

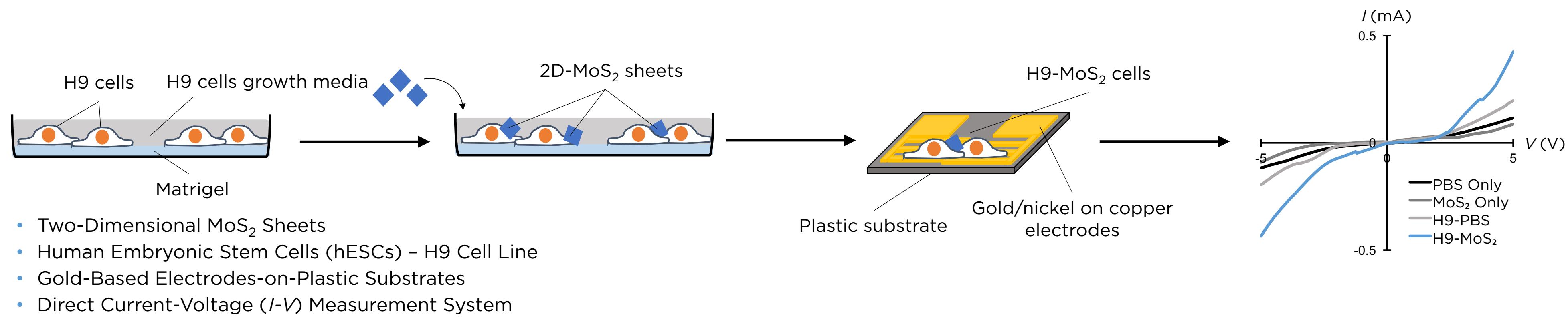
Sophia S. Y. Chan¹, Yaw Sing Tan², Kan-Xing Wu³, Christine Cheung^{3,4}, Desmond K. Loke¹

¹Science Faculty, Singapore University of Technology and Design ²Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR) ³Lee Kong Chian School of Medicine, Nanyang Technological University ⁴Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR)

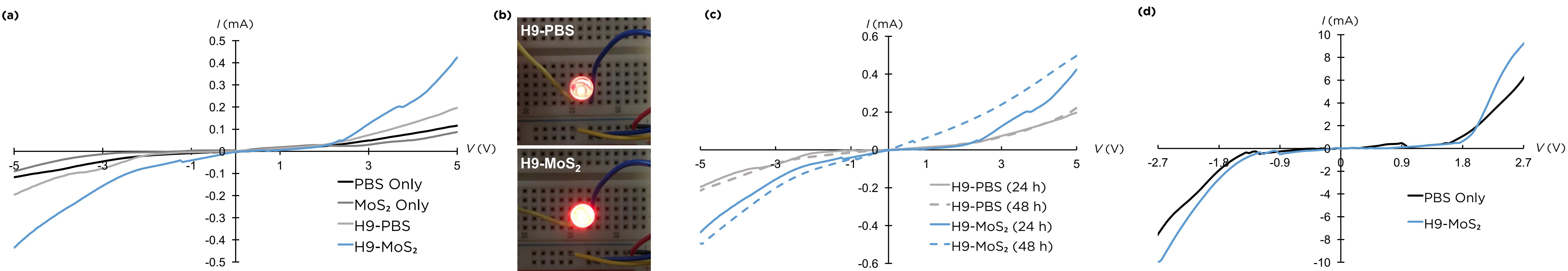
Introduction

Human embryonic stem cells (hESCs) are excellent starting resources to derive functional cells and tissues for regenerative medicine. A major challenge is the ability to remove remaining pluripotent stem cells which causes teratomas after stem cell transplantation.¹ Traditional detection methods are inadequate as they are expensive and destructive. Electrical-based detection (EBD) methods provide a less invasive method to monitor and validate pluripotent stem cells.² However, electrochemical signals produced are too low to allow for easy commercialisation.^{3, 4} Here, we observed a unique bioelectric signal of human embryonic stem cells using direct current-voltage measurements facilitated by few-layered 2D-MoS₂ sheets.⁵

