Hydrogel Microwells for Human Hair Follicle Regeneration

Pan Jing and Kang Lifeng

Alopecia is a global health problem, which affects approximately 50% of men and 20% to 53% of women by age 50 years. Currently, hair follicle transplantation is a common clinical treatment for alopecia. However, there is a shortage of human hair follicles for surgical transplantation, since the only source of hair follicles is from human donors themselves. To this end, tissue engineering may provide an approach to generate human hair follicles in large quantity to meet the clinical needs.

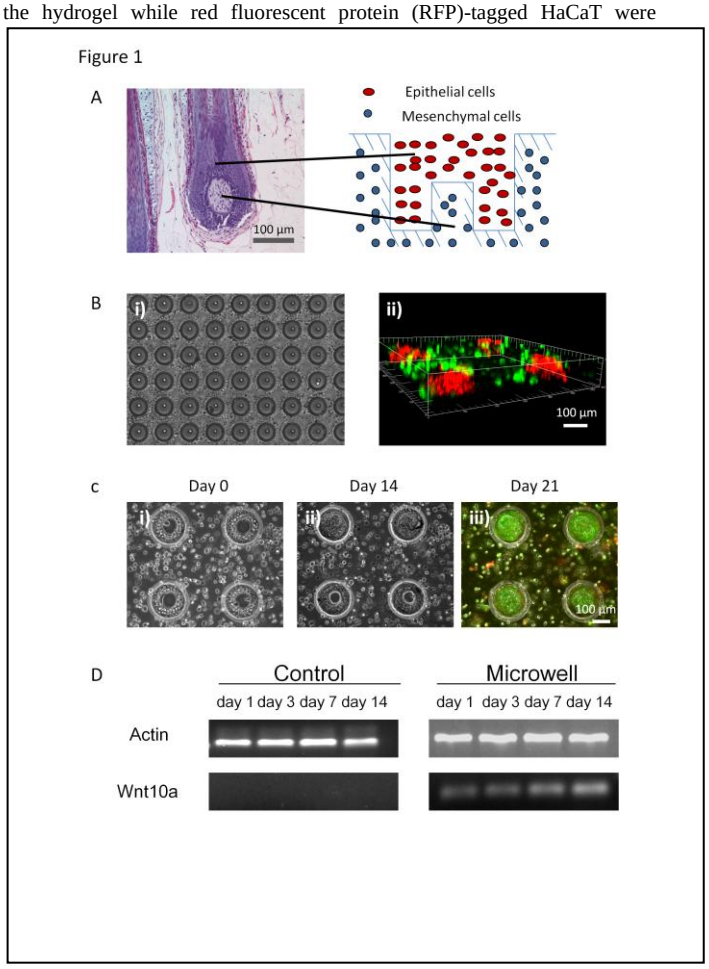
In our study, we developed a hair-follicle like hydrogel microstructure and immobilized keratinocytes and mesenchymal cells at its different compartments of the microstructure, mimicking their spatial distribution *in vivo* for hair follicle engineering in vitro (Fig. 1A). The hydrogel microwells were fabricated in an array format, comprising of 400 microwells per array (Fig. 1B-i).

Human dermal fibroblasts (HDFs) were encapsulated inside the hydrogels while human adult low calcium high temperature (HaCaT) cells were seeded on the surface of the microwells. From fluorescent imaging, green fluorescent protein (GFP)-tagged HDFs were uniformly dispersed inside the hydrogels while the red fluorescent protein (RFP)-tagged HaCaT cells were immobilized inside the microwells (Fig. 1B-ii).

Cell development in the microwell was monitored over 21 days. HaCat cells became aggregates after 3 days inside the microwells. The aggregates grew bigger till the center inlets were all covered by Day 21 (Fig. 1C). By analyzing the specific genes involved in the hair follicle development process, it was demonstrated that the hydrogel microwells had advantages over normal co-culture of HDF and HaCat cells (Fig. 1D).

In summary, the hydrogel microwells enabled epithelial and mesenchymal cells to be positioned in a similar way as the cells *in vivo*, and supported their growth for 3 weeks. Potentially, the co-culture system provided a new tool to regenerate hair follicles *in vitro* for clinical applications.

Figure 1A. Schematic representation of hair-follicle like mould fabrication. A) There are two types of cells which are necessary for hair follicle generation. Blue dots represent mesenchymal cells which can induce the proliferation of epithelial cells (red dots). **Figure 1B**. i) A portion of the whole hydrogel microwell array. ii) Confocal images of 4 microwells, with green fluorescent protein (GFP)-tagged HDFs were uniformly dispersed in the hydrogel while red fluorescent protein (RFP)-tagged HaCaT were



immobilized in the microwells. **Figure 1C**. HaCaT cells formed cell aggregates in the well and grew bigger till center inlets were all covered by Day 21. The cells were stained with a fluorescent Live/Dead kit at Day 21, where the cells stained green in color were live and red were dead. **Figure 1D**. PCR analysis indicated that Wnt 10a gene was expressed from cell co-culture of HDF and HaCat cells in hydrogel microwells but not for control groups of a normal co-culture.

ACKNOWLEDGMENT

The authors thank Singapore Nikon Imaging Center for their assistance.

REFERENCES

[1] J. Pan, S.Y. Chan, J.E.A. Common, S. Amini S, A. Miserez, B.E. Lane and L. Kang. Fabrication of a 3D hair follicle-like hydrogel mould by soft lithography. *J. Biomed. Mater. Res. A.* 101(11):3159-69, Nov 2013.

[2] L. Kang, M. Hancock, M. Brigham and A. Khademhosseini. Cell confinement within patterned nanoliter droplets in a microwell array. *J. Biomed. Mater. Res. A.* 2010;93:547-557, May, 2010

*Research supported by a NMRC-NIG grant.

Pan J. and Kang L. are from Department of Pharmacy, National University of Singapore. Kang L. email: lkang@nus.edu.sg