Soft substrate boosts proliferative potential and maintains keratinocytes functionality during in-vitro expansion compared to plastic.

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Abstract

Annually 11 M cases of skin burns are reported worldwide, resulting 0.1-0.3M patients deaths. Autologous or allogenic skin grafts are the mainstay treatment strategy for burns above the critical size. To generate sufficient number of cells for grafting, keratinocytes from isolated tissue samples are extensively cultured in vitro on plastic petriplates. However, in this method of cell culture, long-term proliferation potential, differentiation capabilities, and responsiveness to immune effector molecules gets diminished with increasing population doubling. In this study, we demonstrate that substrates made of soft polyacrylamide gels mimicking skin elasticity can maintain proliferation rate over passages and generate 3 fold higher numbers of keratinocytes (within the same time) compared to plastic petridishes. Faster cell growth ensures availability of enough number of cells for skin grafts in shorter time. This may significantly reduce the chance of infection, a major cause of death due to burn. Moreover, cells cultured on gels had less proportion of senescent cells in later passages. They showed higher lentiviral vector-based transfection compared to cells from plastic demonstrating their usability for gene-based therapies. They also preserved their expression of IFN-γ responsive genes and the wound healing capability, thus remaining functionally intact. In summary, this research highlights the role of substrate stiffness in maintaining cellular functions in long term culture. The keratinocytes grown on soft polyacrylamide gels can be utilized for treating epidermal disorders such as junctional epidermolysis bullosa, and partial thickness wound in burn or metabolic disorders, reducing the suffering of patients.