Cell clusters are indicative of stem cell activity in the degenerate intervertebral disc: can their properties be manipulated to improve intrinsic repair of the disc?

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**Short running title:** Cell clusters and intervertebral disc degeneration

**Abstract**

The aim of this study was to examine the complexity of the stem cell populations in the intervertebral disc (IVD) and understand their role in disc degeneration with a view to determining whether the resident stem cells could be developed for therapeutic purposes to combat IVD degeneration. Stem cells have been isolated from disc and paradiscal tissues including the notochord, annulus fibrosus (AF), nucleus pulposus (NP), cartilaginous endplate (CEP), ligamentum flavum and vertebral body.  Resident AF and NP cells are relatively sparsely distributed occurring as single or occasional doublet cells surrounded by an extensive extracellular matrix (ECM). Small clusters of 4-12 cells also occur close to annular lesions in experimental ovine and canine disc degeneration, these are indicative of an attempted repair response by resident stem cells.  The rat IVD also has notochordal and peripheral cell populations in the outer AF which express CS sulphation motifs (7-D-4, 4-C-3, 3-B-3[-]) characteristic of activated stem cells, the murine IVD also has a cell population in the outer AF adjacent to the vertebral growth plate with characteristics of a progenitor cell population.  These have also been observed in rabbit, mini-pig, ovine and human IVDs. Chondroid cell nests in the ovine NP may represent a progenitor/stem cell reserve.   Such human chondroid cells, express CS sulphation motifs (7-D-4, 4-C-3, 3-B-3[-]), cytokeratin-8 and 19 and CD cell surface markers typical of stem cells including OCT3/4, CD105, CD90, STRO-1, NOTCH1 and JAGGED1. Similar stem cell populations are present in grade IV degenerate human IVDs. A greater understanding of the biology of this chondroid cell population may identify them as a therapeutic resource. A resident therapeutic cell type adapted to the demanding IVD environment may be advantageous in repair strategies.

**Introduction**

            The intervertebral disc (IVD) is a tough visco-elastic cushion which provides weight bearing properties to the spine during axial loading and mechanical stability during flexion-extension and torsion ([1](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_1),[2](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_2)). The IVD is composed of a disparate arrangement of tissues of different form and function which equip the composite IVD with remarkable biomechanical properties ([3](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_3)). The peripheral IVD is an outer collagen rich annulus fibrosus (AF), consisting of lamellae designed to withstand tensional hoop stresses generated in the IVD during axial spinal loading ([1](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_1),[4](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_4)). These enclose a central nucleus pulposus (NP) which, with its gelatinous proteoglycan rich matrix, imbibes water and provides hydrodynamic and weight bearing properties. Hyaline cartilaginous endplates (CEPs) merge with the fibrocartilaginous NP and AF interlocking these with the bone of the adjacent vertebral bodies([5](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_5)). Each of the tissues of the composite IVD differ in their molecular make-up and in their cellular populations. The cell density of the IVD is one of the lowest of any cartilaginous tissue in the human body ([6-8](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_6)).   A number of cellular morphologies are evident in the IVD, the AF contains cells of an elongated fibroblastic morphology laid down along the collagen fibre bundles in the annular lamellae occurring as interconnected strings of cells of similar morphology to those described in tendon ([9](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_9)). The cells of the CEP and NP display a rounded chondrocytic morphology.  In the mature IVD the cells show little evidence of cell division and occur as sparsely distributed single or occasional doublet cells in an extensive extracellular matrix (ECM).  A population of notochordal cells have also been described in the IVD which persist into adulthood to variable degree depending on animal species ([10](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_10)).

Notochordal cells occur centrally in the NP of rodent IVDs and in also in other species (Fig 1h-p). These appear to have a protective effect on IVD tissues, as is evident in chondrodystrophoid (ChD) dogs where  the age-dependent disappearance of notochordal cells correlates with a higher incidence of disc degeneration while in non-ChD canines notochordal cells persist into adulthood and these breeds have both a later onset and lower incidence of IVD degeneration (IVDD) ([11](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_11),[12](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_12)).  In the human IVD, cells of a typical physalipherous notochordal morphology disappear by 11 years of age however cells have also been described in the adult IVD which nevertheless express notochordal cell markers despite their atypical appearance compared to the notochordal cells found in immature IVD tissues ([13](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_13)).

 The aim of this study was to review the literature on stem/progenitor cells in the IVD and to examine how these might be manipulated to provide a therapeutic resource to combat IVDD and promote IVD repair and regeneration.  In order to do this the literature which describes how stem/progenitor cells are regulated in-situ was also reviewed and extrinsic methods which have been used to stimulate stem cells in other systems were discussed to provide insights into their therapeutic application to IVD stem cells in tissue repair processes.

