

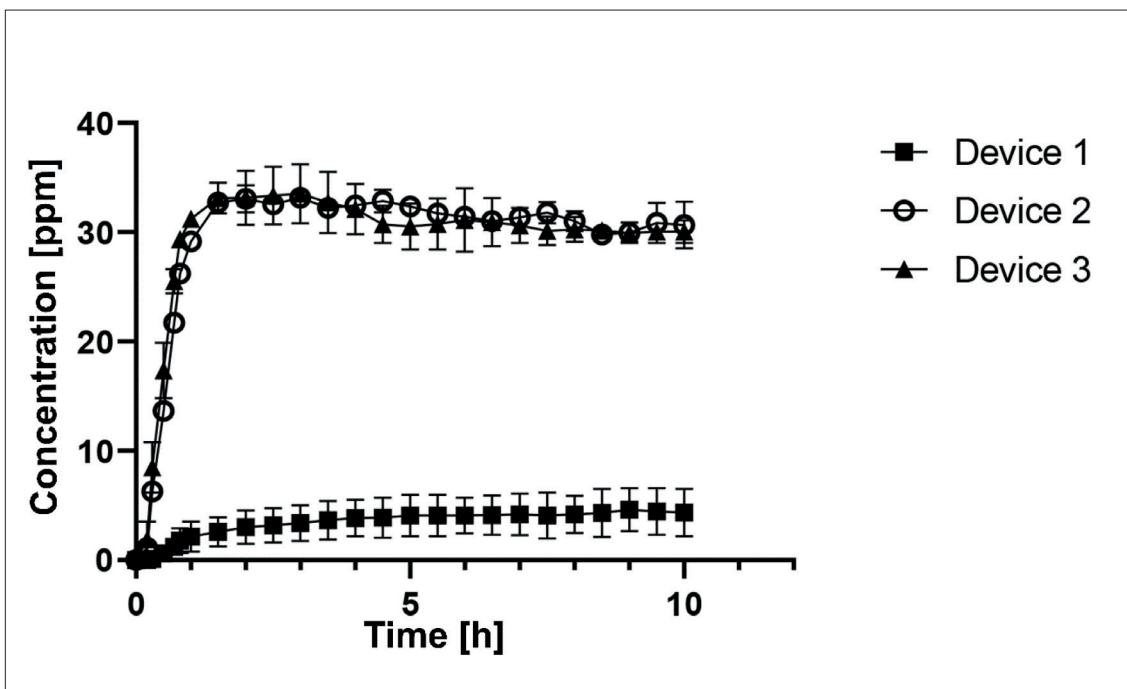
RESEARCH ARTICLE

3D-printed devices for optimized generation of cold atmospheric plasma to improve decontamination of surfaces from respiratory pathogens

