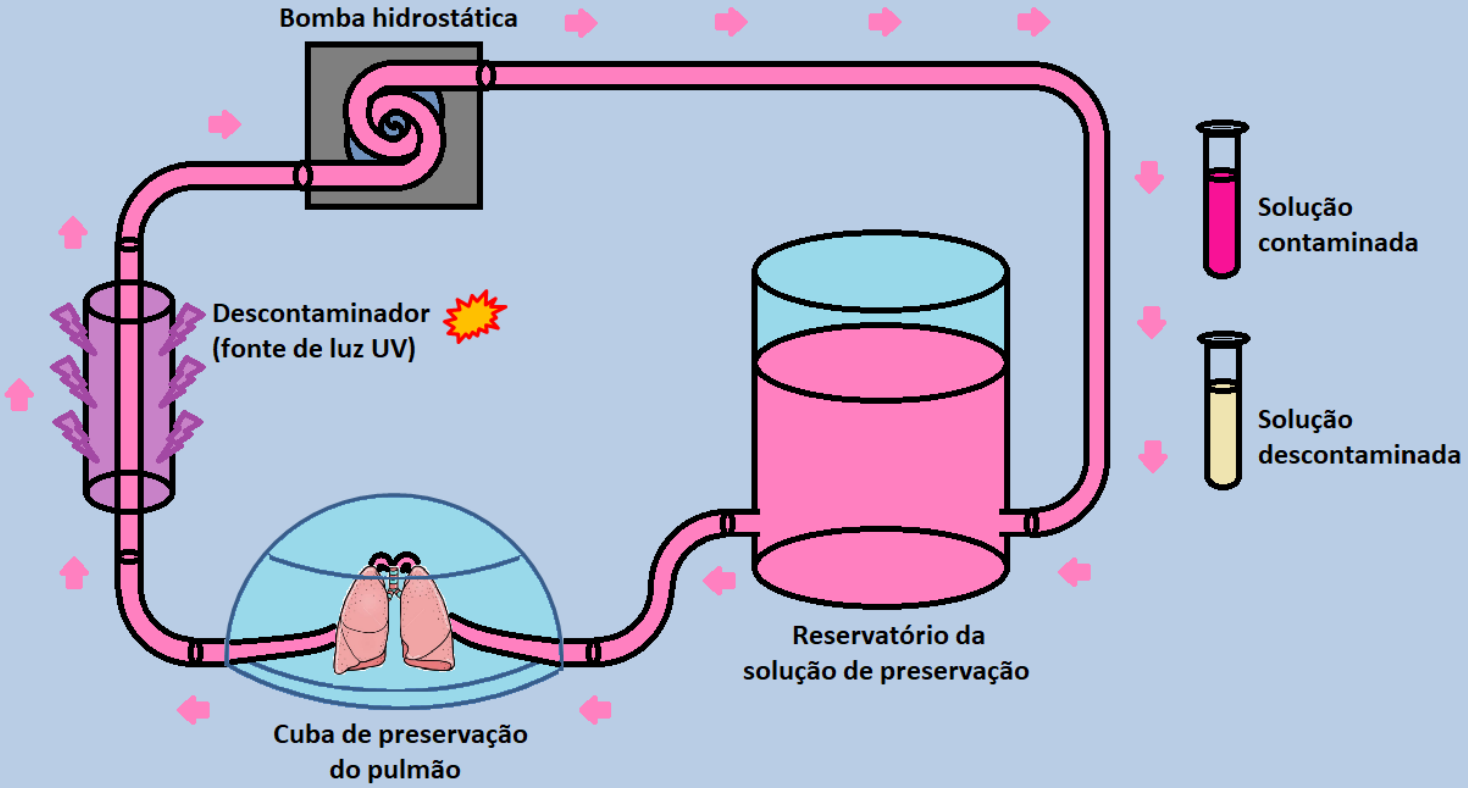
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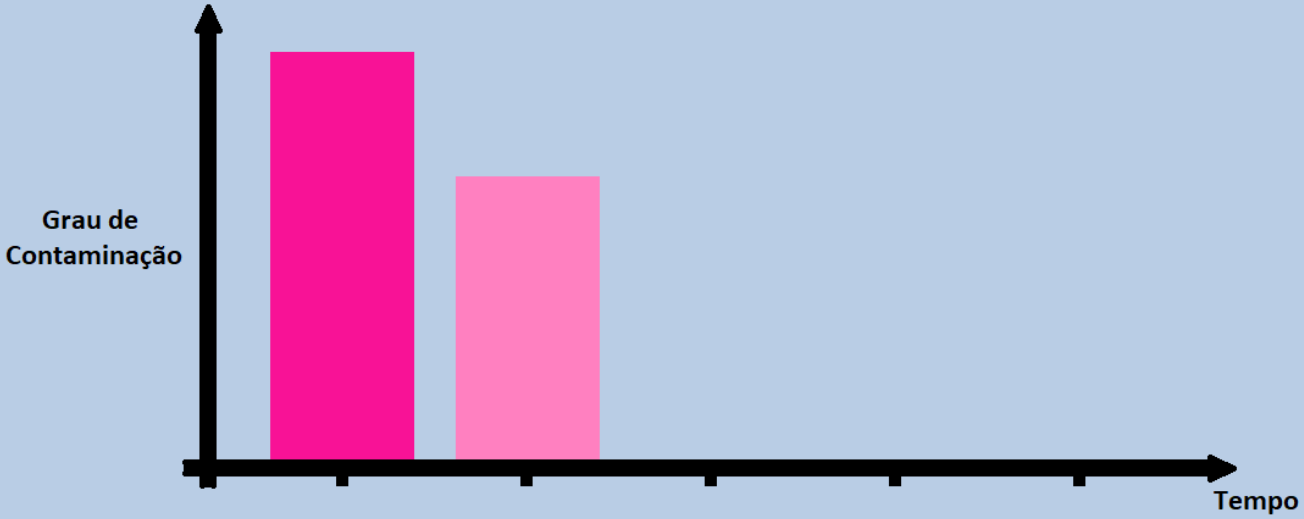
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