Physical Limitations to Tissue Engineering of Intervertebral Disc:

Limited Effect of Transforming Growth Factor (TGF)-β, Fibroblast Growth Factor (FGF)-2 and Osteogenic Protein (OP)-1

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Financial Disclosure: None
There is increasing interest in the using biological methods to repair degenerate discs

- Insert new cells or tissue engineered constructs into the disc
- Stimulate cells to repair the matrix by growth factor injection
- New cells manufacture and accumulate matrix
- Restore aggregcan, disc height and function

Bullough and Boache-Adjei 1988
In recent times, TGF-β, FGF-2 and BMP-7 have demonstrated a great potential as disc anabolic factors because of their ability to induce matrix synthesis and promote repair in degenerative disc.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Effect</th>
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<tbody>
<tr>
<td>PG synthesis ↑, Cell proliferation ↑</td>
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<td>PG synthesis ↑ or ↓</td>
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### TGF-β
- Li X, et al. (2008)

<table>
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<tr>
<td>Canine NP, AF Bovine NP</td>
<td>PG synthesis ↑, Cell proliferation ↑</td>
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### FGF-2
- Li X, et al. (2008)

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### BMP-7 (OP-1)

<table>
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<th>Effect</th>
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<tbody>
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<td>Rabbit NP, AF Bovine NP, AF Rabbit NP, AF Degenerated human NP, AF</td>
<td>PG accumulation ↑</td>
</tr>
<tr>
<td>Bovine NP, AF</td>
<td>PG synthesis ↑, PG accumulation ↑</td>
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<tr>
<td>Bovine NP, AF</td>
<td>PG synthesis ↑, Cell proliferation ↑</td>
</tr>
<tr>
<td>Rabbit NP, AF</td>
<td>Collagen synthesis ↑</td>
</tr>
<tr>
<td>Degenerated human NP, AF</td>
<td>PG synthesis ↑, PG accumulation ↑</td>
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AF: Annulus fibrosus cells, NP: Nucleus pulposus cells, PG: proteoglycan
In this study, we examine how FGF-2 and BMP-7 influence the rate at which proteoglycans can be accumulated in a three dimensional cell culture system.

Is it possible to repair the matrix by FGF-2 and BMP-7 injection under degenerated disc?
MATERIALS and METHODS

**Alginate culture system**

- Cells were isolated from the nucleus pulposus of 18-24 month bovine caudal discs.

<table>
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<tr>
<th>Control</th>
<th>TGF-2 (20 μg/ml)</th>
<th>FGF-2 (50 μg/ml)</th>
<th>BMP-7(OP-1) (100 ng/ml)</th>
</tr>
</thead>
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- Incubate at 37°C/5% CO₂
- 5 beads /well

![Diagram showing cell cultures in alginate beads]

- **Healthy Disc Environments**
  - DMEM containing 6% FBS at densities of 4 million cells/ml under a normal osmotic condition (400 mOsm) and 5% oxygen

They were cultured for 5 days in alginate beads

**Examination:** GAG accumulation (DMB assay)
Amount of GAG/bead increased with time in culture (+: p<0.05), and was increased about 2–3 times by addition of TGF-β, FGF-2 or BMP-7 after 5 days culture (*, #, +: p<0.05).

(Pooled data from seven separate experiments are shown.)
Lactate production was increased by about 1.5-2 times by addition of TGF-β, FGF-2 or OP-1 compared with the control group (*) p<0.05 compared with control.

(Pooled data from seven separate experiments are shown.)
After 2 days cultures, sulphate incorporation rates was increased about 2-3 times by addition of TGF-β, FGF-2 or BMP-7.

However, sulphate incorporation rates decreased with in culture (+: p<0.05 compared with day 2).

(Pooled data from seven separate experiments are shown.)
DISCUSSION

- Proteoglycan loss is one of the first signs of disc degeneration.

- Regeneration of disk tissue with sufficient mechanical strength particularly requires the production of glycosaminoglycan (GAG), which accounts for 7% of disk tissue.

  Adequate GAG per tissue volume to resist compression (7%-GAG/wet wt: viz. 70 mgs/ml)

  2.8-13 kN

  disk tissue

GAG SWELLING PRESSURE
GAG production of the control group was about 0.07 mg/ml/day under the healthy disc environments.

GAG production was increased about 2-3 times by addition of TGF-β, FGF-2 or OP-1, respectively (*, #, +: p<0.05)
Calculated times 7% GAG per wet weight (viz. 70 mgs/ml)

- Calculated times to produce a concentration equal to the in vivo concentrations of 70 mgs/ml assuming initial rates were maintained and there was no loss of GAG, were > 900 days.
- This concentration could be increased to 0.11, 0.15 and 0.19 mg/ml/day by TGF-β (20 μg/ml/day), FGF-2 (50 μg/ml/day) or BMP-7 (100 ng/ml/day), respectively. Thus, growth factors support could increase rates of GAG production by up to 2-3 fold.
- However the theoretical time necessary to produce a construct with the 7%-GAG as the disc matrix even under ideal conditions would still be >>1 year.
Matrix turnover is very slow – around 20yrs for proteoglycan (Roughley PJ. Spine, 2004), >100 yrs for collagen (Verziji N, J Biol Chem, 2000).

Biological repair depends on the disc maintaining a population of viable and active cells

- Degenerative lesions of the disc are not confined to the discs, but also affect end-plates to undergo osteosclerosis.
- Even if regenerated disc cells are successfully transplanted, the cells may not be nourished adequately, possibly causing repeat degeneration and allowing degenerative change to progress again.

Blood: 10%O₂, pH 7.4, 285 mOsm
CONCLUSIONS

- In this study, addition of FGF-2 or BMP-7(OP-1) to constructs was found to have big effect on the concentration of accumulated GAG under healthy disc environments.

- However, matrix turnover is very slow even if the growth factors use, and increasing cell metabolism potentially should increase GAG deposition, but leads to a more nutrients demands.

- Thus, the clinical application of disc regeneration medicine needs to be advanced by providing appropriate physiological conditions with consideration of age-related disc changes.

Acknowledgements: I would like to thank Dr.Jill Urban for supervising me in Physiology Laboratory, Oxford University, UK. I thanks also to the rest of my lab group.