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Self-assembling peptide hydrogel for intervertebral disc tissue engineering

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ABSTRACT

Cell-based therapies for regeneration of intervertebral discs are regarded to hold promise for degenerative disc disease treatment, a condition that is strongly linked to lower back pain. A *de novo* self-assembling peptide hydrogel (SAPH), chosen for its biocompatibility, tailorable properties and nanofibrous architecture, was investigated as a cell carrier and scaffold for nucleus pulposus (NP) tissue engineering. Oscillatory rheology determined that the system would likely be deliverable via minimally invasive procedure and mechanical properties could be optimised to match the stiffness of the native human NP. After three-dimensional culture of NP cells (NPCs) in the SAPH, upregulation of NP-specific genes (*KRT8*, *KRT18*, *FOXF1*) confirmed that the system could restore the NP phenotype following de-differentiation during monolayer culture. Cell viability was high throughout culture whilst, similarly to NPCs *in vivo*, the viable cell population remained stable. Finally, the SAPH stimulated time-dependent increases in aggrecan and type II collagen deposition, two important NP extracellular matrix components. Results supported the hypothesis that the SAPH could be used as a cell delivery system and scaffold for the treatment of degenerative disc disease.

Statement of Significance

Lower back pain (LBP) prevalence is widespread due to an aging population and the limited efficacy of current treatments. As LBP is strongly associated with intervertebral disc (IVD) degeneration, it is thought that cell-based therapies could alleviate LBP by repairing IVD tissue.

Various natural and synthetic biomaterials have been investigated as potential IVD tissue engineering scaffolds. Self-assembling peptide hydrogels (SAPHs) combine advantages of both natural and synthetic biomaterials; for example they are biocompatible and have easily modifiable properties.

The present study demonstrated that a *de novo* SAPH had comparable strength to the native tissue, was injectable, restored the IVD cell phenotype and stimulated deposition of appropriate matrix components. Results illustrated the promise of SAPHs as scaffolds for IVD tissue engineering.

1. Introduction

Lower back pain (LBP) affects as much as 84% of people in their lifetime [1] with the cost to the UK economy estimated at £12 billion per annum [2]. LBP aetiology is multi-factorial, with genetics [3], mechanical injury [4] and aging all contributing to the condition. However intervertebral disc (IVD) degeneration, also known as degenerative disc disease (DDD), is strongly associated with LBP in over 40% of cases [5].

The IVD consists of 3 distinct but interdependent tissues; the fibrous annulus fibrosus (AF) which surrounds the gelatinous nucleus pulposus (NP) [6] with cartilage end-plates on the superior and inferior surfaces [7]. The main roles of the IVD are to act as a shock absorbing system by transferring loads and dissipating

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energy that the spine is subjected to [8], and to act as joints [9]. DDD occurs when there is an imbalance between IVD extracellular matrix (ECM) catabolic and anabolic events [10] which often leads to an inflammatory reaction [11].

Current LBP treatments have limited long-term efficacy [12] and crucially are symptomatic rather than curative. LBP prevalence is rising due to an aging population [13] so there is an urgent need for an entirely new approach DDD treatment.

DDD originates within the NP [14] therefore it has been hypothesised that restoration of a degenerated NP when a healthy AF is present may slow DDD, provide analgesic effects and restore mobility of the back [7] whilst still preserving the capacity of the IVD to remodel [15,16]. A number of animal model trials that investigated the transplantation of NP cells (NPCs) into artificially degenerated IVDs have produced promising results [17] with increases in ECM production and maintenance of disc height reported [18–20]. Injection of autologous human IVD derived chondrocytes into the NPs of LBP sufferers determined that, after a 2 year follow-up, cell therapy provided superior analgesic effects and higher fluid levels than the control population [21].

Due to the disadvantages of current biomaterials, such as lack of biocompatibility and issues with matching the biomechanics of native tissue [22,23], self-assembling peptide hydrogels (SAPHs) have been investigated as potential NP tissue engineering cell carriers and scaffolds. SAPHs have the inherent ability to self-assemble into complex supramolecular structures after exposure to the appropriate stimuli [24,25]. The simple self-assembly mechanism produces hydrogels suitable for the culture of a number of cell lines [22,26,27]. In theory, SAPHs combine the advantages of natural and synthetic biomaterials whilst overcoming their deficiencies [23,28]. For example, the peptide sequence can be easily modified to control the final mechanical and structural properties [22] whilst SAPHs are likely to display good biocompatibility as their breakdown products will be the same as those of proteins synthesised *in vivo*.