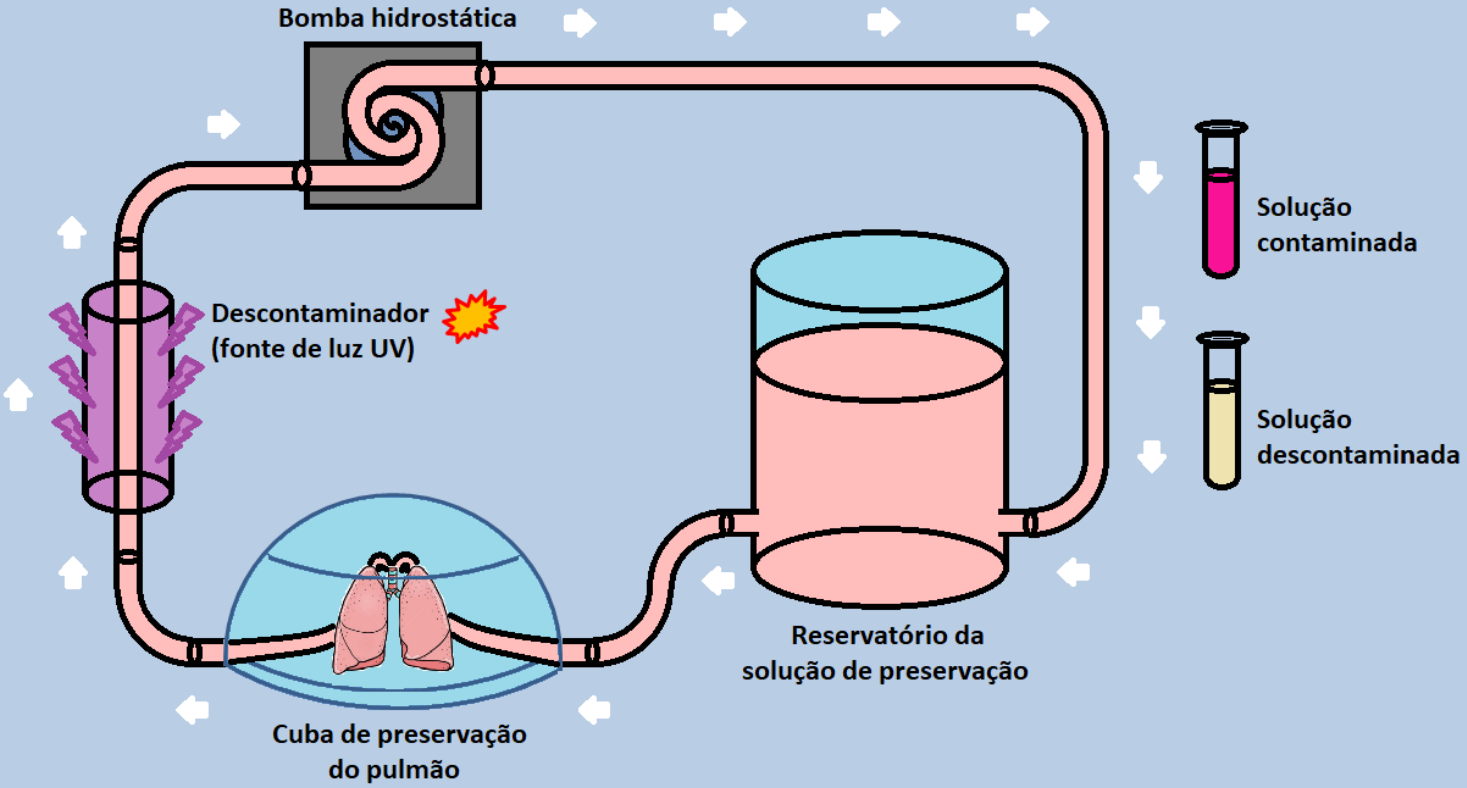
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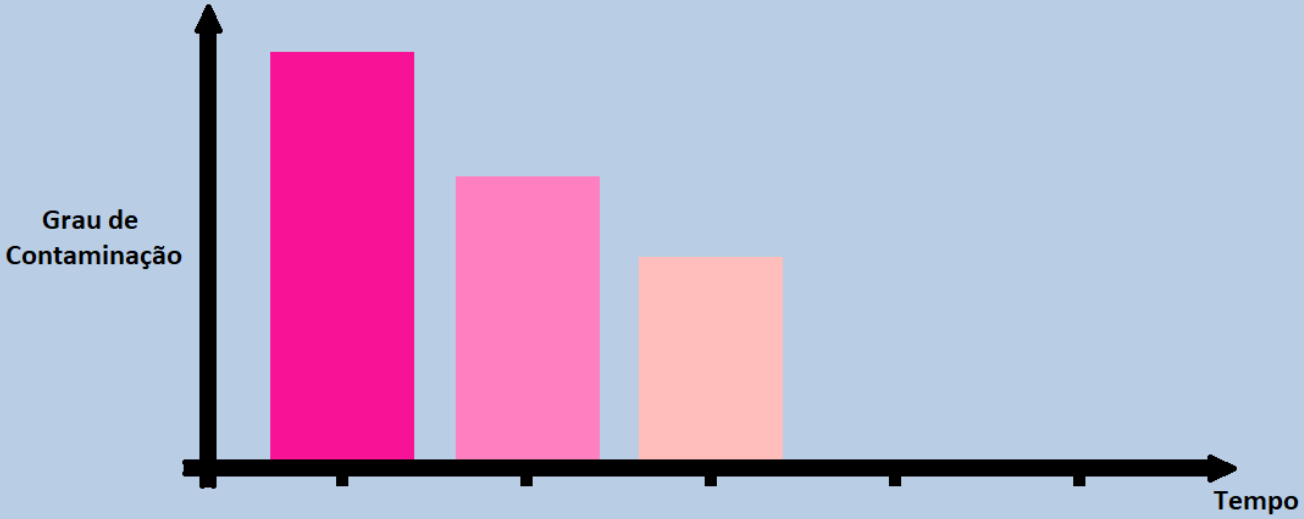
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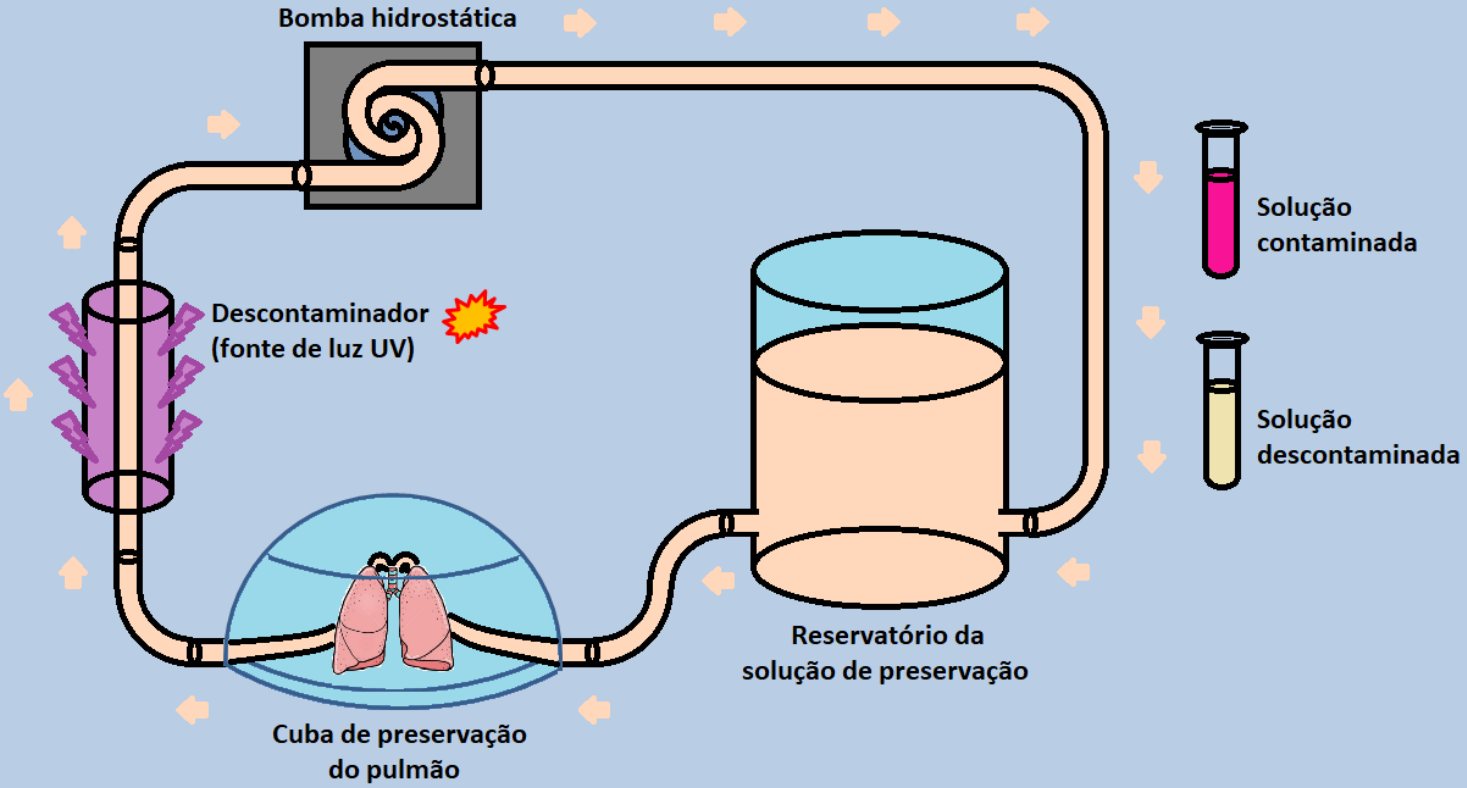