 **Notochordal cells and IVD development**

            Figure 1a shows a segment of human foetal spine with the notochordal remnant in the NP and notochordal tract through the superior cartilaginous vertebral rudiment evident.  The cartilaginous nature of the spine is clearly evident at this stage of spinal development (12 weeks gestational age), hypertrophic chondrocytes also evident in the central vertebral cartilaginous rudiment (Fig 1a) are forerunners to the ossification centres which will eventually lead to bone formation and the vertebral body ([14](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_14)).  While all animals share common pathways of IVD embryonic development, major differences exist in the persistence of notochordal cells in postnatal IVDs ([10](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_10),[15-17](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_15)).  Rat, mouse, pig, cat and rabbit IVDs retain notochordal cells postnatally but to a variable extent and some animal strains retain notochordal cells throughout adulthood ([10](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_10)).  Cattle and sheep resemble human IVDs, displaying an early postnatal disappearance of notochordal cells. The canine IVD is particularly interesting; breeding pressures for small dog traits has led to dogs which display early epiphyseal long-bone growth plate closure in chondrodystrophoid (ChD) canine breeds such as the Dachshund, French bulldog, Beagle and Pekingese ([18-20](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_18)). These dwarf-like features are accompanied by an early replacement of notochordal cells within the first year of life and a premature onset of IVD degeneration ([11](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_11), [21](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_11)-25). Non-ChD canine breeds (Greyhound, Saluki, Borshoi) display a much later onset and significantly lower incidence rates of degenerative disc disease (DDD) ([12](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_12),[19](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_19),[23](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_23),[25](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_25),[26](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_26)).  The persistence of notochordal cells in the non-ChD canine breeds has led to a realisation that this cell type has a protective role to play over other NP cells preserving IVD integrity ([27](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_27)).  The decline of notochordal cells early in life contributes to a relatively early onset of IVD degeneration in ChD breeds ([11](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_11),[23-25](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_23)).  Many authors have proposed that notochordal cells produce trophic factors in a similar manner to mesenchymal stem cells (MSCs), and a recent study has for the first time, characterized and identified the necessary and soluble factors that notochordal cells produce to maintain IVD homeostasis ([27](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_27),[28](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_28)).  Notochordal cells have essential roles to play in embryonic IVD development in all species; however, their rate of disappearance from adult IVD tissues is highly variable. Isolation and culture of single cells from chondroid cell nests in adult human IVDs, showed they proliferated and expressed cytokeratin (CK)-8 and CK-19 and a number of notochordal CD markers ([29](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_29)), another study showed these cells expressed OCT3/4, CD105, CD90, STRO-1, NOTCH1 and JAGGED1 identifying them as originating from a stem cell lineage. An important study by Kim established that notochordal cells can display a number of morphologies thus the adult IVD cells which have been described which express notochordal markers may be evidence of the development of the notochordal population into a resident adult progenitor cell population  ([30](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_30)). Rabbit notochordal cells imaged via an automated live cell imaging system *in-vitro*display three distinct morphologies (large vacuolated, small extensively interconnected and round chondrocyte-like), including a typical chondrocyte-like cell morphology demonstrating these have the capacity to differentiate into cell types typical of the cells observed in mature IVD tissues ([30](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_30)).

Figure 1 b-e shows toluidine blue stained IVD sections from newborn and 12 week old Chloe B6 MMP knock-in and C57 BL/6 mice and the immunolocalisation of perlecan HSPG2 (Fig 1 f, g) which is a prominent proteoglycan in many stem cell niches([31](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_31)).  A discrete cell population (enclosed by dotted lines in Fig 1c, e) adjacent to the peripheral vertebral growth plate (VGP) and outer annulus fibrosus (AF) in the murine IVD have characteristics of a progenitor or stem cell population. Henrikkson et al have identified stem cell niches at this location in a number of animal species including rabbits, minipigs, rats and man ([32](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_32),[33](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_33)).  Shu et al also identified a discrete progenitor cell population in the same location in newborn ovine and foetal human IVDs ([34](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_34)).  Perlecan is prominently produced by these progenitor cells and is a common component of many stem cell niches ([31](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_31),[35-40](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_35)) including perichondrial niches in the developmental human foetal knee, hip and elbow ([41](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_41)). Activated stem cells released from the niche environment express a number of native CS sulphation motifs including 4-C-3, 7-D-4, 3-B-3(-) and Notch-1 ([42](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_42)).  Immunolocalisation of these motifs are indicated in Fig 1 (j-l) in E19 rat IVDs ([43](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_43)).  Notochordal cells are evident in the non-ChD canine IVD (Fig 1m), these have a similar morphology to murine notochordal cells (Fig 1h, i) and contrast with the ChD canine IVD where discrete chondroid cell colonies are present (Fig 1n). Newborn fallow deer (Fig 1o) and newborn sheep IVDs contain small groups of cells in the NP with morphologies more closely resembling the ChD canine than rodent notochordal cells (Fig 1o, p).