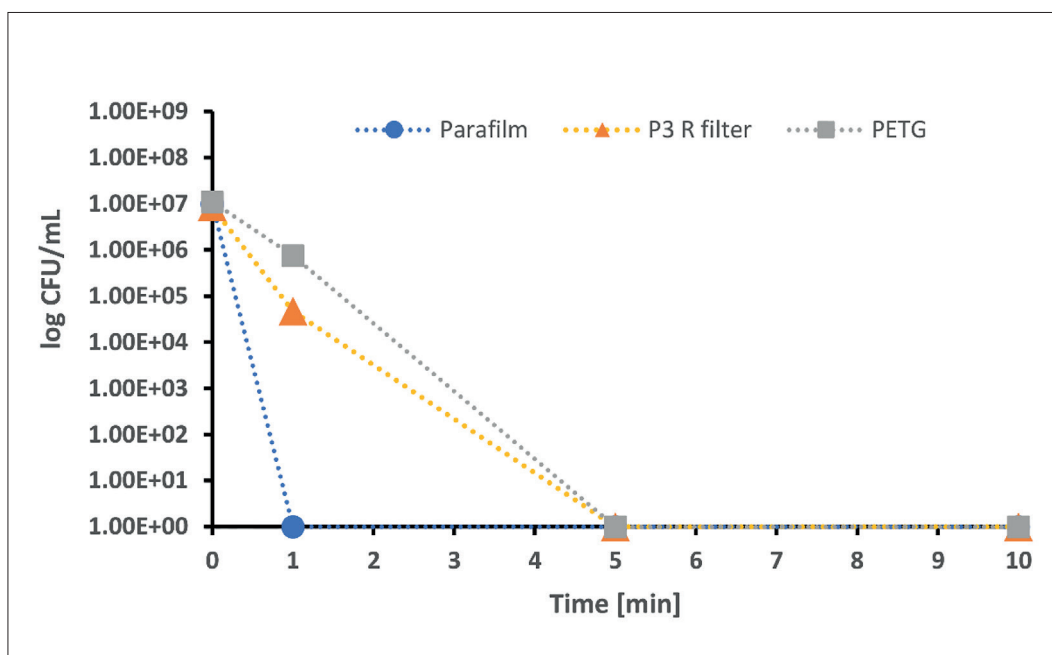
Supplementary File



**Figure S1.** Photographs displaying the external appearance of 3D-printed cold atmospheric plasma (CAP)-generating devices: side view (top) and front view (bottom). Device 1: original version; Device 2: optimized version enclosed in a chamber with a double discharge; Device 3: XXX. The optimized devices (i.e., devices 2 and 3) have holes around the discharges to enhance air and particle flow. They also feature a wall that separates the discharge part from the airflow, making the discharge chamber smaller and the particle atmosphere more concentrated.



**Figure S2.** Concentration (c) of ozone (O<sub>3</sub>) in the gaseous chamber of devices 1, 2, and 3 over time (t). The measurements were taken using an ultraviolet (UV)-100 ozone analyzer (range: 0.01–999 ppm; accuracy: 2%; Eco Sensors, USA), utilizing a 254 nm wavelength absorption on the UV spectral line. The results are consistent with those obtained from the Fourier transform infrared spectroscopy (FTIR) spectra (Figure 3), indicating that devices 2 and 3 produce substantially more O<sub>3</sub> than device 1.



**Figure S3.** Cold atmospheric plasma (CAP)-mediated decontamination of 3D-printed materials. To validate the ability of CAP to decontaminate 3D-printed objects, polyethylene terephthalate glycol-modified (PETG) and other control materials (parafilm and P3 R filter) were exposed to *E. coli*. The CAP exposure time required to eliminate *E. coli* from 3D-printed PETG was 5 min, identical to the decontamination time of the P3 R filter, but slower compared to parafilm (1 min).

Table S1. SARS-CoV-2 infectivity and genome copy number reduction after cold atmospheric plasma (CAP) treatment

SARS-CoV-2	Time (min)	Device 1				Device 2				Device 3			
		CAP		Control samples		CAP		Control samples		CAP		Control samples	
		Average	SEM	Average	SEM	Average	SEM	Average	SEM	Average	SEM	Average	SEM
Infectious titer (PFU/mL)	0	-	-	4.64E+05	5.25E+04	-	-	4.91E+05	1.95E+04	-	-	4.64E+05	5.25E+04
	10	1.85E+05	2.41E+04	3.27E+05	3.97E+04	1.75E+05	4.82E+03	4.89E+05	1.81E+04	7.23E+04	8.42E+03	3.27E+05	3.97E+04
	30	8.00E+04	1.34E+04	2.82E+05	3.68E+03	1.11E+03	5.56E+02	4.25E+05	1.50E+04	2.78E+03	3.67E+02	2.82E+05	3.68E+03
	90	5.56E+02	3.67E+02	2.18E+05	3.73E+04	0.00E+00	0.00E+00	4.20E+05	2.24E+04	0.00E+00	0.00E+00	2.18E+05	3.73E+04
RNA levels (Ct value)	120	0.00E+00	0.00E+00	3.17E+05	2.55E+04	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	0	-	-	17.54	0.09	-	-	17.29	0.03	-	-	17.54	0.09
	10	17.58	0.04	17.28	0.03	17.49	0.07	17.35	0.03	17.51	0.10	17.28	0.03
	30	17.61	0.02	17.38	0.03	19.80	0.09	17.22	0.09	19.24	0.01	17.38	0.03
Infectious titer (PFU/mL)	90	20.08	0.00	17.38	0.11	26.61	0.05	17.12	0.05	25.60	0.10	17.38	0.11
	120	21.99	0.01	17.25	0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Note: The viral inoculum was spotted on parafilm, air-dried, and subsequently exposed to CAP (devices 1–3) or incubated in a laminar flow box (control samples) for the indicated time. Residual virus infectivity was determined by plaque assay and genomic RNA levels by quantitative polymerase chain reaction (qPCR). Absolute levels are depicted as the average of three biological replicates and the standard error of the mean (SEM). Increasing CAP exposure time translated into decreased virus infectivity (infectious titer), with the optimized devices outperforming the original device. Abbreviation: n.a.: Not analyzed.