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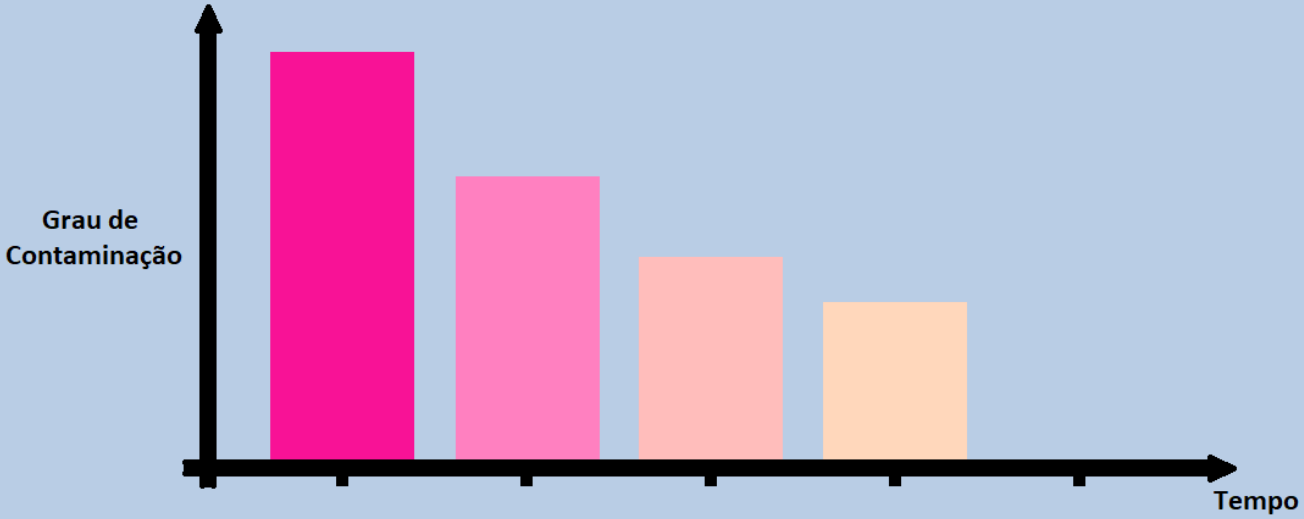
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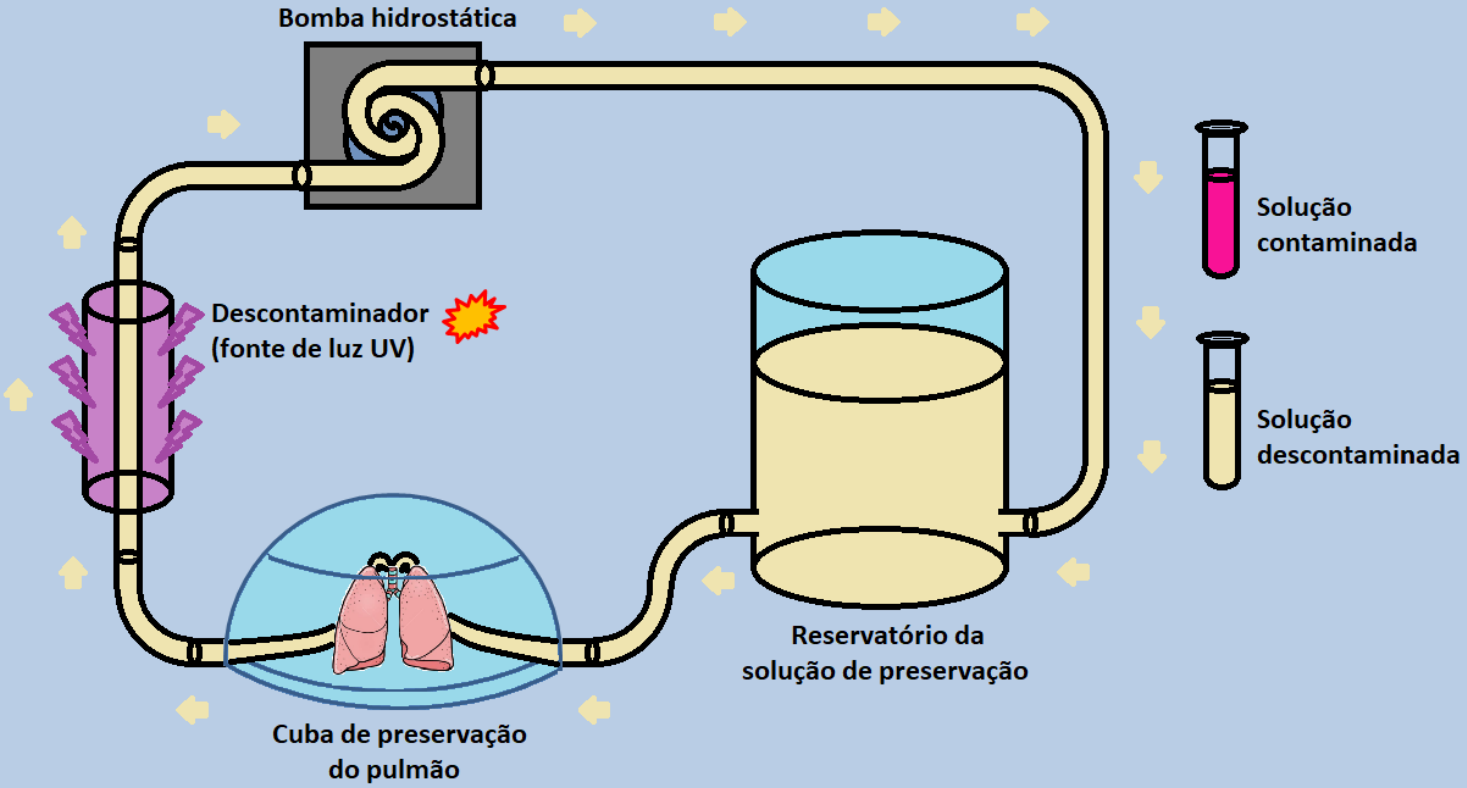
