

**Intervertebral Disc Cell Therapy**  
**Satellite meeting held by Back to Back**  
**Cardiff University**  
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**Introduction**

There is considerable and increasing research interest in a biological approach to address degeneration of the intervertebral disc. Some of this interest has probably stemmed from the work carried out in the disc's related-tissue, articular cartilage, with some reported success of autologous cell therapy in the clinic. Indeed, autologous cell therapy has been used in disc herniation patients in Germany. Whilst there are undoubtedly similarities between the intervertebral disc and articular cartilage, there are also many differences and the application of cell therapy to the spine has factors which do not feature when considering biological repair of cartilage in a synovial joint. The object of this meeting was not to discuss particular tissue engineering projects *per se* but rather to consider some of the peculiarities and challenges of cell therapy for the intervertebral disc by bringing together experts in the field of articular cartilage, intervertebral disc, tissue engineering and spinal surgeons who could be expected to take such a treatment into the clinic.

The meeting began with an excellent overview of the situation given by **Rita Kandel** from Toronto who was invited as a guest speaker and kindly sponsored by the BSMB. In her talk entitled 'The Current State of Disc Cell Therapies' Professor Kandel reviewed the potential of cellular therapies in light of when they might be used to treat degenerative disc disease. Early on in the disease process when resident cells remain and are responsive it might be appropriate to use bioactive molecules delivered either by intradiscal injection or gene therapy to stimulate them; the degenerative disease process could be modulated by either up-regulating production of matrix molecules, down-regulating molecules involved in the degradative process, or up-regulating regulatory molecules such as TIMP1. In more advanced disease when there is cell loss, delivery of cells on their own or seeded onto a scaffold could be used. This approach could be utilized to treat a degenerate nucleus pulposus or to generate a patch for annulus fibrosus repair. When extensive structural abnormalities are present it might be more appropriate to replace the entire disc with a biological implant composed of nucleus pulposus, annulus fibrosus, and cartilage endplate tissues integrated to the top surface of a bone substitute. The bone substitute is a necessary component to replace the sclerotic endplate to ensure revascularization; it is also likely to provide effective attachment to native tissue. Cellular therapies to repair the degenerate disc are still in the experimental stage. At present the only clinical trial in this area is the one in Germany consisting of injecting cells isolated from herniated tissue, whose numbers were expanded in cell culture, into single level degenerate discs. The long term outcome of this study is pending although the authors claim promising early results. However given the pathological changes that occur in the degenerate disc and what we know about the pathogenesis of this disease, the question was raised as to whether biological disc replacement was the only option if the goal was long term efficacy. The problems limiting clinical application of these treatments were also discussed as were some approaches that could be taken to overcome them, such as the use of stem cells. Given that this field is in its infancy there was general agreement that development of these treatments will likely teach us a lot about the biology of the tissues and cells that make up the disc.

One area which certainly differs from the articular cartilage field is who should be treated and which patients might benefit from cell therapies. Whilst autologous chondrocyte implantation (ACI) was originally used to treat patients with isolated chondral defects in the knee, there is no one group of individuals for whom disc cell therapy would be clearly suitable. **Jeremy Fairbank**, Oxford, provided a clinical perspective by emphasising the complexity of back pain and our poor understanding of the factors actually causing it. Whilst there is some relationship between disc degeneration and back pain, psychological distress is actually the best predictor of both chronic back pain and outcome following treatment. However Professor Fairbank suggested that there is evidence (eg from Hong Kong) that there are factors and techniques which might be suitable to use in the identification of appropriate patients for biological repair. For example, perhaps patients with disc degeneration occurring earlier in life than might normally be expected could be targeted

initially. One might aim to treat discs at a single level initially, but actually multiple level treatment may be a more promising area. An intact outer annulus might be a necessity, at least based on some results to date. Typical exclusion factors might be all patients with a normal level of back pain for their age group and 'yellow flags'. Diagnostic techniques to be used would be likely to include MR scans to show the spinal level at which changes occur; identifying which level is painful may need to rely on techniques such as discography and local anaesthesia. Other assessments may be necessary, particularly relating to demonstrating appropriate nutritive pathways to support the needs of any cells to be introduced.

