

Measurement of collagen synthesis by cells grown under different mechanical stimuli

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INTRODUCTION: The use of scaffolds in tissue engineering is essential to provide cells with a matrix for cell proliferation and differentiation resulting in tissue regeneration. Normally this process involves seeding cells onto an artificial biodegradable scaffold providing mechanical support for cells until there is sufficient extracellular matrix deposition (ECM) to replace the artificial scaffold. Collagen is the bulk protein found in the ECM and measurement of its synthesis is the most direct, absolute indicator of ECM production.

METHODS: An HPLC method has been used for assay of hydroxyproline as a measure of collagen synthesis. At this stage 2D cell cultures were used as an initial screen. Four cell types (two CHO cell lines, human adult dermal fibroblasts and human neo natal dermal fibroblasts) were grown for 11 days in well plates. Culture media were sampled every 2-3 days for cumulative production of soluble collagen. At the end point of culture cell pellets were hydrolysed to give total collagen levels. Production rates, total collagen deposition and % deposition efficiencies were derived from these data for the four cell types.

These were compared with fibroblasts grown on polyglycolic acid (PGA) and polyethylene terephthalate (PET) meshes under static conditions, with and without mechanical stimulation in a pulsatile mechano-bioreactor.

RESULTS: The HPLC assay system was effective in quantifying collagen synthesis by cells in 2D culture. Comparison of the four cell types indicated that human adult dermal fibroblasts (HDFa) produced the largest quantity of total collagen (1331 ng) at day 11 but at low deposition efficiency (10.7%). The deposition efficiency of human neo natal dermal fibroblasts (HDFn) was highest (18.5%) from 957 ng of total collagen produced at day 11.

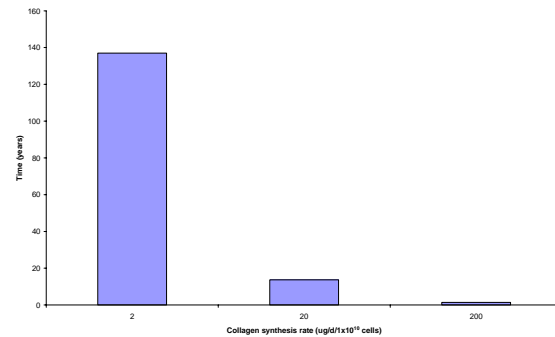


Fig. 1 The time required to produce 100 mg of collagen by cells at synthesis rates of 2, 20 and 200 µg per day.

Based on these results we have calculated the time required to produce 100 mg of collagen if cells were depositing 2, 20 or 200 µg of collagen per day by 1×10^{10} cells (Figure 1). On this basis the HDFn cells would take 54 years and the adult fibroblasts 67 years to produce a 100 mg construct.

Results for collagen synthesis by fibroblasts seeded onto static PGA and PET meshes are ongoing.

DISCUSSION & CONCLUSIONS: The results indicate that the collagen synthesis rates obtained using existing culture systems are insufficient to produce adequate collagen for a tissue engineered construct.

An alternative strategy may be to seed cells onto a collagen scaffold and culture in a bioreactor for expression of other ECM proteins (e.g. elastin, growth factors) in order to speed up the process of fabricating a tissue engineered construct.

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