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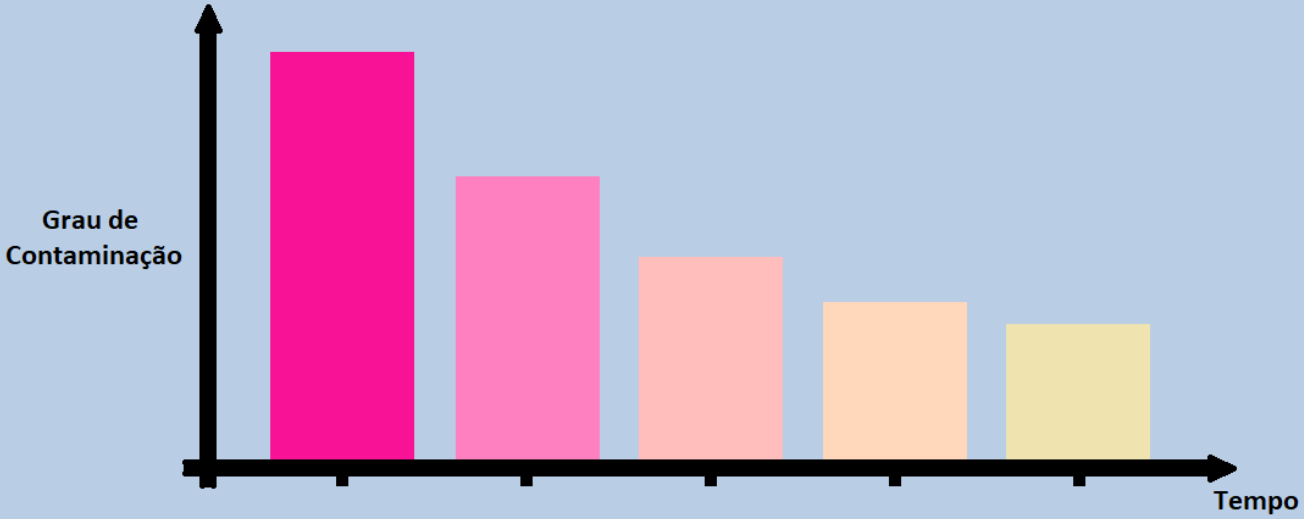
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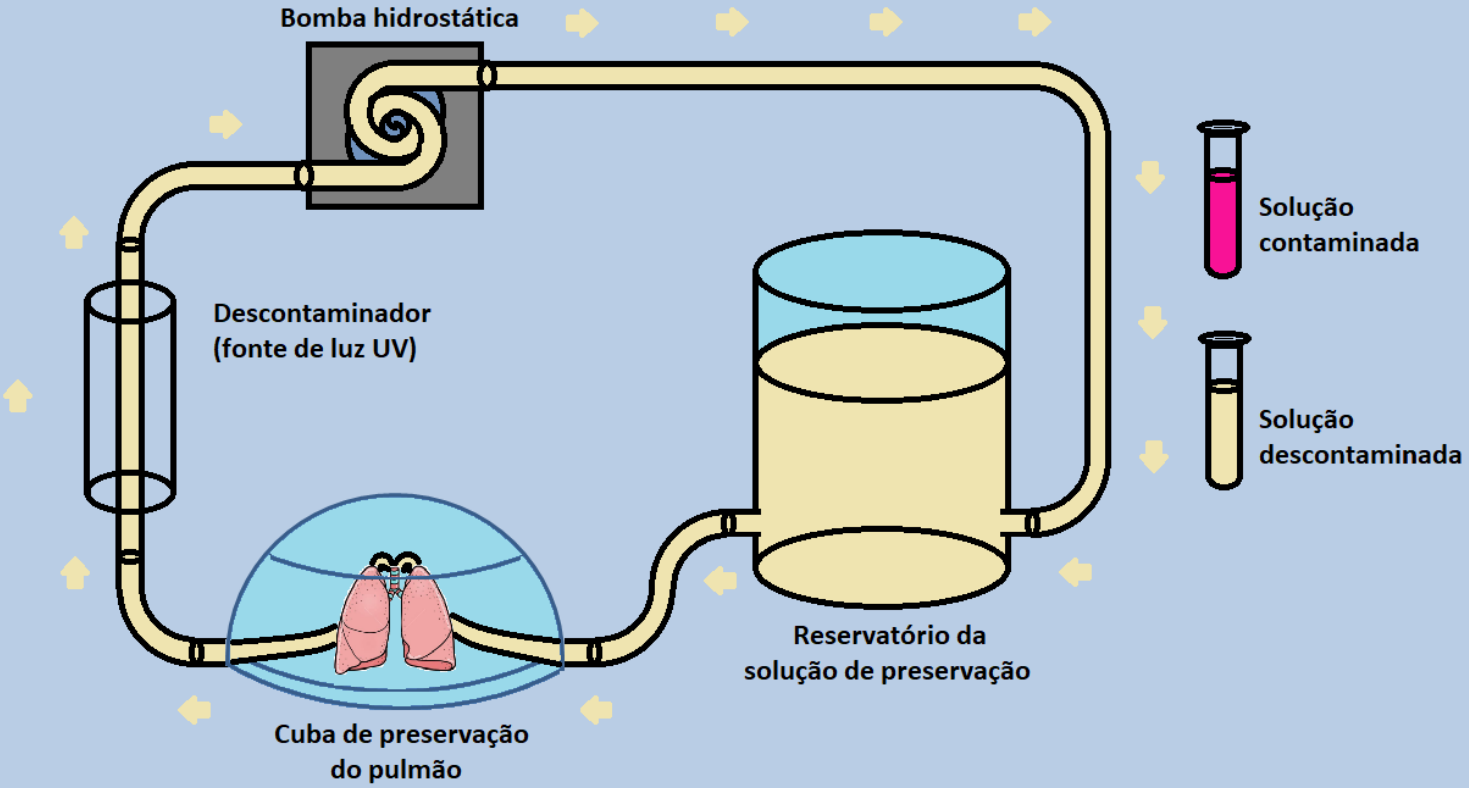