Progenitor cell populations expressing the 4-C-3, 7-D-4 and 3-B-3 CS sulphation motifs are evident  in knee, hip and elbow cartilage rudiments and are actively involved in diarthrodial joint development ([41](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_41),[42](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_42),[44-47](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_44)). These CS sulphation motifs are considered markers of tissue morphogenesis ([48](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_48)).  The positive demonstration that these CS motifs represent markers expressed on the surface of progenitor/stem cells became apparent from a study where chondrocytes from superficial cartilage were sorted by flow cytometry using antibodies to these CS motifs ([42](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_42)).  The isolated 4-C-3, 7-D-4 and 3-B-3(-) positive chondrocytes have been demonstrated to be capable of synthesising a full depth neocartilage which displayed a stratified structure with proteoglycan and collagen epitope distributions in the superficial, mid and deep cartilage zones identical to those observed in native articular cartilage and chondrocyte morphologies typical of each zone appropriately distributed throughout the neocartilage ([42](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_42)).

Bioactive compound(s) have been demonstrated in the conditioned medium from cultured notochordal cells from a number of species which have the potential to promote IVD regeneration ([49](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_49)).  While the relative potency for repair displayed species dependant variations, encouraging results were nevertheless obtained in terms of GAG and type I and II collagen deposition in repair tissue and the promotion of cellular proliferation which would promote IVD repair.  These findings support further research into strategies based on notochordal cell technology employing canine or porcine models for their translation into humans.  A detailed molecular study on the canine IVD secretome employing mass spectrometry and Ingenuity Pathway Analysis has demonstrated the importance of the CTGF-TGF-b axis and *Wnt*signalling pathways by notochordal cells in the prevention of DDD and in IVD repair ([50](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_50)).  Ingenuity Pathway Analysis of the canine NP notochordal secretome revealed TGFβ1 and CTGF as major hubs in protein interaction networks. The importance of TGFβ1 and CTGF in IVD pathobiology was also demonstrated in vitro by promotion of the synthesis of IVD ECM proteins, increased cell proliferation and reduced NP cell death. A single injection of rhTGFβ1 and CTGF in a rat-tail disc injury model mitigated DDD.   These two studies clearly show the potential of bioactive compounds produced by notochordal cells in therapeutic measures aimed at IVD repair and regeneration.

 **Identification of stem cells in the IVD**

            The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed minimal criteria for the definition of human MSCs. (i) They should be a plastic-adherent cell type when maintained in standard culture conditions. (ii) They should express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. (iii) They should be multipotent, capable of differentiation into osteoblasts, adipocytes and chondroblasts in-vitro ([51](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_51)). Using these criteria, a number of studies have demonstrated the presence of IVD stem cells.  Stem cells have been isolated from AF ([52](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_52)), NP ([53](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_53),[54](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_54)),  CEP ([55](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_55)),  ligamentum flavum ([56](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_56)), notochord ([57](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_57),[58](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_58)),  and vertebral bone ([59](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_59)) and from the degenerate human IVD ([60-63](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_60)).  These studies indicate that IVDs contain a number of stem cell niches including the notochord.

 **Tie-2 and its roles in tissue development and MSC differentiation.**

            The recent identification of a Tie2 positive cell population (CD202b) in the IVD is evidence of an additional IVD stem cell population requiring further examination ([64](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_64),[65](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_65)). Tie-2 is a tyrosine kinase cell surface receptor for the angiopoietin vascular growth factor family (Ang 1-4) that have important roles in embryonic and postnatal angiogenic events important in the formation of blood vessels which provide nutrition to the high density of progenitor cells which undertake spinal development ([66-70](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_66)).  Ang-1 promotes vessel maturation, endothelial cell adhesion, migration, and survival while Ang-2, acts antagonistically on these processes by promoting cell death and disrupting vascularization ([66](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_66),[69](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_69)). However, paradoxically, when VEGF is present, Ang-2 can also promote neo-vascularization ([70](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_70)).

