Regeneration of the IVD Using a Bio-nanotechnology Strategy
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Introduction
Low back pain (LBP) is a crippling condition with an incidence estimated to be in excess of 70% in industrialised nations1. Degeneration of the intervertebral disc (IVD) has been identified as a leading factor of LBP based on imaging studies2. Current therapies alleviate the pain temporarily but do not restore disc function. The contained, avascular IVD environment provides an ideal setting for intradiscal injection of therapeutic biomolecules. The objective of this study is the development of a targeting mechanism for disc regeneration using scFv antibody fragments generated using phage display technology to target scaffolds containing therapeutic factors to specific cells within the disc.

Materials and Methods
Candidate target genes were chosen based on microarray and RT-PCR analysis. This led to identification of NCAM, which was cloned using overlap PCR to add N- and C-terminal tags for protein transport and purification. DNA sequencing was followed by subcloning into an expression vector, protein production and purification of the recombinant protein using immobilised metal affinity chromatography (IMAC) for panning with a human scFv library3.

Results
Following overlap PCR and cloning of the product, the sequence of the construct was confirmed prior to cloning into an expression vector. Protein expression was carried out in the E. coli periplasm and optimisation of expression was followed by purification using IMAC to take advantage of the added C-terminal hexahistidine tag (Fig.1). Initial phage display screening led to an obvious increase in anti-NCAM1 binding (Fig.2). Individual phage clones were identified for scFv production and linking to model hyaluronan-based systems for in vitro targeting studies.

Discussion and Conclusions
Analysis of phage populations from each panning round is underway. Antibodies with high affinities for NCAM will be expressed in E. coli and purified for linking to scaffolds. This is expected to lead to increased delivery of therapeutic biomolecules to target cells.

References

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Disclosures
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