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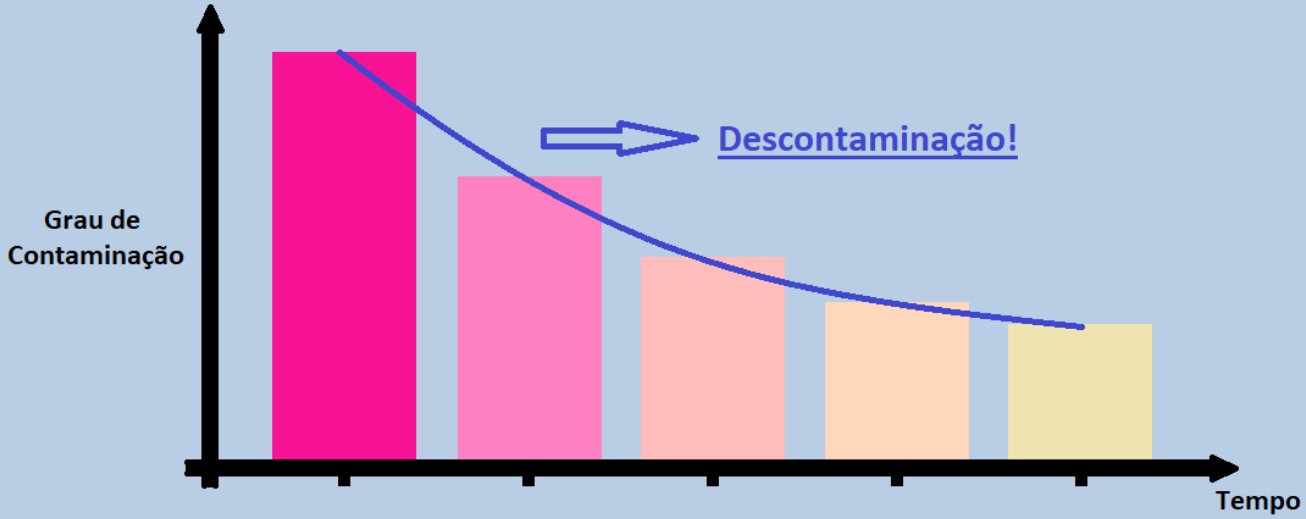
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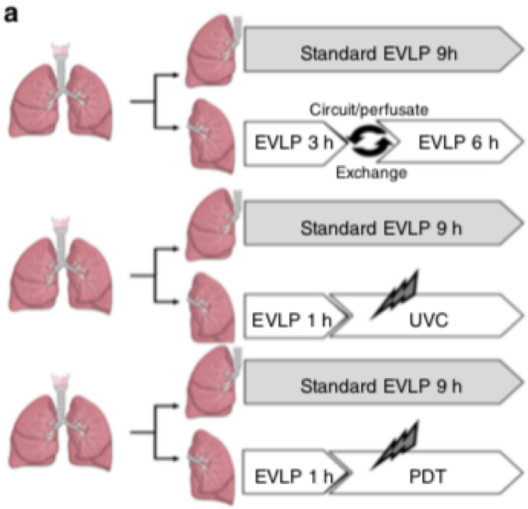