In the stem cell niche environment MSCs are held tightly together through adherens junction adhesion formations provided by the cell surface glycoproteins E-cadherin and N-cadherin which also provide inhibitory signals which prevent cell proliferation ([71](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_71),[72](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_72)).  Stem cells in the niche environment are subject to mechanical feedback cues which influence Hedgehog-Wnt-cadherin-catenin cell signalling which regulates stem cell proliferation ([71-76](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_71)). When MSCs are activated there is a shift in E-cadherin expression to N-cadherin and a marked alteration in the expression of a number of cell surface carbohydrate epitopes on the stem cells.  Tie-2/Ang-1 signalling in MSCs also regulates β1-[integrin](https://en.wikipedia.org/wiki/Integrin) and N-cadherin mediated interactions maintaining the stem cells in the niche environment in a quiescent slowly recycling state which ensures their long term viability.

Pluripotent stem cells express a number of cell surface molecules which display non-sulphated KS chains (poly N-acetyl lactosamine) or low sulphation KS chains.  These are not detected by antibodies such as 5-D-4 which detect highly sulphated KS chains such as those found in mature connective tissues.  Pluripotent stem cells thus express a number of developmentally regulated carbohydrate motifs with roles in cellular regulation and proliferation and cell-ECM interactions which regulate differentiation, cell aggregation and attainment of pluripotency.  MAbs to embryonic mucin core antigen (EMCA-2, EMCA-3); human embryonal ‘Battle of Trafalgar’ marker antigen TRA-1-60 and TRA-1-81 detect low sulphation or non-sulphated KS epitopes ([77-80](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_77)) which identify pluripotent human embryonic stem cells and induced pluripotent cells ([77](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_77)).  Upon stem cell activation not only is there a shift in the surface carbohydrate  expression profiles but there also is a change in E-cadherin expression to N-cadherin and the activated stem cells also cease to display the EMCA-2,3 and TRA-1-60 and 1-81 motifs.  Furthermore, activated stem cells express cell surface proteoglycans bearing CS sulphation motifs identified using MAbs 4-C-3, 7-D-4, 3-B-3(-) and 6-C-3, these have been used to demonstrate activated stem cells in the surface regions of cartilaginous rudiments in diarthrodial joint and spinal development.  These sulphation motifs are not only markers of tissue morphogenesis during development but have also been detected in remodelling connective tissues in pathological tissue degeneration which may be evidence of adult stem cell activity.

 **Perlecan and its roles in the regulation of MSC activation, proliferation, differentiation and the attainment of stem cell pluripotency.**

Perlecan is a cell regulatory and matrix organisational proteoglycan which has been observed as an ECM proteoglycan delineating stem cell niches in human foetal cartilaginous rudiments in hip and knee diarthrodial joint development and in the perichondrium of the foetal elbow as well as the vertebral cartilaginous rudiments and intervertebral disc anlagen in spinal development ([14](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_14),[31](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_31),[41](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_41),[81](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_81),[82](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_82)).  Ang-2 and VEGF are both ligands for perlecan ([83](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_83)), their sequestration at the margins of stem cell niches in these developmental tissues may therefore provide regulatory properties over stem cell activation.   FGF-2 is also a major perlecan ligand which has roles in the maintenance of long-term stem cell viability ([84-86](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_84)).  Moreover, CS and HS glycosaminoglycans such as those of the side chains of perlecan have been shown to be capable of disrupting stem cell adherens junction formations provided by E-cadherin and this is considered essential for the attainment of stem cell pluripotency ([87](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_87)) thus perlecan has a number of important contributions to make regarding the regulation of stem cell activation and differentiation.  Cell surface expression of Ang-1 and Tie-2 identifies multipotent progenitor cells in the NP. Populations of Tie2 positive (Tie2+) and disialoganglioside 2 positive (GD2+) IVD progenitor cells in the human and murine NP have therapeutic utility for IVD regeneration but their numbers decrease markedly with aging and degeneration of the IVD, indicating that their regenerative capacity gets exhausted ([64](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_64)). Tie2+GD2+ progenitor cells can be induced from Tie2+GD2- precursor cells under simple culture conditions indicating potential therapeutic interventional options. Moreover, Ang-1, is also crucial for the survival of the resident NP cells which is relevant to IVD tissue homeostasis.

