Research article

Preliminary report of use of a novel gel designed for Nucleus Augmentation

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Abstract

A new option for the treatment of Low Back Pain has been developed consisting in the injection of a novel gel (DXM), featuring a high water content and great capacity to sustain load.

Prior to in-vivo investigation, the gel has successfully undergone ISO biocompatibility and biomechanical tests.

To replicate the intended use in human patients, an animal model (aged sheep) was used for the investigation of the injection of DXM into intervertebral discs. Tissues from 2 animals were harvested and prepared first to assess the spontaneous degeneration and the appropriateness of the model, then for assessing material behaviour with the injected discs, compared to control and sham discs. X ray films showed uniformly, a small increase in disc height over the "degenerate" control.

The injected gel filled natural tears available in the degenerated IVD material. Histology analysis is in favour of an excellent tolerance, no inflammatory cells, or foreign body reaction, no morphological changes, no reduction of the number of cells or cell viability.

has been noted histologically. The study confirms the injected material will interact as expected with the residual Nucleus Pulposus material.

Back pain is a common problem that affects most people at some point in their life. At any one time > 600 million people worldwide are experiencing back pain¹. The majority of patients typically improve rapidly in the first month. However up to a third report persistent back pain one year after an acute episode, resulting in substantial limitations in activity. The resulting economic cost of low back pain in the US and UK is $85.9 billion and
£1.6 billion respectively, a figure higher than any other condition including arthritis, cardiovascular disease and cancer\(^2\).

The options currently available for evaluation and management of low back pain include exercise therapy, pharmacological approaches, nerve blocks, radiofrequency and electro-thermal denervation procedures and surgery. Despite this there is little consensus on an effective general treatment programme.

The integration of fundamental research into the pathogenesis of low back pain at the molecular level and the physical parameters which affect the biology of the intervertebral disc may lead to the development of more successful treatment programmes.

Prof Anthony Freemont (AJF – Clinical Professor in Regenerative Medicine) at the University of Manchester has researched the basic biology of back pain and has shown that shrinkage of the centre of the intervertebral disc (a hydrogel called the nucleus pulposus) is directly linked to back pain.

Since 2004 AJF and Prof Brian Saunders (Biomaterials, University of Manchester), advised on clinical requirements by spine surgeons, have been collaborating in the development of a gel to replace that lost as the disc shrinks. This has resulted in the development of a novel class of biomaterial - “Doubly Crosslinked Microgels” - (DXM) to replace the nucleus pulposus in the intervertebral disc. This material is unique in its properties in comparison to other hydrogels available for this application due to its ability to retain water independently of load bearing and osmotic pressure. Biomechanically it is a perfect mimic of the nucleus pulposus and, excitingly, it is a smart material that can be delivered by injection because it is a liquid outside the body, but forms a firm, load bearing hydrogel following injection. This negates the need for surgery, meaning it can be used as a day case treatment.

Material and Method
Animal selection
To replicate the intended use in human patients, an animal model was used for injection of the DXM. Various animal and ‘degenerative’ models have been suggested for studies on lumbar discs\(^3,4,5,6\), however these models involve inducing ‘artificial’ degenerative disc tissues thus may not be fully representative. As the intended use of the DXM is to repair aged degenerate disc (DD) we used a model of naturally occurring age-related degeneration in the sheep. For this purpose 10-12 years old sheep were selected.

The initial study discussed here involved three animals, a further study with eight will be conducted in 2015. The study has been approved by the Ethical committee (Notification of Authorisation received on February 2014) Animals are provided by the CIT /CIC – Bordeaux (www.cic-it-bordeaux.fr)
**Material composition**
Methyl methacrylate (MMA, 99%), methacrylic acid (MAA, 99%), ethyleneglycol dimethacrylate (EGDMA, 98%), ammonium persulfate (APS, 98%), Sodium dodecyl sulfate (SDS, 98.5%), potassium phosphate dibasic (K_2HPO_4, 99%) and glycidyl methacrylate (GMA, 97%) were purchased from Sigma Aldrich and used as received. L-ascorbic acid (AS, 99%) was purchased from scientific lab supplies and used as received. Chloroform and Sodium hydroxide (NaOH, 98%) were purchased from Fisher Scientific and used as received.

Poly (MMA/MAA/EGD) microgel was synthesised as reported in a previous publication. The material was functionalised with GMA to enable formation of a double crosslinked microgel (DXM) at physiological pH via use of a dual syringe system.

The DXM material has successfully undergone biocompatibility and biomechanical tests according to ISO 10993 and ISO 18192-2 respectively. To proceed from these tests an animal study was conducted, as is the focus of this paper.

Medmix® 4:1 ratio (5ml) double syringes were prepared using dual system of filter sterilised microgel and buffer solution containing initiators that induced polymerisation upon mixing. A 16:2, 4:1 mixing chamber and 18 gauge needle was used to deliver the DXM into the NP.

**Procedure**
An advantage of using sheep lumbar spine is availability of 5 discs for use; one disc was used as a ‘native’ control left unaltered, one disc was needle punctured only, to be used as a second control, one disc was injected with PBS, two discs were injected with DXM gel.

A trained Interventional radiologist from the University Hospital of Bordeaux conducted the injection into the selected discs using the discography approach under X Ray navigation.