Methods, Materials & Equipment

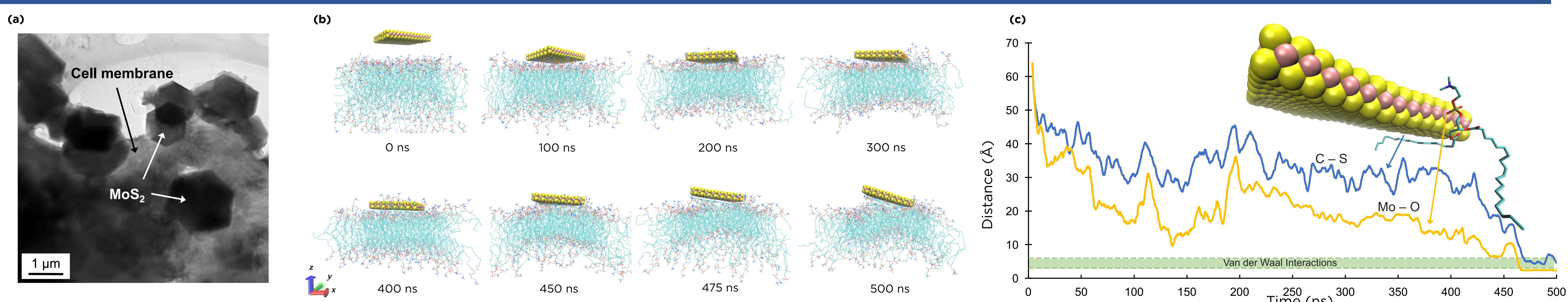


2D-MoS₂ Sheets Enhance Native H9 Bioelectric Signals



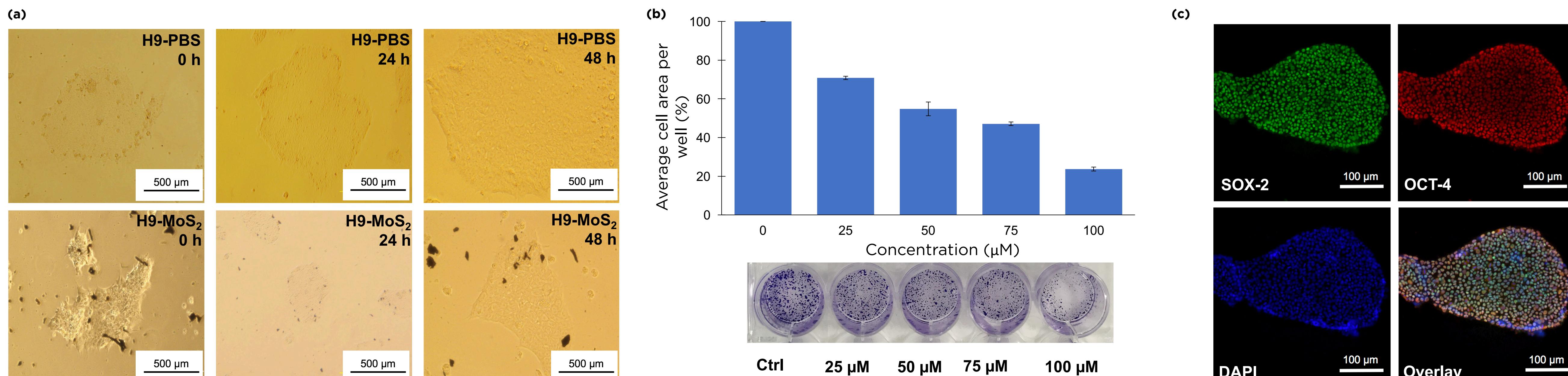
- a) Direct I - V measurements were performed on devices. **H9-MoS₂ produced a significantly higher signal (blue line).**
b) Devices with H9-PBS and H9-MoS₂ were connected to LED with 4 V applied. **H9-MoS₂ produced a more intense light.**
c) Incubation time-dependent current measurements. **48 h incubation lead to a higher measured current.**
d) Direct I - V measurements in incubation-like conditions; instead of drying on the device, the cells are kept wet and supplemented with PBS. **H9-MoS₂ produces a signal of 1.828 mA at 2 V.**

Cell-Material Interactions



- a) TEM image: 2D-MoS₂ sheets (white arrows) are embedded within the cell membrane (black arrow).
b) MD simulation of monolayer of MoS₂ and lipid bilayer comprising of POPC. Snapshots were taken between 0 – 500 ns.
c) Dynamic bond distance between the MoS₂ monolayer and the lipid bilayer. Towards the end of the simulation, S-atom and C-atom bond distance fluctuates between 3 – 6 Å (green area), the average bond distance of **van der Waal interactions** (blue line). The distance between Mo-atom and O-atom become consistently 2 Å, indicative of the formation of **electrostatic forces** (yellow line).

2D-MoS₂ Sheets Exhibit Low Cytotoxicity



- a) Light microscope images of cells incubated without and with 2D-MoS₂ sheets. **Cell morphology of H9-MoS₂ does not significantly differ from H9-PBS.**
b) Cells incubated with varying concentration of 2D-MoS₂ sheets e.g. 0 – 100 μM were fixed with PFA and stained with crystal violet (purple). **Low concentration of MoS₂ resulted in high cell populations**, similar to previous papers. Normalised area of living cells were calculated as a percentage against control as shown in the graph.
c) Fluorescence images for H9 cells immunostained for typical pluripotent transcription factors SOX-2 (green) and OCT-4 (red) against control (DAPI, blue). **MoS₂ did not significantly affect stem cell pluripotency.**

Conclusion

- Using direct I - V measurements and 2D-MoS₂ sheets, we demonstrated **enhanced bioelectrical signals of H9 cells**.
- Enhanced current flow could be due to the **spontaneous formation of van der Waal and electrostatic interactions** observed in the MD simulation.
- Low cytotoxic effects** of 2D-MoS₂ sheets on H9 cells was also demonstrated.

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