 **CD146 identifies migratory progenitor cells with high therapeutic potential**

CD 146 is another cell surface marker displayed by IVD progenitor cells. CD146 identifies cells of a migratory phenotype with higher therapeutic potential and which have the ability to home to IVD defects to promote repair processes ([88](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_88),[89](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_89)).  CD146 isa multifunctional protein ([90](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_90)) and is also known as the melanoma cell adhesion molecule (MCAM) or cell surface glycoprotein MUC18.  CD146 is a 113kDa cell adhesion molecule which binds to laminin 411 and 421 and interacts with a6b1 integrin to regulate cell migration ([91](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_91)).  CD146 is used as a receptor by Wnt5a to regulate cell motility by redistributing and coordinating the expression of adhesion receptors to regulate directional cellular movement ([92](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_92),[93](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_93)).  CD146 is expressed by MSCs isolated from multiple adult and foetal organs ([90](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_90),[94](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_94)), its expression is linked to multipotency and delineates cells with greater differentiation potential ([95](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_95)) including chondrocytes ([96](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_96),[97](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_97)) and IVD cells ([88](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_88),[95](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_95)).  CD146 is used by trophoblasts ([98](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_98)), ovarian cancer and melanoma cells ([99](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_99),[100](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_100)), monocytes and T cell progenitors ([101](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_101)) to facilitate their migratory properties.  Knock-down of CD146 reduces the migration and proliferation of endothelial cells ([102](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_102)).   CD146 is a pericyte marker and is also expressed by chondroprogenitor cells with an enhanced capacity for cartilaginous repair.  This cell population also expresses the Wnt 5a receptor, tyrosine kinase-like orphan receptor 2 (ROR2) as a cell surface marker, this is predictive of a chondroprogenitor/MSC population having an enhanced capacity for chondrogenic differentiation ([103](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_103)).

 Advances in imaging methodology including a 3D non-invasive vital imaging method and a live automated cell-imaging system ([30](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_30),[104](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_104)) has improved the imaging of cell clustering in the IVD. Positron emission Tomography has been applied to the imaging of MSCs injected into canine IVDs in an annular lesion IVD degeneration model, these remained viable for only 3 weeks in-situ (105) demonstrating a relatively short therapeutic window using this cell type.

A murine cell population in the VGP margins adjacent to the outer AF (Fig 1 c, e) insertion is particularly prominent in *Hspg2* exon 3 null mice (Fig 2 a-c) where domain-1 of perlecan is deleted thus the perlecan which populates this niche environment  is HS deficient.  Perlecan is prominently expressed by this cell population (Fig 1 f, g).   The cell population contained in this niche is also CD146 positive which is a marker of a migratory progenitor cell type of enhanced multipotency and greater differentiation and therapeutic potential (manuscript under preparation)([88](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_88),[96-98](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_96)).  Fig 2b and c show that these cells occupy the whole beadth of the VGP and also migrate towards the NP and are associated with a greater deposition of GAG in these tissues and greater maturation rates in the *Hspg2* exon 3 null mouse.  Kim et al 2003 indicated that CEP cells migrated into the murine NP and were responsible for the transformation of the notochord to a fibrocartilaginous tissue during murine IVD maturation ([106](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_106)).  The migratory CEP cells Kim et al 2003 described may actually be from this peripheral VGF-AF cell population (Fig 2). Perlecan-HS has repressive effects on cell proliferation, migration and matrix deposition in-vitro ([107-109](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_107)).  Treatment of cultured rat articular chondrocytes with heparitinase-I resulted in enhanced proliferation and accumulation of GAG ([108](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_108),[109](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_109)).

Furthermore, the use of an activity inhibiting antibody to perlecan in these cultures increased the production of type II collagen indicating that perlecan-HS in some contexts can have a negative regulatory role over chondrocyte differentiation in-vitro ([108](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_108)).  This may explain the observed morphological differences in VGP development in *Hspg2* exon 3 null mice, and Chloe B6 and C57BL/6 mice (Fig 2a, b, d).