Animals were raised by a certified farmer experienced in research on animals. One month after the interventions the animals were killed and their lumbar spines harvested, placed in the tissue fixative of 10% neutral buffered formalin and sent to the molecular pathology laboratories of the IVD group in Manchester, a recognised world leader in the understanding of the biology of IVD disease and discogenic back pain, and their management.

The first sheep was used to train the interventional radiologist and not included in the study.

Spines from the remaining 2 sheep were harvested (Figure 1) and the intervertebral...
disc and the associated bones removed (Spinal Functional Unit [SPU]). The bone attached to the disc (Bony End Plate) was pared away to leave enough to support the disc, but insufficient to affect disc function, bone immediately next to the disc (the vertebral bony end plate).

These pieces of tissue were turned into appropriate sagittal "blocks" of IVD tissue (and associated bone). These are the supported and selected tissue from which histological sections are made.

Because of concerns about the integrity of the gel under the acid conditions usually used to decalcify bony tissue for histological examination an old, but still appropriate technique was used to cut the sections "undecalcified". This required that Selotape is applied to the surface of the block and then sections are taken from below the Selotape which are then lifted from the surface of the block intact, attached to the Selotape.

Tissue sections were then stained with Haematoxylin and Eosin stains; the conventional stain used for morphological examination of all tissues.

Tissue sections were then subjectively assessed, quantitative measurements were obtained for important parameters. These included:

- Disc height (distance between endplates)
- Number of cells per sq millimetre
- % of viable cells adjacent to the IVD

**Results**

First tissues were assessed for evidence of spontaneous degeneration before assessing the discs that had received different interventions.

Briefly the findings were:

- The elderly animals used in this study have IVD with morphological evidence of degeneration (Fig 2) similar to that seen in humans.

- It is not clear whether the IVD in the lumbar spine vary in size naturally as it does in human spine but the IVD from nearer the cranial end of the lumbar spine were narrower than those from the caudal end.

- It was not always possible to see the gel within the IVD because it tended to be physically removed during sectioning, but where it remained it was clearly seen (Fig 3). Elsewhere spaces were left where it had been removed from the fixed (and...
therefore morphologically intact) tissue (Fig 4).

When the gel was injected, it filled natural tears available in the degenerated IVD material (Figure 5)

The discs that had received "sham" procedures were the lowest of all the IVD, where macro histology shows an empty disc and torn tissues. (Figure 6)

Whether due to the gel or not, those discs in which gel had been injected all showed uniformly, a small increase in disc height over the "degenerate" control (i.e. untreated disc), which was the most caudal of all the discs examined. (Table 1)

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Disc</th>
<th>Height</th>
<th>Number of cells / sq mm % of viable cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/2</td>
<td>Puncture</td>
<td>1.7</td>
<td>45</td>
</tr>
<tr>
<td>L1/2</td>
<td>Puncture</td>
<td>1.8</td>
<td>41</td>
</tr>
<tr>
<td>L3/4</td>
<td>Serum</td>
<td>1.9</td>
<td>36</td>
</tr>
<tr>
<td>L3/4</td>
<td>Gel</td>
<td>2.2</td>
<td>47</td>
</tr>
<tr>
<td>L3/4</td>
<td>Gel</td>
<td>2.3</td>
<td>48</td>
</tr>
<tr>
<td>L4/5</td>
<td>Gel</td>
<td>2.4</td>
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<tr>
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<td>Gel</td>
<td>2.4</td>
<td>48</td>
</tr>
<tr>
<td>L5/6</td>
<td>Control</td>
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<td>38</td>
</tr>
<tr>
<td>L5/6</td>
<td>Control</td>
<td>2.5</td>
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The degree of "natural" degeneration, in which there is cell death and cell proliferation to form IVD cell clusters, made assessing changes in total and viable cell
counts very difficult. There is no evidence that the gel reduces cell counts/unit area (Fig 4), with cell counts within these discs in the same range as the non-gel discs (however, the non-gel discs have a very wide range of cell counts because of degeneration).

- Even though there are differences in total cell numbers between all discs, there was no evidence of the gel reducing the number of cells or cell viability.

**Discussion and remarks**

This preliminary study shows that aged sheep constitute an acceptable animal model for the study of degenerative intervertebral disc. They develop similar biomarkers to those that characterise degeneration of human discs.

The study reveals it is possible to inject a biocompatible, radio opaque biomaterial into the lumbar disc using the well-known and safe approach used to perform discography.

Numerous investigations have reported the low incidence of complication using the standard route used in a discography procedure⁹.

Material was visible upon injection; however a more radio-dense material is recommended for routine clinical use.

The study confirms the injected material will interact as expected with the residual Nucleus Pulposus material to maintain the disc height, compared to control and shame disc.

The injected gel did not cause any adverse reaction within the disc as no inflammatory cells, or foreign body reaction has been noted histologically. The number of morphologically viable cells are in favour of an excellent tolerance of the material a month after injection.

The Nucleus Augmentation procedure is intrinsically safer than Nucleus replacement procedure as the gel is injected through a needle hole and hardens within the material of the IVD, preventing extrusion and migration as shown in the study.
References


4) Mauro Alini et Al, Are animal models useful for studying human disc disorders/degeneration? *Eur Spine J* (2008) 17 pp.2-19,


