ASSESSMENT OF MICROBIOLOGICAL CONTAMINATION IN A CELL PROCESSING CENTER FOR A HUMANOID ROBOT

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○The presenter has no conflict of interest.

Conclusion

- A robotic cell processing facility (R-CPF), incorporating a versatile humanoid robot system “Maholo” (Robotic Biology Institute Inc.) and an All-in-one cell processing unit (Technical Research Laboratory Innovation Division, DAI-DAN Co., Ltd), was set up for cell processing for a clinical research.
- The air ventilation system with fan filter units kept an atmospheric pressure and the age of air following the PIC/S-GMP guidelines.
- Assessment of microbiological monitoring demonstrated that R-CPF maintained the required cleanliness and an aseptic environment essential for cell processing.

Discussion

Advantages

- Cell culture protocols based on basic science research are directly transfers to a clinical grade cell processing using this system
- This system reduces labors’ costs for cell culture trainings and cell processing.
- This system reduces involvement of human operators, a primary contaminant, reducing a risk of microbial contamination during cell processing.

Limitations

- Culturing multiple types of cells under the current system have a risk of cross contamination.
- Several human cell culture skills are difficult to be transferred.

Background and Aim

The use of robots in CPF has an issue regarding the impact of robotic structure and operations on an aseptic environment essential for cell processing. In this study, we conducted microbial monitoring to assess cleanliness in R-CPF during cell culture operations by a humanoid robot system “Maholo” for a clinical research of allogenic iPS-derived retinal pigment epithelial cells transplantation.

Method

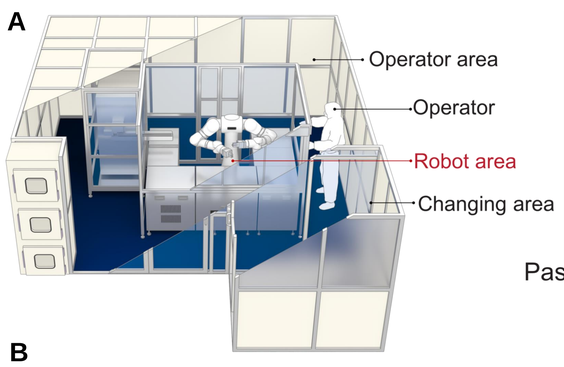
- Monitoring periods

Microbial monitoring was performed in R-CPF in Kobe Eye Center hospital (Fig.1 and Fig. 2) for a clinical research from August 2021 to June 2022 (Fig. 3). From August to December in 2021, operation development, operator education and operator training were conducted as Phase 1. From January to May 2022, trial operation was conducted as Phase 2. In June 2022, full-scale operation was conducted as Phase 3.

- Monitoring methods

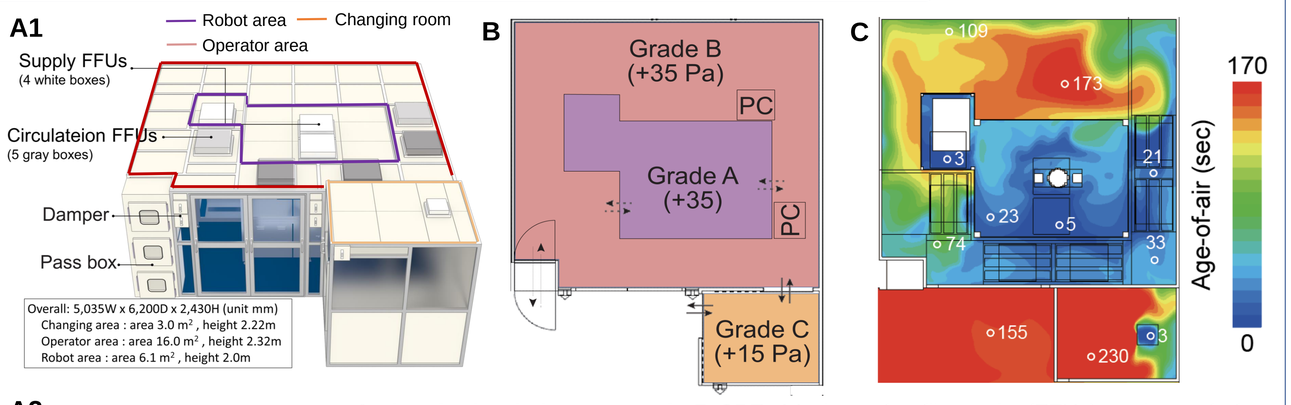
Microorganisms were monitored in R-CPF as described in Fig.3. All testing locations are shown in Fig.3.Floating microorganisms were sampled onto culture plates using an air-sampler at a flow rate of 100 L/min for 10 min. Falling microorganisms were sampled onto settle plates placed at testing locations for a duration of up to 4 hours. Adhering microorganisms were sampled with sterile swabs or contact plates at testing locations. All samples were placed in an incubator at 27.5 ℃ for 5 – 7 days. After the incubation, colony-forming units (CFUs) in culture plates were counted. For the swab test, microbial contaminations were determined by either negative or positive of microorganisms in the culture medium.