Examination of  CS sulphation motif expression in the E19 developmental rat IVD ([43](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_43)) has demonstrated focal expression of the 4-C-3, 7-D-4 and 3-B-3(-) CS sulphation motifs in and around the notochord undergoing a fibrocartilaginous transformation (Fig 1 j-l).  This is consistent with these CS sulphation motifs as markers of the tissue morphogenesis which occurs in development by activated stem/progenitor cells.  The non-ChD canine IVD contains an extensive network of notochordal cells in the NP while the ChD canine does not (Fig 1m).  The ChD IVD has small clusters of chondroid like cells of similar morphology to those seen in the ovine IVD ([9](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_9)).  Chondroid-like cell nests in the NP of adult ovine IVDs have a differing morphology to those of the resident NP or AF cells (Fig 3c, m).  These chondroid-like cellular arrangements occurred as groups of cells undergoing division thus had a proliferative phenotype (Fig 3d) and were therefore dissimilar to the resident NP and AF cells which occurred singly with occasional doublet cells evident ([9](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_9),[110](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_110)).  Chondroid cell nests can be easily missed particularly when evaluating low power toluidine blue stained tissue sections (Fig 3a) however these become apparent in higher power images (Fig 3c, d).  The chondroid-like cells occur in a fine granular basophilic matrix apparently devoid of the fibrillar material which is visualised by toluidine blue staining of the surrounding NP (Fig 3c, e, m).  The NP cells are smaller than the chondroid cells and have a more oval appearance (Fig 3c, m).  By manually adjusting the contrast/brightness of toluidine blue stained images, the chondroid cell clusters can be more readily differentiated in young adult IVDs which have a relatively high GAG content compared to 4 year old IVDs (Fig 3a).  Substantial cell numbers have been observed in these chondroid cell nests in excess of 700 cells counted in a single microscopic field (Fig 3f).  Cell numbers in these chondroid cell nests are maintained with ageing unlike cells in the AF and NP which decline due to apoptosis and autophagy, a few dead cells have been observed in the chondroid cell nests but the vast majority of these cells appear viable and healthy.

Immunolocalisation of the CS-proteoglycans aggrecan and versican  demonstrated that aggrecan was immunolocalised both in the surrounding NP and within the chondroid cell nests except in a pericellular region around small chondroid cell clusters (Fig 3g, h). Versican however was immunolocalised in the NP surrounding the chondroid cell nests but not within the nest (Fig 3i, k).  Hyaluronan was also immunolocalised pericellularly around the chondroid cell-clusters within the cell-nest and had a localisation pattern similar to the aggrecan exclusion zone around the chondroid cell-clusters (Fig 3n).

 Hyaluronan is a common ECM component surrounding stem cell niches and has roles in maintaining stem cell viability and a quiescent slowly recycling phenotype in the niche ([111-116](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_111)) but may also participate in the differentiation of stem cells to a migratory activated phenotype ([41](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_41),[117](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_117)).  Isolated chondroid cells from such cell nests in human IVDs have been cultured (Fig 4 a, b) ([29](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_29)) and shown to synthesise the CS sulphation motifs 3-B-3(-), 4-C-3, and 7-D-4 (Fig 4c-e). These cells also express cytokeratin-8 and 19 (Fig 4g, h) and display a stem cell CD profile by flow cytometry consistent with their identity as MSCs ([29](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_29)).  In an independent study Brisby and colleagues (2013) also isolated cells from degenerate human IVDs aged 34-69 years and demonstrated these expressed OCT3/4, CD105, CD90, STRO-1, NOTCH1 and JAGGED1 confirming their identity as cells of a stem cell lineage ([118](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_118)).

 Cell clusters have also been observed associated with annular lesions in experimental IVD degeneration in an ovine annular lesion model ([119](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_119)) (Fig 5c, e).  Furthermore, chondroid-like cell nests in the NP were observed in close proximity to these cell clusters in the inner AF where normal disc structure had been disrupted by an annular lesion propagating in from the outer AF (Fig 5f).  Cell clusters are not normally seen in this region of the intact healthy AF which typically contains strings of interconnected elongated fibroblast-like cells layed down parallel to the fibrillar collagen in the AF([120](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_120))**.**We postulate that these circular cell clusters in the inner AF arise as cells recruited from chondroid cell nests in the adjacent NP in the destabilized IVD ([119](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_119)).  These chondroid cell nests had a high GAG content and stained intensely with toluidine blue, manual adjustment of the contrast/brightness of the image facilitated the visualisation of such cell nests (Fig 5f).  Cell clusters in the inner AF have not been reported in non-operated control IVDs.  Cell clusters of similar morphology have however been observed in the AF and NP of grade IV degenerate human IVDs (Fig 5g, h).

 **What is the trigger for stem cell activation in OA and IVDD**

               Stem cells are exposed to microenvironmental cues including a myriad of soluble factors, adhesive interactions with ECM components, and mechanical stresses from the ECM and respond by eliciting differentiation responses in-situ ([121-123](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_121)). The molecular basis of how mechanical signals regulate gene transcription and cellular differentiation and become translated into biochemical signals which regulate cell signalling cascades is now being unravelled ([121-123](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_121)). Mechanical forces specifically target the activity and expression of transcription factors and chromatin remodelling enzymes directly involved in gene expression([124-129](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_124)). Thus surface fibrillations in OA, and annular defects in experimental disc degeneration, focal depletion of ECM proteoglycans and attendant changes in the biomechanical microenvironment are important determinants in the activation of stem cells in their niches ([121-123](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_121)).