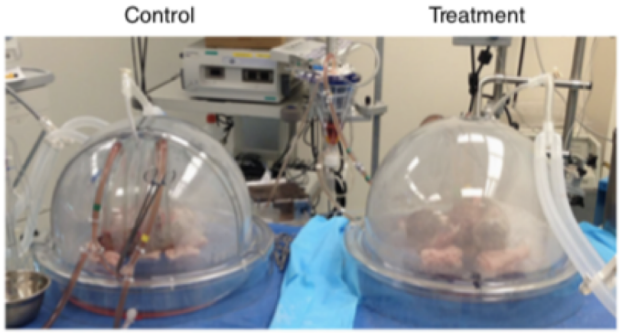

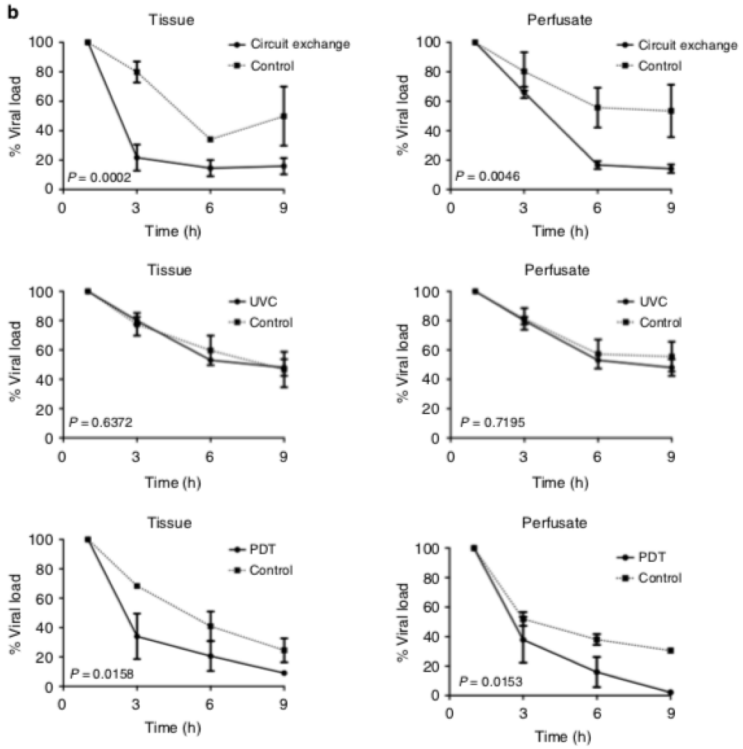

Fig. 2 Effect of EVLP and light-based therapies (LbT) on HCV RNA levels in HCV NAT + human donor lungs. **a** Paired study design: Lungs from same donor were separated to 2 distinct EVLP systems under different treatment conditions (n = 3, each): standard EVLP (control) vs. treatment (circuit exchange, UVC or PDT). **b** Effect of EVLP and associated treatments towards perfusate and lung tissue HCV levels measured by qPCR during 9 h of treatment. Lung tissue and EVLP perfusate measurement results were normalized for percentage of viral load decrease from baseline and presented as mean ± SEM. The two-way ANOVA statistical test was used for analysis. EVLP: ex vivo lung perfusion