Table S2. Influenza A H1N1 virus infectivity and genome copy number reduction after cold atmospheric plasma (CAP) treatment

Influenza A H1N1 virus	Time (min)	Device 1		Device 2		Device 3		Control samples	
		Average	SEM	Average	SEM	Average	SEM	Average	SEM
Infectious titer (PFU/mL)	0	-	-	-	-	-	-	1.14E+06	2.68E+04
	10	1.33E+05	1.07E+04	8.44E+04	1.11E+03	7.00E+04	3.33E+03	9.22E+05	2.94E+04
	30	4.10E+04	4.89E+03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	9.06E+05	9.46E+04
	90	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	8.86E+05	8.82E+04
RNA levels (Ct value)	0	-	-	-	-	-	-	16.47	0.04
	10	16.59	0.05	17.72	0.04	21.81	0.07	16.68	0.05
	30	17.20	0.02	17.80	0.06	20.21	0.05	16.32	0.03
	90	17.79	0.11	22.58	0.16	20.21	0.07	16.55	0.05

Note: The viral inoculum was spotted on parafilm, air-dried, and subsequently exposed to CAP (devices 1–3) or incubated in a laminar flow box (control samples) for the indicated time. Residual virus infectivity was determined by plaque assay and genomic RNA levels by quantitative polymerase chain reaction (qPCR). Absolute levels are depicted as the average of three biological replicates and the standard error of the mean (SEM). Increasing CAP exposure time translated into decreased virus infectivity (infectious titer), with the optimized devices outperforming the original device.

Table S3. Human adenovirus infectivity and genome copy number reduction after cold atmospheric plasma (CAP) treatment

Human adenovirus	Time (min)	Device 1			Device 2			Device 3					
		CAP		Control samples	CAP		Control samples	CAP		Control samples			
		Average	SEM	Average	SEM	Average	SEM	Average	SEM	Average	SEM		
Infectious titer (TCID <sub>50</sub> /mL)	0	-	-	1.18E+04	3.99E+03	-	-	1.17E+04	6.03E+03	-	-	4.09E+04	2.00E+04
	10	4.08E+03	1.71E+03	6.25E+03	6.25E+02	9.48E+03	3.85E+03	5.16E+03	1.50E+03	8.68E+03	4.95E+03	2.77E+04	1.54E+04
	30	1.63E+03	1.48E+02	3.52E+03	1.12E+03	1.91E+02	1.20E+02	4.62E+03	2.01E+03	1.27E+03	9.46E+02	8.94E+03	7.40E+03
	90	5.16E+02	1.50E+02	3.45E+03	1.08E+03	2.12E+01	1.76E+01	5.15E+03	4.69E+02	1.24E+02	9.74E+01	5.83E+03	3.80E+03
	120	6.28E+02	2.57E+02	3.11E+03	1.29E+03	0.00E+00	0.00E+00	2.90E+03	8.42E+02	0.00E+00	0.00E+00	3.43E+03	2.11E+03
	Genome copies	0	-	-	6.50E+04	4.45E+04	-	-	1.80E+05	1.74E+05	-	-	2.89E+04
	10	4.31E+04	3.64E+04	5.98E+04	4.70E+04	6.73E+04	6.13E+04	1.21E+05	1.18E+05	3.90E+04	2.39E+04	8.50E+04	4.12E+04
	30	3.78E+04	3.16E+04	6.12E+04	5.87E+04	6.76E+04	6.62E+04	8.17E+04	7.91E+04	1.50E+04	1.04E+04	3.24E+04	2.30E+04
	90	2.56E+04	2.41E+04	3.63E+04	3.36E+04	7.56E+04	7.13E+04	1.27E+05	1.25E+05	1.03E+04	7.90E+03	3.17E+04	1.99E+04
	120	2.49E+04	2.24E+04	2.65E+04	2.23E+04	6.65E+03	3.33E+03	8.28E+04	8.10E+04	1.01E+04	8.88E+03	2.08E+04	1.40E+04