The requirements for a successful disc repair also differ from those seen in ACI, both in regard to the mechanical requirements and tissue organisation. **Bob Mulholland**, Nottingham demonstrated in some dramatic videos (courtesy of John Sheppard, Hastings) of how both normal and pathological discs deform when mechanically loaded. It is thus evident that any repair must be permissive of this complex range of movements and hence will require appropriate structural organisation of the repair tissue; consideration must be made of both the annulus and the nucleus. He also discussed experiments which demonstrated that abnormal and non-uniform load-carriage appear to lead to pain; in any repair tissue, it is thus important that the nucleus loads hydrostatically (ie both horizontally and vertically) and that no stress peaks arise because of the properties of the repair tissue. Disc height gain appears less important as there is no evidence linking loss of disc height to pain. **Vic Duance** and **Bruce Caterson**, Cardiff, and **Sally Roberts**, Oswestry then discussed the question of what tissue will constitute an adequate repair; would a generalised fibrocartilage be necessary or would hyaline cartilage be adequate? If one is trying to match the biochemistry, then the vitreous of the eye is more similar to the nucleus pulposus than are other cartilages! They pointed out that at present, unlike the situation with ACI, we have little understanding of the type of tissue organisation which is required for a successful repair. However it is evident that whatever tissue is formed, good integration between repair and resident tissue is likely to be necessary. Mosaicplasty, a method of repairing cartilage defects with isolated plugs of autologous cartilage has shown that integration with newly synthesised repair tissue can be achieved and can be stimulated by techniques such as removing glycosaminoglycans from the interface, growth factors or the introduction of cells. In discussion it was put forward that while it might not be necessary to recapitulate the fine structure of the disc exactly, any repair tissue must be able to provide adequate biomechanical strength, flexibility and load-carriage. Other approaches were also discussed such as combining cell therapy with a 'ready-made' tissue, (as in allografts already being carried out in China), or using an acellular therapy.

Several speakers then discussed the constraints arising from biological or physical factors which must be considered if repair is to be successful. **Brian Johnstone**, Portland, gave an overview of some biological constraints. He pointed out that since the aetiology of disc degeneration was unknown, any repair runs the risk of recapitulating the degenerative pathway. Delineation of the goal is crucial as strategies for repair of the nucleus are very different from those for repair of the annulus or the whole disc with its adjacent bone. Choice of an appropriate source of cells for repairing the disc and ensuring cell survival and appropriate matrix production is hindered by poor knowledge of disc cell phenotype and behaviour. He stressed the difficulties involved in repairing a large structure like the human disc, illustrating this by his experience where he found that successful repair of a rabbit meniscus had not translated at all to repair of goat meniscus which had failed dramatically. The problems of repairing large avascular tissues were also pointed out by **Jill Urban**, Oxford who discussed the constraints imposed by the limited nutrient supply. Because low molecular weight nutrients such as oxygen and glucose are supplied to the disc cells by diffusion, the cell density which can be supported is limited and is inversely proportional to disc height (eg a disc of 2mm in thickness can support >30 million cells/ml, whereas a 6mm thick disc can only support <7.5 million cells/ml. Repair in a mouse or rabbit disc may be reasonably rapid; however, even in a healthy human disc, the low cell density which can be supported means that production of sufficient matrix to repair the tissue will be very slow (years). In the degenerate discs which are likely to be the target of cellular repair strategies, end plate calcification is common and further limits transport of solutes to the cells; any implanted cells may not survive. **Peter Winlove**, Exeter, pointed out that current diagnostic techniques cannot give the information necessary for assessing which patients would benefit from cellular repair therapies nor can they assess cellular function. MRI can only provide structural information at a fairly crude level and hence can only assess gross changes. However delivery of contrast agents into the disc as proxies for nutrients could provide a method for assessing in which patients cellular therapies are viable once the measurements are better understood. Methods such as spectroscopy, though useful for imaging markers of cell metabolism and matrix turnover in more cellular tissues, may not be sensitive enough in the disc which is bedevilled by its

low cell density. Development of needle optical or electrochemical sensors may provide the best means both of diagnosing the current metabolic state of a disc and of its repair, but taking these developments from their use *in vitro* to *in vivo* (and understanding what the measurements mean) is challenging.

**David Eyre**, Seattle, concentrated on collagen phenotype, particularly relating to type II collagen. He showed that it is constructed on a template of collagen V and XI  $\alpha$  chains, with different combinations and subtle differences between tissues. For example, in the nucleus pulposus it is [ $\alpha 1(XI)\alpha 1(V)\alpha 2(V)$ ], whilst adult articular cartilage is [ $\alpha 1(XI)\alpha 1(V)\alpha 3(XI)$ ] and the vitreous of the eye has 2  $\alpha XI$  chains and 1  $\alpha V$  chain. It is likely to be important that the correct splice variant or post-translational modifications are produced for the tissue, for example the type IX collagen associated with type II in young discs (but absent from old disc tissue). The collagen II network differs in these tissues with the collagen II network in articular cartilage being a constrained, cross-linked network whereas in disc and vitreous it is loose and extensible once removed from mechanical constraints. Although there is as yet no direct evidence it is likely that network organisation is linked to molecular structure, it would thus be necessary that cells producing any engineered or repaired disc, produced disc-like collagen II rather than cartilage-like collagen II. In addition, perhaps the collagen phenotypes could be used as markers or indicators of achieving success. **Rita Kandel**, Toronto, then pointed out some potential concerns of cell therapy for disc regeneration. Inappropriate repair strategies or leakage of growth factors injected into the disc to facilitate repair could lead to abnormal differentiation of stem cells. She stated that the possibilities of inducing tumours from stem cells or chordomas via notochordal cells must be considered in any potential treatment. Control of osteogenesis via the use of implanted cells (eg MSCs) or growth factors would be important: too much could prove a problem whilst not enough may provide insufficient fusion if implanting a whole disc/motion segment with bone to attach to host vertebrae. She also pointed out that we need to consider what complaint we are aiming to treat. Treatment should be aimed towards relieving pain which will require research into its pathogenesis. Because of the mismatch between pain and disc degeneration and because of the large psycho-social overlay seen in back pain in particular, we need to develop methods for selecting patients who are likely to benefit from biological therapies. If they are used inappropriately they may fall into disrepute.