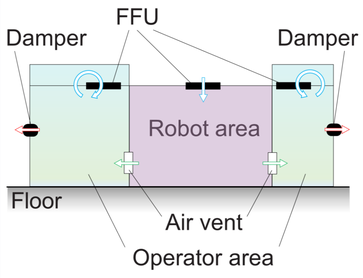
Fig.1, LabDroid Maholo and All-in-one CP unit in Robotic CPF



A, the structural images of R-CPF. Left panel shows the 3D image. A human operator supports cell culture operations by LabDroid. Right panel represents a top view of R-CPF. Solid arrows indicate flow lines of human. Dot arrows indicate flow lines of items for cell cultures. **B, a top view of LabDroid and layout of the equipment**. The components: ① dual-arm humanoid; ② CO_2 incubator; ③plate carrier slider; ④ refrigerator; ⑤ micropipettes; ⑥ freezer; ⑦ dustbin; ⑧ aspirator; ⑨ tip sensor; ⑩ micropipette tips’ stocker; ⑪ micropipette tips; ⑫workspace; ⑬ block incubator; ⑭ microscope; ⑮labware’s stocker ⑯ centrifuge. Dural-arm humanoid performs cell culture with these peripheral equipment. **C, a front picture of LabDroid**.

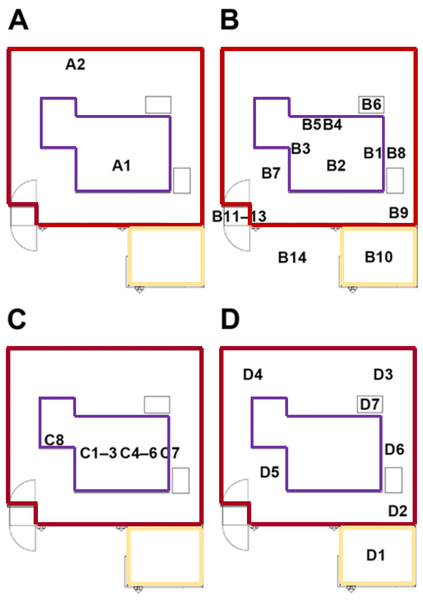
Fig.2, Cleanliness control and fluid dynamic simulation





A, the air circulation system in R-CPF. A1 shows fan filter units (FFUs) on the roof of —robot area, —operator area, and —changing room. A2 shows the air flows in R-CPF as follows: the flow from outside air to the robot area through the supply-FFUs (straight blue arrow), the flows from the robot to the operator area through an air vent (green arrows), the flows in the operator area purified through the circulation FFUs (circular blue arrows), the flow from the operator area to outside through a damper (red arrows). **B, a cleanliness grade map.** Atmospheric pressures were set for Grade A-C area in R-CPF according to PIC/S-GMP indications. **C, Computational fluid dynamics (CFDs) simulation of the airflow inside and outside the R-CPF.** CFDs simulation calculates a computation of the age-of-air, a metric denoting the duration necessary for fresh air to replace existing air. The lowest (3 sec) and highest value (173 sec) of the age-of-air in R-CPF meet the minimum requirement of the air change rate for PIC/S-GMP indications. Numbers represent the age-of-air (sec). CFDs are simulated at a height of 1,000 mm from the floor.

