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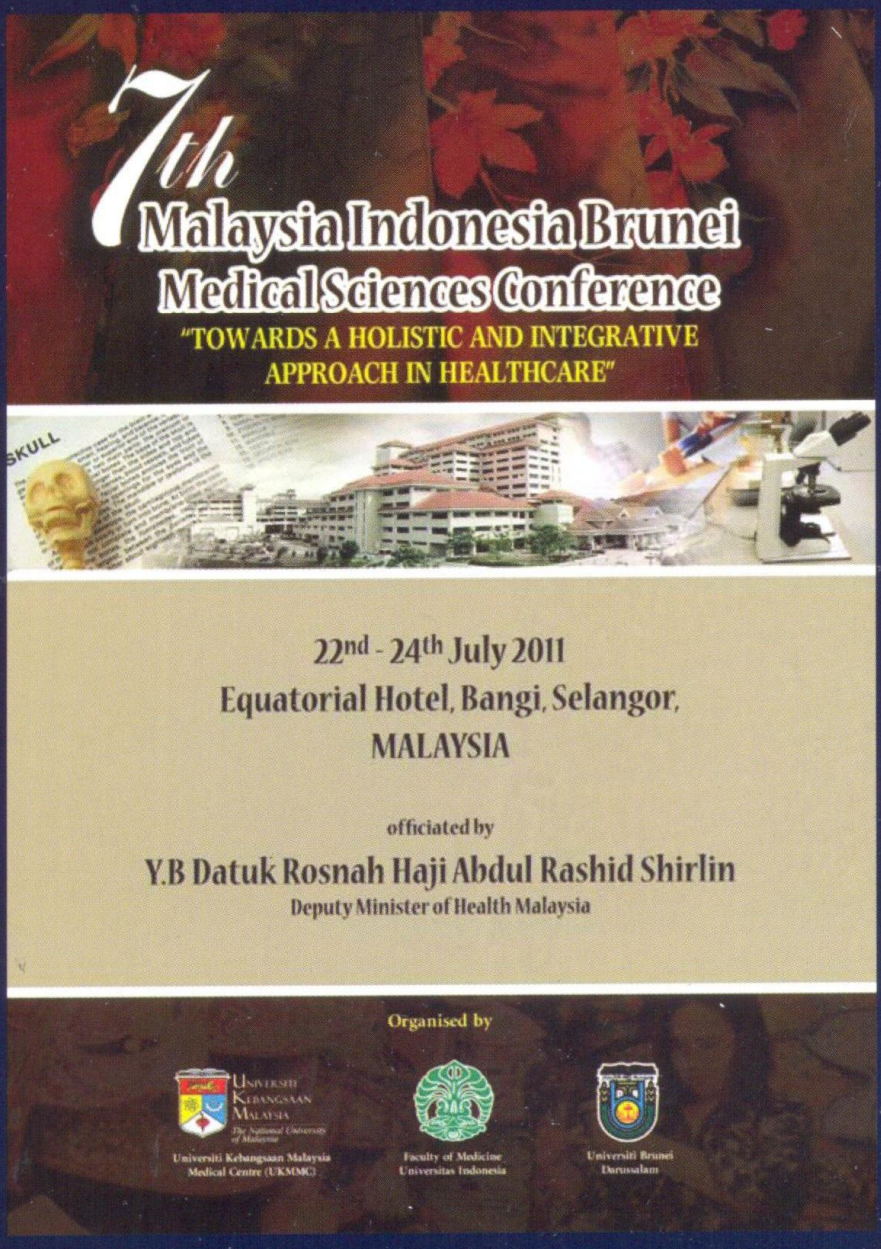

MODIFICATION OF THE WOUNDING TECHNIQUE FOR THE SCRATCH WOUND ASSAY FOR THE EVALUATION OF WOUND HEALING PROPERTIES

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Background:
Wound healing is a complicated and highly regulated cellular and biochemical events that involve the interaction between various cell types, intermediate cytokines and extracellular matrix (ECM). Scratch wound healing assay is developed to study directional cell migration *in vitro* that mimics the *in vivo* process. Most wounding assays are performed using a mechanical means to generate a break in a confluent monolayer of cells that resulted variable wound size, which was reported up to 33%. Moreover, the secretion of growth factors from damaged cells during scratching also influence the cellular activity at the wound site.

Materials and Methods:
In this study, we used silicon rubber to prepare the wound model, where silicon rubber for wound size was incorporated into the culture dish prior to cell seeding. For testing wound healing, dermal fibroblasts isolated from human skin were seeded at 1.0×10^5 cells/cm^2 in silicon rubber embedded culture dish along with the control group. After two days culture, the wound was created by removing silicon rubber from the test group and scratching by pipette tip in control group, and wound size were measured. In addition, migration was also observed using live cell imaging technique. The experiment was performed in triplicate.

Results:
It was found that the wound created by silicon rubber have uniform size and shape between experiments. The average wound size for test sample was measured to be 0.90±0.001, while for the control group was 0.38±0.03. The variability of wounds among different experiment for test group was evaluated on average 0.2%, while for control group was 16%. Moreover, use of silicon rubber didn't affect the migration of cells on the wound area created by silicon rubber.

Conclusion:
These results suggest that this new technique for wounding can be used to create a standardized wound to investigate the effect of various substances on wound healing.

Keywords:
tissue engineering, wound assay, wounding technique, silicon rubber