A number of SAPH classes have been investigated for applications in NP tissue engineering with promising biocompatibility results reported however these studies did not investigate the rheological behaviour of the SAPHs despite the important biomechanical role of the NP [29–31]. Furthermore, these (and similar) NP SAPH studies used a limited and non-NP specific set of marker genes [29–32] therefore the phenotype of the encapsulated cells could not be confirmed. We have previously identified a number of novel genes that characterize the bovine IVD transcriptional profiles, allowing determination of a NPC phenotype [33].

FEFEFKFK (F; phenylalanine, E; glutamic acid, L; lysine) chains have a hydrophobic and hydrophilic face [24,27] so an increase in solution ionic strength and pH causes the chains to self-assemble into anti-parallel β-sheets nanofibres. Above a critical gelation concentration, the nanofibres aggregate into a self-supporting hydrogel accompanied by a significant increase in viscosity and slight reduction in volume [22]. FEFEFKFK SAPHs have been successfully exploited as drug delivery vehicles [34] and cell culture scaffolds [27,35]. Bovine articular chondrocytes encapsulated within FEFEFKFK SAPH adopted rounded morphology, proliferated and produced the cartilage ECM component type II collagen which suggested that cells retained their phenotype. It was concluded that the system was a suitable bovine chondrocyte culture environment and could serve as a template for cartilage tissue engineering [36]. Previous studies have highlighted a strong similarity in cell morphology and ECM production between chondrocytes and NPCs [33,37]. We postulated that the FEFEFKFK SAPH should therefore be capable of supporting NPC culture, preserving their phenotype and stimulating production of appropriate ECM components.

In this study, FEFEFKFK SAPH was investigated for its potential as a cell carrier and scaffold for NP tissue engineering. The system was characterised then optimised to match the native NP strength and to determine its injectability. Finally, the 3D culture of primary bovine NPCs was analysed for cell viability, gene expression of encapsulated cells and production of ECM components to determine whether the FEFEFKFK SAPH could restore and preserve the NP phenotype.

2. Materials & methods

2.1. Preparation of SAPH

Table 1 describes the FEFEFKFK SAPH production process where the predetermined amount of peptide powder (>95% purity, batch LR294429) (*Biomatik, USA*) was dissolved in distilled water (dH_2O). The samples (pH 1.9–2.1) were heated at 80 °C for 2 h to ensure complete dissolution. The gelation process was induced by the addition of 1 M NaOH and 100 μl of 10× Dulbecco's phosphate buffered solution (DPBS) (*HyClone, USA*). Further heating at 80 °C for 12 h aided mixing of constituents. Cooling to room temperature (RT) produced a homogenous clear hydrogel with a pH of between 9.3 and 9.6.

2.2. Oscillatory rheology to determine viscoelastic properties

Viscoelastic behaviour was determined using an AR-G2 rheometer with 20 mm parallel plates on days 1, 7 and 14 after plating FEFEFKFK SAPH (Section 2.4). For each run, 150 μl of sample was loaded onto the stage and the upper plate was lowered to a 0.25 mm gap. A strain amplitude sweep was performed at constant frequency (1 Hz) between 0.01 and 100% strain to determine the linear viscoelastic region of the material. The storage modulus (G′), the deformation energy stored during the shear process of the sample [38], and loss modulus (G″), the amount of energy dissipated during shear [24], was recorded at 1% strain (frequency sweep). A recovery cycle experiment on day 1, designed to mimic the injection process, involved breaking the SAPH by applying 160% strain then reducing the strain to 1% and recording G′ and G″ values. All samples were tested at 37 °C and a solvent trap was used to minimise solvent evaporation.

2.3. Bovine NPC isolation and culture

Bovine NPCs (bNPCs) were isolated from bovine tails (18–36 months) purchased from a local abattoir. Each sample was enzymatically digested in serum-free media containing 0.5% pronase (*Merck Chemicals Ltd, Nottingham, UK*) for one hour and transferred to serum free media containing 0.5% collagenase type II (*Invitrogen, Paisley, UK*) and 0.1% hyaluronidase (*Sigma, Poole, UK*) for two to three hours on an orbital shaker at 37 °C. Supernatant

Table 1
Constituents required to produce 25, 30 and 35 mg ml^{-1} FEFEFKFK SAPHs.

Final SAPH concentration/mg ml^{-1}	Peptide powder/mg	dH_2O/μl	1M NaOH/μl	10× DPBS/μl	Cell suspension or medium/μl
25	31.25	811	89	100	250
30	37.5	802	98	100	250
35	43.75	786	114	100	250