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The Mechanical Strength of Collagen Gels Containing Glycosaminoglycans and Populated with Fibroblasts

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INTRODUCTION: Collagen based scaffolds are widely used for tissue engineering strategies; many are designed to biodegrade gradually and become populated with host cells. The effect of infiltration by host cells on the mechanical properties of the scaffolds is unclear. We have used fibroblast populated collagen lattices to study the effects of the presence of 3T3 mouse fibroblasts on the mechanical properties of collagen gels containing glycosaminoglycans (GAGs) crosslinked with the carbodiimide, 1,1-carbonyldiimidazole (CDI), and the polyamine, putrescine (Put).

METHODS: The gel substrates were prepared as described by Osborne et al [1]. Briefly, gels containing 0.3% (w/v) type I collagen ± the GAG, chondroitin-6-sulphate (0.6mg/ml), were allowed to set for 4h before some were treated with 1M CDI and/or 0.5M Put for 18h. Gels were set in a rectangular Perspex casting plate 25mm x 60mm and a polymer mesh (PVC, 38 hole/cm^2) was incorporated at either end of the gel to facilitate gripping the mesh in the Instron tensile testing machine. Load, stress, strain at failure and Young's modulus (Mod on Table 1) were calculated for each gel composition in the absence and presence of 3T3 cells. Where 3T3 cells were added, the cell density was 2.5 x 10^4 cells/cm^2 and cell viability in the gels was determined by confocal microscopy.Both cell seeded and unseeded gels were incubated for 6 days in Dulbecco's medium containing 10% foetal calf serum at 37^{o}C in air/5%CO_2 before being tested.

RESULTS and DISCUSSION: In the absence of cells, incorporation of either GAG and/or the crosslinkers transformed the gel into a stronger stiffer gel, as shown on Table 1A by the increases in the values for the load, stress, and Young's modulus compared with those measured in plain collagen gels. Regardless of chemical composition, the presence of 3T3 cells weakened the gel considerably, and decreased the Young's modulus. This marked effect can be observed by comparing the values for each parameter in gels of the same composition in Tables 1A and B. The mechanical advantages attained by the addition of GAG and crosslinkers to the gels, were overcome by the effect of the fibroblasts. In these gels the process of scaffold degradation, which *in vivo* would allow cell migration and proliferation, predominates over synthesis of new matrix by the cells. It may be possible to modify the balance between these two processes and influence scaffold remodelling.

*Table 1. The mechanical properties of collagen (Coll) gels in the absence (A) and presence of 3T3cells (B) after 6 days incubation. Results are mean±S.D. of n=5. *P<0.05 comparing the treated collagen gels with plain collagen gels in each Table, by ANOVA followed by Dunnett's test. All parameters were statistically different when cell seeded and unseeded gels with the same chemical composition were compared by unpaired Student's t-test.*

A. Gels without cells

P	Coll	GAG/ Coll	Coll + CDI + Put	GAG / Coll + CDI+Put
Load (N)	0.9± 0.1	1.2± 0.1*	0.9± 0.1	1.09± 0.09*
Strain %	11.6 ±1.3	8.4± 0.6*	11.4± 1.1	11.78± 0.88
Stress (kPa)	13.9 ±4.6	19.7± 3.3*	25.2± 1.7*	22.11± 3.5*
Mod (MPa)	0.1± 0.04	0.2± 0.02*	0.2± 0.03*	0.15± 0.03*

B. Gels with 3T3 cells

P	Coll	GAG/ coll	Coll + CDI + Put	GAG / Coll + CDI+Put
Load (N)	0.7± 0.1	0.6± 0.1	0.7± 0.2	0.5± 0.1*
Strain %	13.8± 2.6	13.3± 1.8	10.8± 1.1*	10.9± 2.2
Stress (kPa)	7.8±0. 9	7.1± 1.1	8.0± 0.7	5.8± 0.4*
Mod (MPa)	0.05± 0.01	0.04± 0.01	0.05± 0.01	0.04± 0.004

REFERENCES:[1]C.S. Osborne, W.H. Reid, M.H. Grant (1999) *Biomaterials* **20**: 283-290.