Animal models for testing repair were then discussed. **Jim Ralphs**, Cardiff reviewed development of the different regions of the disc comparing stages in the rat with those of human. While the annulus of human and rat are similar in structure and cell type there are large differences in the nucleus. Notochordal cells and a semi-fluid nucleus persist in rodents, pigs, cats and in some dog breeds. However these cells disappear before adulthood in man, ruminants, horses and chondrodystrophoid dogs and are replaced by the more chondrocyte-like cells of unknown provenance which synthesize the firmer more collagenous mature disc nucleus. Notochordal cells also disappear in rabbits, but the resulting nucleus is fibrous and not similar to that found in man. Thus rodents may be suitable as a model for humans up until, but possibly not after, birth, due to the inherent differences in cell type of the nucleus and the growth plate and secondary epiphysis. **Jim Melrose**, Sydney, described a sheep model of disc degeneration which might be appropriate for testing cellular disc repair. It involves a stab injury, performed antero-laterally. The sheep could be considered suitable because it is of relatively large size, and its disc matrix and disc mechanical behaviour have been well characterized. In addition, the cell populations of the different regions appear similar to those seen in man. The larger stab injury model proposed has the advantage that degenerative changes are induced within 3 months as opposed to the 12-18 months in the present sheep degeneration model. **Peter Winlove**, Exeter, explained how repair will probably involve matching the mechanical and biochemical environment of repair tissue with the environment found *in vivo*. Macroscopic mechanical properties of the nucleus differ between species and with age and pathology and might be difficult to match in repair tissue. Keeping cells in repair tissue 'happy' will depend on an adequate supply of nutrients from the microcirculation and also will rely on the microcirculation for removing metabolites and matrix breakdown fragments. However even in ruminants and in dogs with the appropriate cell type, there are differences in the critical blood supply route to the nucleus; moreover the relatively short transport distances to the disc cells even in larger animals such as sheep might give false indications of whether cell based treatments could succeed in man. **Rita Kandel**, Toronto, and **Sally Roberts**, Oswestry, who have some experience in assessing cartilage repair in ACI, then discussed how disc repair tissue could be assessed as in disc, unlike in ACI, it would not be ethical to take biopsies at defined stages of repair. Assessing the biological function of the disc (ie potential load bearing etc) can be done at different levels ranging from *in vitro* monolayer culture systems, through 3-D cultures, to *in vivo* animal models or perhaps the best system, the human patient. There was discussion as to how much laboratory testing needed to be carried out, in the light of all the

limitations of these methods and the acceptance that some other treatments, such as hip arthroplasty, were not fully evaluated before going to the patient. In effect a phase I trial for cell therapy in the disc in humans has already been carried out in Germany.

**Jeremy Fairbank**, Oxford finally gave an indication of how success of cellular repair treatments could be assessed. Clinical tests and imaging would not be sufficient. Since pain is the symptom suffered by virtually all disc patients, some measure of changes in pain score would be essential. A number of standardised scores such as the visual-analogue pain score could be used. Changes in the degree of disability could be assessed through well-validated questionnaires such as SF-36 and also the Oswestry disability index which applies specifically to the spine. MRI could show up any gross structural changes. Finally, the health-economics of cellular repair therapies require evaluation. The cost of intervention in regard to potential clinical outcome should be considered by all those developing cellular repair strategies.

Throughout the meeting there was much interaction and discussion between participants, with various other issues being raised. For example, **Dick Heinegard**, Lund, questioned the involvement of the immune system, inflammation and activation of Toll-like receptors in the disc and whether this might mediate against disc repair unless the issue was addressed. The previous use of chymopapain injections for treating disc prolapse was referred to on several occasions. **Dave Buttle**, Sheffield, pointed out that this treatment provided a good model showing that the disc, at least in a healthy animal, was capable of repair, albeit slowly.

### **Conclusions and Recommendations – where do we go from here?**

This workshop demonstrated that there are several aspects relating to cellular repair of the disc, which differ from cell therapy currently being applied to articular cartilage. Apart from serious technical difficulties which have yet to be solved, one particularly challenging area is the identification of patients who will potentially benefit from these treatments; another is how the outcome can be assessed. A further obstacle to the development of cell therapy is the cost per patient. Not only does this impact on commercial interest and investment, but for such treatment to be recommended as routine health care by NICE within the UK it must be shown to provide 1 QALY of benefit for £30000.