

CONTRACTILE BEHAVIOUR OF 3T3 MOUSE FIBROBLASTS IN ARTIFICIAL SKIN SUBSTITUTES: EFFECT OF DIFFERENT CELL-SEEDING METHODS

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INTRODUCTION: With the rapid development of tissue engineering and gene therapy, collagen-based biomaterials are frequently used as cell transplant devices; an example is bio-artificial skin substitutes [1-3]. In this study, we determined the effects of two different cell-seeding methods, monolayer and suspension seeding, on the rate of contraction of free-floating collagen and collagen-glycosaminoglycan (GAG) gel matrices in vitro. 3T3 mouse fibroblasts were cultured in/on the matrices for up to 7 days.

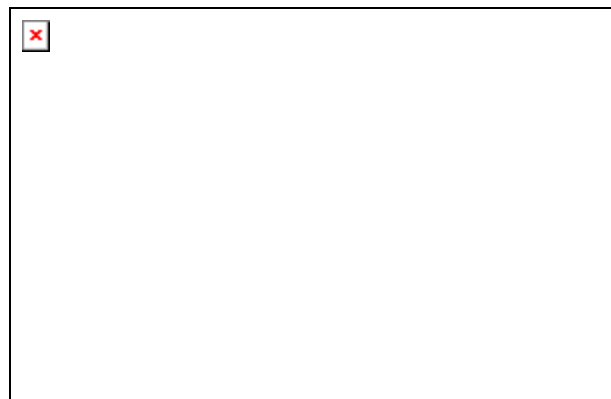
METHODS: In monolayer cell seeding, 5ml of 0.3% (w/v) collagen gel matrices (24mm x 60mm) floating on 3ml of 10x complete Dulbecco's Modified Eagle's Medium (DMEM) in a hydrophobic cell chamber of 24mm x 60mm x 11mm were washed once in DMEM prior to seeding. To modify the gel matrices with GAG, chondroitin-6-sulphate (CH₆SO₄) was prepared at 3 mg/ml in 1x serum-free DMEM and incorporated into the collagen solution at 20%. 3T3 mouse fibroblasts in medium were then pipetted on the collagen and collagen-GAG gel matrices at a cell seeding density of 9×10^3 cells/cm². In suspension cell seeding, the 3T3 cells were seeded in suspension within the collagen gel matrices at a density of 1.3×10^5 cells/ml. The numbers of cells in both methods of seeding for both the collagen and collagen-GAG gel matrices were identical. Medium was changed daily. The area of contraction of the matrices was measured daily using a light-box over which the cell chambers were placed on a standard millimeter grid. Measurements were then calculated using digital planimetry and Scion imaging software (Scion Corporation, U.S.A.).

RESULTS: Fibroblasts seeded on the gel matrices as a monolayer culture induce significantly less contraction than fibroblasts seeded in suspension within the gel matrices (Fig. 1). It was shown that generally the extent of contraction was less in the

GAG-treated collagen gel matrices. This difference was not significant.

CONCLUSIONS: These results showed that different cell-seeding methods do have an effect(s) on the contraction of collagen-based artificial skin substitutes.

REFERENCES: ¹ S.T. Boyce, D.J. Christianson and J.F. Hansbrough (1988) Structure of a collagen-GAG dermal skin substitute optimized for cultured human epidermal keratinocytes, *J Biomed Mater Res* **22**(10):939-57. ² S.T. Boyce, R.J. Kagan, N.A. Meyer, et al (1999) The 1999 clinical research award. Cultured skin substitutes combined with integra artificial skin to replace native skin autograft and allograft for the closure of excised full-thickness burns, *J Burn Care Rehabil* **20**(6):453-61. ³ W.H. Eaglstein, O.M. Alvarez, M. Auletta, et al (1999) Acute excisional wounds treated with a tissue-engineered skin (Apligraf), *Dermatol Surg* **25**(3):195-201.



*Fig. 1: Effect(s) of different cell-seeding methods on the contraction of collagen-based artificial skin substitutes. Results are mean \pm S.E.M., n=5. Using ANOVA, * $p < 0.05$, compared with monolayer cell seeding on collagen gel matrices.*

