DIFFERENT ISOLATION STRATEGIES FOR EPIDERMAL KERATINOCYTES - EFFECTS ON CELL COUNTS, VIABILITY AND STEM CELL MARKERS (117)

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Questions: The clinical use of cultured keratinocytes to treat massive burns is in some cases essential, but results are often sub-optimal. Here, we test the hypothesis that methods that will cleave cell surface proteins/glycoproteins are, in part, the cause of the sub-optimal repair. More gentle methods that leave the cell membrane proteins, such as integrins intact, allowing for rapid attachment, will result in better overall coverage.

Methods: Skin was obtained from 8 patients undergoing abdominoplasties. The epidermis was digested using either Trypsin (0.05 % Trypsin-EDTA, Invitrogen, Carlsbad, CA); TrypLE Select (1X, Invitrogen, Carlsbad, CA); StemPro® Accutase Cell dissociation Reagent, (Invitrogen, Carlsbad, CA); Cell dissociation solution, 1X, Non enzymatic, (Sigma-Aldrich, Schnelldorf, Germany). Crude cell count and viability was measured using Luna automatic cell counter (Logos Biosystems Inc, Annandale, USA). The cells were also analyzed for the presence of cell surface proteins (CD29, CD49f, CD117, Delta P 63) and intracellular marker (CK19) using Flowcytometry. After 7 days of culture, the extent of proliferation was assessed using crude cell counts.

Results: Trypsin was found to generate the highest average cell yield (30% higher than the Tryple select) (1.6x10⁶ cells/mL) as well as the highest viability (66%) after 15 min incubation (Kruskal-Wallis ANOVA p = 0.04,). However, after 24 hours of incubation, TrypLE Select, StemPro® Accutase and Cell Dissociation Solution 1X, Non Enzymatic all generated higher viable cell yields compared to Trypsin (25% in average), indicating a more gentle tissue dissociation process. After one week of culture, StemPro® Accutase treatment for 1 hour showed the highest average cell yield double the average yield by trypsin isolated cells (1.5x10⁶ cells). (p=0.04) The expression of cell surface markers was different among different used enzymes, Tryple select was generated highest expression of CD29+ (p<0.05), Trypsin generated highest expression of CD117+ (p<0.05) and Non-enzymatic cell dissociation buffer generated highest expression of CK19 (p<0.05)

Conclusion: Depending on the immediate aim of the dissociation process, i.e., whether to obtain an early single cell suspension or a high cell viability it is important to select the proper dissociation substance and technical approach.