Note: The viral inoculum was spotted on parafilm, air-dried, and subsequently exposed to CAP (devices 1–3) or incubated in a laminar flow box (control samples) for the indicated time. Residual virus infectivity was determined by the 50% tissue culture infectious dose (TCID<sub>50</sub>) and genome copy numbers by quantitative polymerase chain reaction (qPCR). Absolute levels are depicted as the average of three biological replicates and the standard error of the mean (SEM). Increasing CAP exposure time translated into decreased virus infectivity (infectious titer), with the optimized devices outperforming the original device.

Table S4. Human rhinovirus infectivity and genome copy number reduction after cold atmospheric plasma (CAP) treatment

Human rhinovirus	Time (min)	Device 1						Device 2						Device 3						
		CAP			Control samples			CAP			Control samples			CAP			Control samples			
		Average	SEM	SEM	Average	SEM	SEM	Average	SEM	SEM	Average	SEM	SEM	Average	SEM	SEM	Average	SEM	SEM	
Infectious titer (TCID <sub>50</sub> /mL)	0	-	-	4.64E+04	2.77E+04	-	-	1.33E+03	4.00E-02	-	-	-	3.25E+03	5.35E+02	-	-	-	-	-	
	10	1.43E+04	9.00E+03	1.64E+04	7.66E+03	1.03E+02	3.78E+01	1.33E+03	4.00E-02	1.37E+02	4.05E+01	1.63E+03	4.74E+02	-	-	-	-	-	-	
	30	4.57E+03	2.81E+03	3.74E+04	3.13E+04	0.00E+00	0.00E+00	7.24E+02	1.67E+02	0.00E+00	0.00E+00	1.60E+03	8.33E+02	-	-	-	-	-	-	
	90	8.54E+01	1.46E+01	5.99E+03	3.83E+03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	120	4.37E+01	2.25E+01	3.89E+03	1.73E+03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Genome copies	0	-	-	9.86E+03	5.19E+03	-	-	3.93E+04	3.06E+04	-	-	2.99E+03	9.90E+02	-	-	-	-	-	-	
	10	9.60E+03	7.22E+03	1.12E+04	5.13E+03	2.31E+04	1.03E+04	7.26E+04	5.92E+04	5.18E+03	2.40E+03	5.77E+03	1.02E+03	-	-	-	-	-	-	
	30	9.62E+03	4.95E+03	9.34E+03	5.86E+03	6.36E+03	4.02E+03	7.84E+04	3.54E+04	4.05E+03	3.20E+03	1.33E+04	6.40E+03	-	-	-	-	-	-	
	90	5.64E+03	5.05E+03	9.24E+03	5.90E+03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	120	5.00E+03	4.87E+03	9.02E+03	5.66E+03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Note: The viral inoculum was spotted on parafilm, air-dried, and subsequently exposed to CAP (devices 1-3) or incubated in a laminar flow box (control samples) for the indicated times. Residual virus infectivity was determined by 50% tissue culture infectious dose (TCID<sub>50</sub>) and genome copy numbers by quantitative polymerase chain reaction (qPCR). Absolute levels are depicted as the average of three biological replicates and the standard error of the mean (SEM). Increasing CAP exposure time translated into decreased virus infectivity (infectious titer), with the optimized devices outperforming the original device. Abbreviation: n.a.: Not analyzed.