

Scaffold Characterisation for Nucleus Pulposus Regeneration

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INTRODUCTION: The overall goal of this project is to develop an in-situ cross-linkable, biodegradable cell-seeded scaffold for the recapitulation of degenerated intervertebral discs. In a previous study we have shown that microbial Transglutaminase (mTGase) is an efficacious enzymatic cross-linking agent for protein based substrates [1]. The specific aims of this study are to i.) investigate the efficacy of mTGase in cross-linking type II collagen and glycosaminoglycans (GAGs) and ii.) generate biomechanical control data from bovine nucleus pulposus (NP) explants under creep loading in confined compression.

The hypotheses behind these objectives are that mTGase cross-linking will enhance the physicochemical properties of the scaffold and that compression modulus data obtained from NP explants will elucidate biomechanical properties necessary for tissue engineering of the NP.

METHODS: Scaffolds were prepared with collagen (C-9301, Sigma) to GAG (C-4384, Sigma) dry weight ratios of 2:2 and 2:5 respectively. Ca²⁺ independent mTGase (Activa[®]WM, Ajinomoto Co. Inc., Japan) was purified by cation-exchange chromatography (specific activity of 27000nmol putrescine incorporated/mg/hour). Collagen was cross-linked with 0-250µg/ml mTGase and the amount of cross-link formation was quantified by amino acid analysis.

A confined compression fixture made from polytetrafluoroethylene was designed for use with a Dynamic Mechanical Thermal Analyser (DMTA[®]2980, TA Instruments Inc., DE, USA). Creep loading (constant load of 18N) of bovine NP tissue was carried out over a three hour period at 37°C.

RESULTS: The amount of ε(γ-glutamyl)lysine increased rapidly with increasing mTGase concentrations up to 50-100µg/ml, with a plateau at higher mTGase concentrations (Fig. 1). Preliminary data obtained from creep loading bovine lumbar NP tissue (Fig.2) showed a compression modulus of 0.475MPa after 180 minutes.

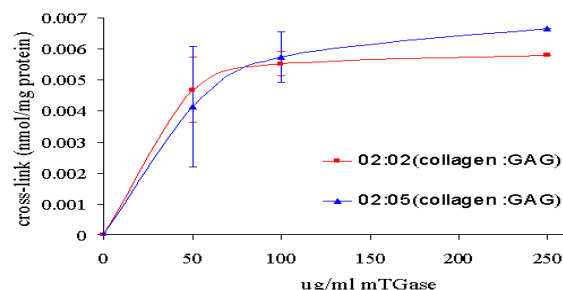


Fig. 1: Amount of cross-link formed in mTGase cross-linked collagen-GAG scaffolds (n=2).

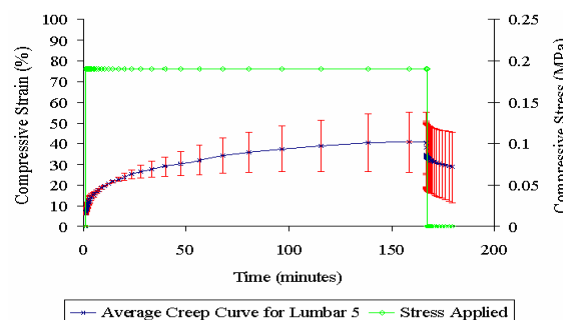


Fig. 2: Creep curve obtained from bovine lumbar (L4-L5) NP tissue. (n=3).

DISCUSSION & CONCLUSIONS: Optimal cross-link formation was found to occur at 50-100µg/ml of mTGase. Future biomechanical studies will compare the strength of mTGase cross-linked scaffolds to bovine NP tissue. Since the extracellular matrix protects the cells in the NP from stresses, recreating the biomechanical environment that the cells are exposed to *in vivo* could potentially induce more effective synthesis of disc tissue.

REFERENCES: ¹ E. Broderick, et al (2005) *J Biomed Mater Res* **72B**:37-42.

ACKNOWLEDGEMENTS: Irish Research Council for Science Engineering and Technology, funded by the National Development Plan. NUI Galway Millennium Fund.