Evaluation of Different Tissue Processing Methods in Bama Pig Skin as an Animal Model for Topical Delivery Systems

Chao Zhang, Jacob Zhi Chen, Huan He, Shoutao Liu, Lijuan Hou, Yi Tao, Liang Shen
DMPK Service Department, WuXi AppTec, 6F Building 2 of Fumin Phase III, No.818 Wusong Road, Guo Xiang, Wuzhong Economic, Suzhu, Jiangsu 215124, China

药明康德 WuXi AppTec

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Introduction

This research uses minipigs, chosen for their skin's similarity to human skin, as a focus of our study. This poster evaluates the bead milling and traditional homogenization methods for processing pig skin tissues, with an emphasis on efficiency and cross-contamination prevention. We highlight the importance of accurate skin layer separation and uniform, high-quality homogenization samples for successful biological analysis. The detailed process of isolating various skin layers underscores the crucial role of homogenization in ensuring accurate results.

Methods

Instruments

Leica Cryostat | ECLIPSE LV100N Microscopy | POLYTRON ® PT1200E | OMNI Bead Ruptor Elite

Step one: **Measuring the thickness of each layer of skin**
Skin tissue is first fixed with formalin and then vertically sectioned of approximately 2 mm thickness using a Rotary Microtome. These sections are subsequently stained using the Hematoxylin and Eosin (H&E) staining method. After staining and mounting of slides, histopathology images are captured and the length is measured using Image Acquisition Software. Based on the different skin layer thickness values, accurate separation of the epidermis and dermis was achieved by Leica cryostat.

Step two: **Comparing two methods for homogenizing epidermis samples: hand disperser and ball mill homogenizer**
For the test, the epidermis was chosen as an example due to its difficulty in homogenizing and its small quantity. Two epidermal tissue samples were accurately weighed, divided in parallel, and then mixed with the same proportion (1:19 w:v) of homogenization buffer (Methanol/PBS Buffer). The POLYTRON® PT1200E hand disperser was employed with a shear blade at a medium speed of 10,000 rpm for 30 seconds in the first method. The OMNI ball mill homogenizer was utilized in the second method with tailored tubes and 2-3mm zirconia beads. Homogenization was conducted at liquid nitrogen controlled temperature and a set speed of 5m/s for 15 seconds.

Results

Figure 1 shows the histopathology image of the skin layer sections of the Bama mini pigs and the skin layer thickness values measured in our laboratory. According to literature[1] reports, it has been documented that 4-month-old Bama mini pigs have the same thickness as in humans and are suitable for the evaluation of transdermal formulations.

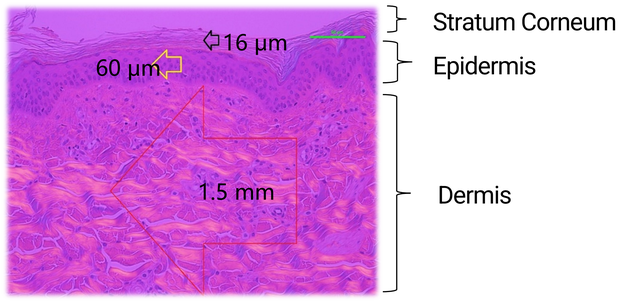

Figure 1. Bama Pig Skin Layer Thickness Through H&E Staining and Microscopic Analysis

Figure 2 shows that the epidermis tissues were observed under LV100N microscopy following homogenization to evaluate the particle size of the homogenized tissue. Table 1 provides the advantages and disadvantages between traditional homogenization and bead milling methods.

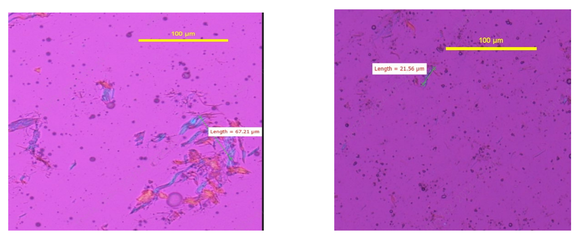

Figure 2. Microscopic images of epidermal tissue samples, magnified 20 times. Left: traditional method. Right: bead milling method.

Table 1. Comparison of Traditional Homogenization and Bead Milling Methods

Method	Traditional	Bead Milling
Dispersion	Uneven with the presence of large aggregates	Uniform
Particle Sizes	50 µm to150 µm	5 µm to15 µm
Temperature Control	Periodically intermittent dry ice cooling	Stable and sustained liquid nitrogen control temperature
Contamination Risk	Potential cross-contamination	No
Process	Manual handling	Semi-automated process
Channel (Efficiency)	Single	24-channel
Minimum Sample Amount Required	≥1000 µL, but a lot evaporates	≥100 µL , with very little lost
Exposed to the environment	Open	Sealed

*In the case of the bead milling method, it is stated that the efficiency is over 20 times higher compared to traditional homogenization.

Conclusion

Based on our extensive investigation, which includes over 70 studies on nearly 600 pigs, we have accumulated abundant experience and technical knowledge, and at the same time we have continuously refined the methods used for homogenizing tissue. All comparative validation results have demonstrated that the bead milling method significantly outperforms the traditional homogenization method. This method makes working with samples faster and easier, needing about 20 times less work and time. It also lowers the chances of contaminating samples by using disposable tubes. When looking closely at skin samples processed this way, milling with beads made smaller, more evenly spread particles (sizes from 5 µm to 15 µm). This is different from the bigger, less regular sizes made by the traditional method (sizes from 50 µm to 150 µm).
With the advancement of materials technology, the bead milling method now offers a wider application. We offer beads and tubes with free RNA, supporting the research and development of nucleic acid-based drugs. In the process, some solvents, such as methanol and acetonitrile , which are volatile and potentially toxic, are used in homogenization. By employing a sealed operation, the bead milling method also ensures both safety for personnel and environmental friendliness.

References

[1] Yu Liu, Junying Chen, Haitao Shang. Light Microscopic, Electron Microscopic, and Immunohistochemical Comparison of Bama Minipig (Sus scrofa domestica) and Human Skin. Comp Med. 2010 Apr; 60(2): 142–148

liu_shoutao@wuxiapptec.com