 *Modulation of stem cell activity by extrinsic forces*

            In addition to biochemical signals which regulate stem cells in-situ, mechanical cues also play regulatory roles in stem cell differentiation. Stem cells are responsive to extrinsic mechanical forces and these are important determinants of stem cell differentiation, fate and lineage commitment. Stem cells contain mechanical sensors which equip them to perceive and respond to mechanical signals. The nucleus is the largest and stiffest cellular organelle and its interaction with cytoskeletal proteins is an essential feature in the mediation of cellular mechanics.  Nuclear mechanics involve sophisticated interactions between lamins, chromatin, nucleoskeletal-proteins and transcription factors through which stem cell differentiation is undertaken in response to mechanotransduction and mechanical cues from the ECM ([130-133](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_130)).

 Transmission of mechanical cues (compression/shear) from the ECM can modulate stem cell activity; stem cell and ECM geometry /topography and substrate rigidity at the nanoscale level all contribute to how stem cell behaviour is affected by their local microenvironment (134, 135). Matrix-mediated signals include mechanical stimuli such as strain, shear stress, forces transmitted through variation in substrate rigidity and matrix topography ([121](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_121),[136-139](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_136)). The mechanome of live stem cells has been mapped by measuring local strain fields at the fluid-cell interface ([140](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_140)). Self-renewal and lineage commitment of stem cells, are also directed by mechanical cues through integrin-mediatedfocal adhesions, which anchor the stem cells in the ECM allowing them to sense their surrounding microenvironment, and allow them to react to these parameters ([141](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_141),[142](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_142)). Substrate-cell and cell-cell interactions both activate specific mechanotransduction pathways that regulate stem cell fate ([143](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_143)) with feed-on mechanical cues to the stem cell influenced by substrate stiffness, surface nanotopography, microgeometry, and extracellular compressive and shear forces ([144](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_144),[145](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_145)). Dynamic pressurization of notochordal cells within the NP induces their transition to a mature phenotype ([146](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_146)), compression (hydrostatic or osmotic pressure) or a combination of shear and dynamic compression induces chondrogenesis of MSCs ([128](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_128),[147](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_147)), while tensile shear forces are important determinants for the induction of osteogenic differentiation ([148-150](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_148)). With this realization that the ECM microenvironment can influence stem cell fate ([151](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_151)) methods have been developed to engineer the physical environments of MSCs in culture systems in order to direct their differentiation for applications in regenerative medicine ([134](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_134),[152-159](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_152)) and high throughput screening procedures have been developed to evaluate the stem cell niche and how this responds in such procedures ([160](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_160)).

 Like most mammalian cells, MSCs contain a single primary cilium ([161-165](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_161)) however its function is relatively poorly understood but by analogy with other cell types its probable function in stem cells can be deduced ([166](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_166)). In other cell types the primary cilium is a cybernetic probe ([167](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_167)) providing information to the cell regarding its orientation in the ECM  which co-ordinates transcription factor responses arising from mechanical stimuli ([168](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_168),[169](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_169)) integrating these into biochemical responses through receptor tyrosine kinase mediated cell signalling ([168](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_168),[170](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_170)). The primary cilium is a sensory microtubule-encased membranous protrusion from the cell surface which regulates the cell cycle, cell proliferation, embryonic development ([171](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_171)) and directional migration of fibroblasts (172, 173). In stem cells the microtubular structure of the primary cilium and its connection with the centriole is consistent with a probable function in the regulation of cell division ([171](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_171)), and cell signalling responses to mechanical stimuli ([161](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_161),[168](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_168),[170](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_170)).

 *Can stem cell populations be stimulated to improve their repair potential*

As already discussed tissue engineering applications have been developed where the microenvironment has been manipulated to induce appropriate proliferative and differentiation responses in stem cells for therapeutic purposes.  Bioscaffolds have also been developed using CS, HA and fibrillar collagens to apply their cell directive capability to promote the differentiation of stem cells to chondrogenic phenotypes conducive for repair of tensional and weight bearing connective tissues ([174-179](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_174)).  One method employing a copolymer of type II collagen, HA and chondroitin-6-sulphate has specifically focused on repair of the NP ([180](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_180)) however much of the methodology developed to induce chondrogenesis of stem cells for cartilage repair may also be adaptable to repair of the IVD.