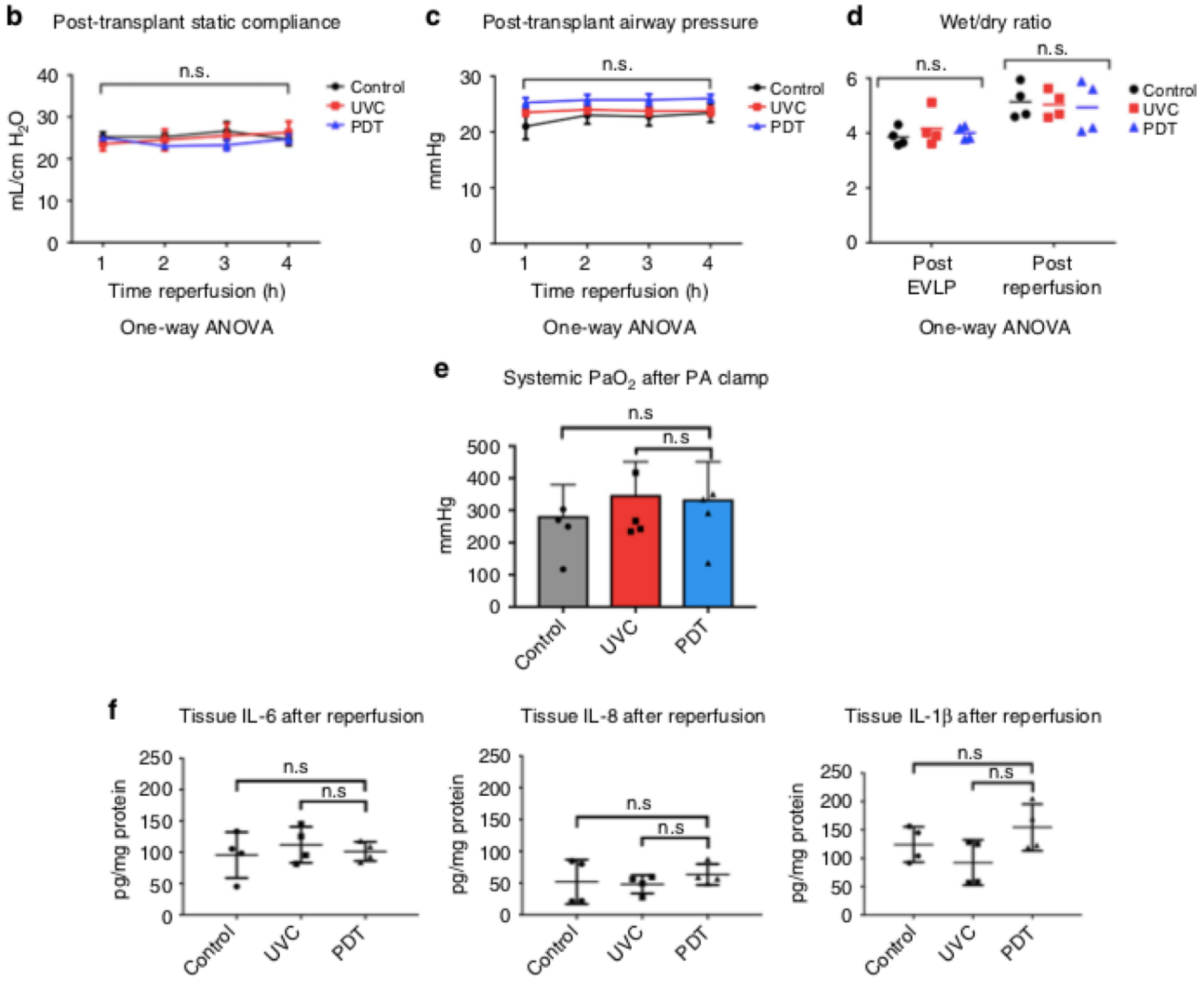

Fig. 5 Pre-clinical large animal safety studies using EVLP/LbT treatments. **a** Schematic of a pre-clinical EVLP and lung transplantation model, designed to assess potential acute lung injury in donor lungs after LbT applied during EVLP ($n = 4$, each group): (1) Control (standard EVLP technique); (2) UVC (254 nm; 31 mW/cm²); (3) PDT, using 1μmol/L MB diluted in the perfusion solution associated with red light irradiation (660 nm; 20 mW/cm²). **b**–**e** Lung function parameters after left lung transplantation (N.S. after one-way ANOVA statistical analysis). **f** Graft inflammatory cytokine assessment in lung tissue after transplantation (N.S. after one-way ANOVA statistical analysis. Error bar indicate standard deviation)

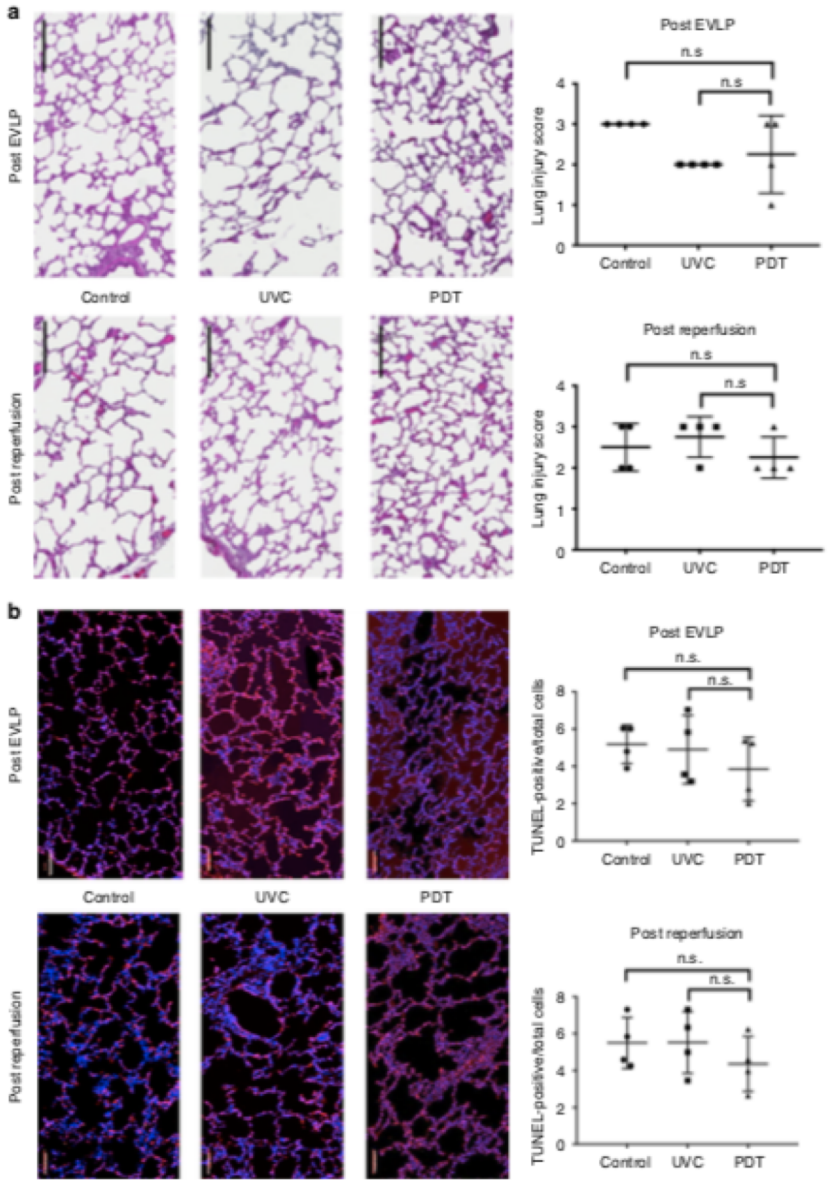

Fig. 6 Pre-clinical large animal safety studies using EVLP/LbT treatments. **a** Lung injury score after transplantation, scale bar = 100 µm. **b** Cell death assessment (TUNEL) after transplantation, scale bar = 400um (N.S. after one-way ANOVA statistical analysis). MB: methylene blue; CIT: cold ischemia time; PDT: photodynamic therapy; Ultraviolet C (UVC) irradiation; EVLP: ex vivo lung perfusion; LbT: Light based therapy. Error bar indicates standard deviation

Prevention of viral transmission during lung transplantation with hepatitis C-viraemic donors: an open-label, single-centre, pilot trial

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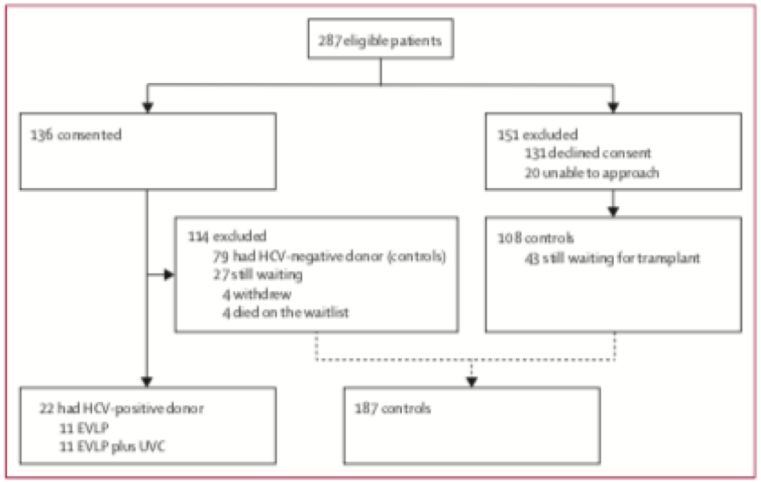


Figure 1: **Trial profile**
EVLP=ex-vivo lung perfusion. HCV=hepatitis C virus. UVC=ultraviolet C.

