TACSM Abstract

Reliable, Semi-Automated Wound Healing Rate Determination in Muscle

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ABSTRACT

In the field of Regenerative and Sport Medicine, there is great interest in the development and validation of compounds and devices with the potential to accelerate wound healing and muscle regeneration. *In vitro*, this effect can be evaluated in a scratch test model, in which a pipette removes a line of cells from a confluent monolayer of cells with high regenerative capacity and the time to close this injury measured. **PURPOSE:** To develop a reliable, dynamic, and quantitative process with a shorter duty cycle and semi-automatic operation for the determination of wound healing rate, as compared to fully manual operation. **METHODS**: C2C12 murine myoblasts were cultured to confluence under standard conditions. A 200 µL pipette tip was used to make a scratch across each well, and 0 and 0.5mM of pro-myogenic Si-ions were added to the media. A Keyence BZX-710 microscope was used to capture images every 183 seconds over 36 hours at 10x magnification with 0.7 pixels/µm and 4 µm pitch. An enclosed cell culture stage contained a cell incubator system keeping cells at 37°C with a 5% CO_2 humidified air. For the manual operation, one image was randomly selected from the automated images every 12hr. ImageJ Macro WH_NJ was used to quantify the percent area of the field (scratched) of interest without cells and was normalized as needed per experimental conditions. **RESULTS:** The manual and automatic slopes for the 0 and 0.5mM Si-ion treatments were -4.87E-06, -4.84E-06, -6.01E-06, and -5.98E-06, respectively, for the full 0-36hr. There was a high degree of correlation between the manual and semi-automatic rates for both the 0 and 0.5mM Si-ions, at r=.84 and .98, respectively. There were no statistically significant differences between healing rates (i.e., closure times) for the automatic or manual 0 or 0.5mM Si-ions, or within either method, following a two-tailed student's *t*-test with alpha level of $p<.05$. Within 12hr periods, the semi-automatic method provided greater detail for the healing rate, such as the faster initial rate seen in 0.5mM Si-ion, not discernible in 12-hour increments for the manual method. **CONCLUSION:** These data support the functionality of our new methodology described here. The descriptive and inferential statistics shown here demonstrate agreement between the two analyses, while the semi-automated method presented additional dynamics and kinetics information beyond the manual method in early-test behavior that could not be measured manually. Further development in this area will focus on continuing to shorten duty cycles for higher fidelity and the quantitative analysis of dynamic behaviors. Potential clinical-translational applications of our new method are to screen libraries of compounds with putative muscle regeneration capacity using human muscle cells. We also plan to test basal differences in muscle cells from biopsies of sedentary and active individuals, as well as healthy individuals vs. those with various metabolic and musculoskeletal and cardiovascular disorders, and aging sarcopenia. Our new methodology coupled with these translational studies will help advance new compounds and devices with early promise for the field of Regenerative and Sport Medicine into the pre-clinical animal phases of validation.