Fig.3, Microbial monitoring results



E

Sampling Method	Position ID	Description	Grade	Unit	Criterion	Year:	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2021	2022	2022	2022	2022	2022	2022
						Month:	9	9	9	9	9	10	10	10	10	10	11	11	11	11	11	11	12	12	1	1	5	5	6	6
						Day:	10	13	17	19	26	1	8	13	26	29	4	10	16	19	22	25	1	6	7	18	13	23	8	17
							Phase 1																		Phase 2				Phase 3	
Air sample	A1	Robot area inside front	A	CFU/m3	<1		0	0	0	0	0	0	8*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A2	Robot area outside back	B		≤10		7	2	7	1	4	6	0*	0	0	15	4	3	3	2	3	2	5	0	0	0	0	0	0	0
Settle plates	B1	Robot area inside left	A	CFU/4 hr d = 90 mm	<1		0	0	0	0	0	INV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B2	Robot area inside front	A		<1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	INV	0
	B3	Robot area inside right	A		<1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B4	Robot area inside back	A		<1		INV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B5	Robot area inside freezer	A		<1		INV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT
	B6	Operation PC	B		≤5		2	4	7	0	0	1	3	0	0	1	1	2	4	0	0	0	1	0	0	0	0	0	0	0
	B7	Robot area outside rignt	B		≤5		0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	NT	0	0	0	0	0	0
	B8	Robot area outside left	B		≤5		1	0	1	0	0	0	3	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0
	B9	Booth entrance	B		≤5		0	3	0	0	0	0	1	INV	0	0	INV	0	0	0	1	0	1	0	0	0	0	0	0	0
	B10	Changing room	C		≤50		5	3	3	0	0	3	2	1	3	8	3	5	0	3	2	2	2	0	0	0	0	3	0	0
	B11	Pass box inside (upper)	B		≤5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B12	Pass box inside (middle)	B		≤5		2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
	B13	Pass box inside (lower)	B		≤5		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	B14	R-CPF outside	(Ref)				3	1	6	0	0	2	2	16	3	8	1	2	4	9	2	0	0	7	2	1	1	4	2	1
Swab	C1	Right robot hand (tube contact area)	A	Binary (+/-)	-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	-	NT	NT	-	-	-	INV
	C2	Right robot hand (plate contact area)	A		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	-	NT	NT	-	-	-	INV
	C3	Right robot hand (supporting part)	A		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	-	NT	NT	-	-	-	INV
	C4	Left robot hand (tube contact area)	A		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	-	NT	NT	-	-	-	INV
	C5	Left robot hand (plate contact area)	A		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	-	NT	NT	-	-	-	INV
	C6	Left robot hand (supporting part)	A		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	-	NT	NT	-	-	-	INV
	C7	Slide door handle	B		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	NT	NT	NT	-	-	-	INV
	C8	Plate carrying slider pedestal	A		-		-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	-	NT	NT	NT	-	-	-	INV
Contact plates	D1	Changing room floor	C	CFU/plate S = 25 cm2	≤25		2	2	2	0	1	8	1	0	2	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0
	D2	Booth entrance floor	B		≤5		1	0	1	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D3	Robot operation PC floor	B		≤5		2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	D4	CO2 incubator floor	B		≤5		0	2	0	0	0	0	4	0	0	1	1	0	2	0	0	0	0	1	0	0	0	0	0	0
	D5	Robot area outside rignt floor	B		≤5		3	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
	D6	Robot area outside left floor	B		≤5		0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	INV
	D7	PC Desk	B		≤5		1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	INV
	D8	Operator's right hand finger	B	CFU /5-fingers	≤5		0	1	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	INV
	D9	Operator's right left finger	B		≤5		0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A–D, positions of microbial monitoring. Box colors: —Grade A, —Grade B, —Grade C. Air sampling, settle plates, swabs, and contact plates sampling positions are shown in Fig. A, B, C, D, respectively. Symbols such as ‘A1’ are identical to the position IDs in Fig. E. **E, monitoring results with air sampling, settle plates, swabs, and contact plates from 2021-09-10 to 2022-06-17.** The position IDs are identical to the symbols in Fig. A–D. The cleanliness classification is based on the PIC/S-GMP standards. Dark gray, deviation, NT, not tested; INV, invalid (tested but failed). In Phase 1, during operation development, operator education and operator training, microbial contamination was detected at various sampling positions in R-CPF. Besides, the CFUs in September 17, October 8, October 29, and November 22 were depart from the accepted standard. In contrast, the acceptable number of microbial colonies were observed at only one position in one day during trial operation periods, Phase 2. In addition, no colonies was observed during full-scale operation periods, Phase 3.

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