	HCV-negative donors (n=187)	HCV-positive donors (n=22)
Age, years	52 (30–65)	33 (29–37)
Donor from the USA	11 (6%)	16 (73%)
P/F, mm Hg	424 (366–489)	383 (315–495)
Donor was a smoker	88 (47%)	20 (90%)
Donor had a cardiac death	50 (27%)	2 (9%)
Ex-vivo lung perfusion	65 (35%)	22 (100%)

Data are median (IQR) or n (%). P/F=partial pressure of oxygen/fraction of inspired oxygen.

Table 1: **Donor characteristics**

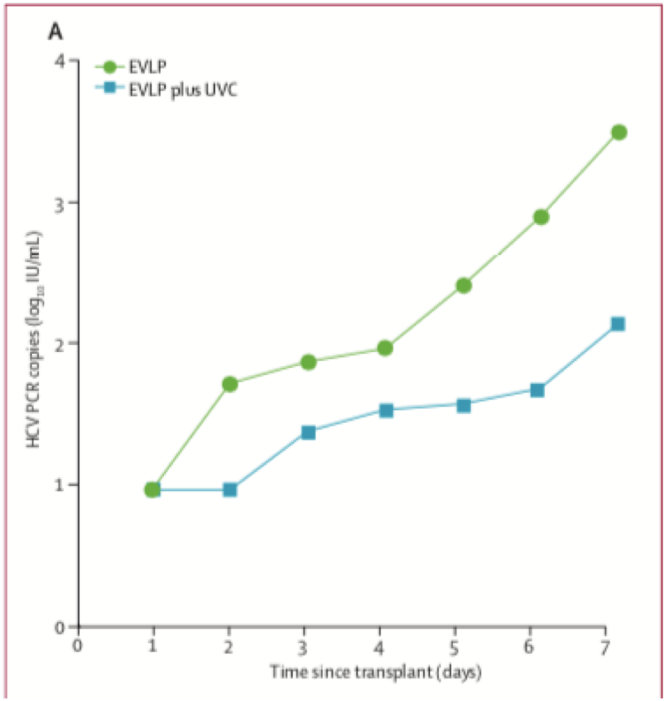
	HCV-negative recipients (n=187)	HCV-positive recipients (n=22)
Age, years	60 (47–67)	65 (60–68)
Reason for transplant		
Interstitial lung disease	108 (58%)	13 (59%)
Chronic obstructive pulmonary disease	47 (25%)	7 (31%)
Cystic fibrosis	24 (13%)	2 (9%)
Status 2–3	125 (67%)	11 (50%)
Positive crossmatch	26 (14%)	4 (18%)
Single lung	30 (16%)	11 (50%)
Waitlist* time, days	42 (13–169)	17 (13–133)

Data are median (IQR) or n (%). *Canadian waitlist status: status 1, patients stable on low concentration of oxygen; status 2, patients requiring high oxygen concentrations or pulmonary hypertension or both; status 3, patients with a rapid decline in lung function, generally hospitalised on ward or in intensive care.

Table 2: **Recipient characteristics**

day-1 viral load, e is the natural logarithm, x is time variable, and a is the slope of the curve when plotted on a logarithmic axis. We assigned viral loads that were less than the limit of quantitation to a nominal value of 1·0 $\log_{10}$ IU/mL. We calculated viral load doubling time for each patient using the equation (ln2)/a. We compared doubling times between groups using the Mann–Whitney *U* test. All statistical analyses were done with GraphPad Prism 8 or R 3.5.0, with summary statistics within groups presented as median values with IQRs (for numerical data) or counts and percentages (for categorical data).

PDT and UVC – lung graft decontamination



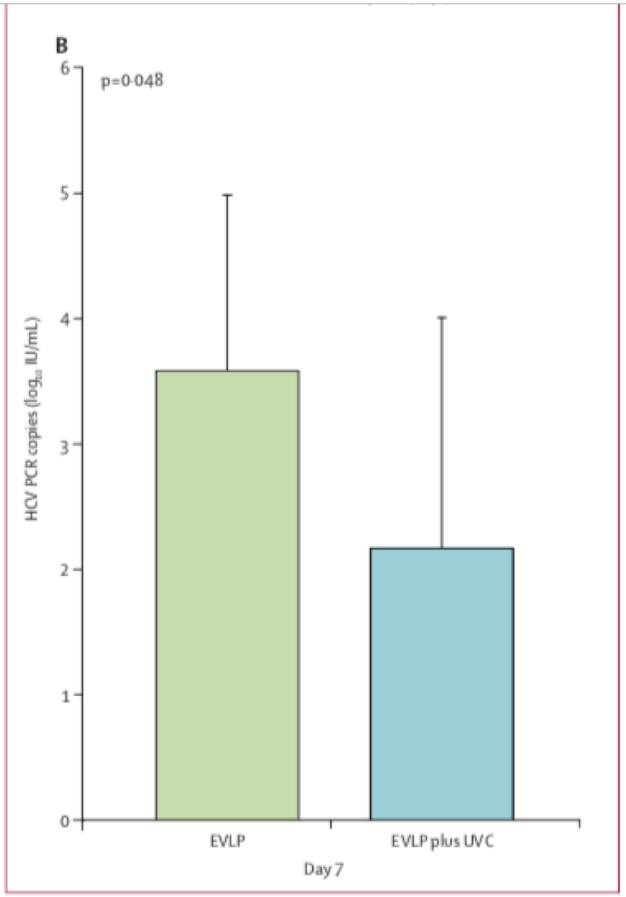


Figure 4: **Serum viral load in the first week and at day 7**
Medians presented with IQRs. (A) Kinetics of HCV viraemia in first week after transplantation in recipients receiving HCV-positive donor lungs. (B) Recipient median viral load at day 7 after transplantation in EVLP or EVLP plus UVC groups. EVLP=ex-vivo lung perfusion. HCV=hepatitis C virus. UVC=ultraviolet C.