 A number of biofactors have been used to stimulate various stem cell preparations in-vitro and in-vivo including, steroids ([181](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_181)), bioactive sphingolipids ([182](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_182)), glycolipid surfactants ([183](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_183)) and small molecules which induce skeletal muscle cell differentiation and embryonic stem cell activation ([184](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_184)).  Plerixafor is an immunostimulant used to mobilize hematopoietic stem cells ([185](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_185)).  Salvianolic acid, an anti-oxidant from the deciduous perennial traditional chinese medicine from *Salvia miltiorrhiza* (Chinese red sage, Danshen) has been used to stimulate adult neural stem/progenitor cells ([186](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_186)).  Small RNAs have been used to stimulate a number of stem cell populations ([187-193](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_187)). Small molecules have been used to stimulate stem cells including the progestogen steroid megestrol acetate which has been used to increase the proliferation, migration, and adipogenic differentiation of adipose-derived stem cells([194](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_194)).  Simvastatin enhances bone marrow stromal cell differentiation into endothelial cells ([195](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_195)).  Necdin controls cellular proliferation and exerts transcriptional control over white adipocyte progenitor cells ([196](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_196)).  Bioactive HS preparations prepared by FGF-2 affinity chromatography have been used to induce proliferation and differentiation of human and rat MSCs to improve their therapeutic potential ([197-199](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_197)).  FGF-2, TGF-b1, PDGF-BB, CTGF have been explored as means of improving stem cell proliferation and differentiation ([200-209](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_200)).  As suggested by Brisby et al (2013) further investigations may uncover a specific stimulatory compound suited to the in-vivo manipulation of IVD stem cell populations to improve their therapeutic properties ([118](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_118)).

 *Promotion of the migratory phenotype of IVD resident stem cells to improve their ability to repair IVD defects*

             The migratory properties of activated IVD stem cells to defects in the IVD to effect repair processes is an important attribute for an effective therapeutic stem cell ([210](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_210),[211](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_211)).  As already discussed, stem cells displaying the CD 146 cell surface marker have a greater cell proliferative capacity and cell migratory phenotype and this marker is predictive of a MSC population of high therapeutic potential ([90](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_90),[96](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_96),[97](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_97),[212](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_212),[213](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_213)).  A number of chemokines/receptors (CXCL3, 5, 6, 10-12, 14, 16, CXCR1-6; XCL1, 2; CX3CL1, CX3CR1; CCL1-5, 7, 8, 18, 19, 21, 24-26,CCR1-4, CCR6-9), cytokines/growth factors (PDGF-BB, PDGF-AB, TGF-β1, TNF-α, VEGF) promote the migration/homing of MSCs ([211](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_211)) and this improves their therapeutic efficacy.  A number of chemotactic factors have also been identified which positively influence stem cell migration.  These include PGE2, which in inflammatory conditions activates the ERK1/2 and FAK cell signalling pathways to promote stem cell migration ([214](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_3ZV44rQQ7oPjJ21Spq22hMsSnDrLixZLXHjBsjL3CmM1CQn8dP4Xis8dhLZcw765fZxct6NKH75TysJGP99fxXTQL1RByiZAN3RjzJEbhymbGgXxqPVR7p6DHrUydZCABtujLj1N1bwXeAcKn4qe5M1pKTNu1ZjDNJATCpXHge1xCy3AFCvK1bR2uGrBk7HeyvnR9E5n9EJiBHvntDnZHf8LbAXwudfktyNWYNPTaPV2i9vWv#_ENREF_214)). MiR-211/STAT 5A signalling also improves the therapeutic utility of stem cells ([215](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_215)). MiR-221 and MiR-26b regulate the chemotactic response of stem cells through activation of the Akt and FAK  signalling pathways ([216](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_216)).  MiR-27b regulates SDF-1a - CXCR4 interactions which are responsible for MSC recruitment ([217](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_217)).  Substance-P ([218](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_218)) promotes MSC migration while activated uPAR-b1 integrin interactions facilitate PDGF dependent stem cell migration ([219](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_219)).  Mechano growth factor E peptide also regulates migration and proliferation of MSCs ([220](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_220)) and C1q has chemoattractant activity  priming chemotactic responses to SDF-1a thereby promoting MSC migration ([221](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_221)).  It will be important to determine which of these are the most suitable agents to apply to the resident IVD stem cells to promote disc repair processes and these offer exciting possibilities.

 **How widespread are cell clusters in tensional and weight bearing connective tissues other than the IVD**

               Cell clusters are not isolated to the degenerate IVD ([222-224](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_222)) but have also been observed in knee articular cartilage ([225-227](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_225)), meniscus ([228-230](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_228)) and fibrocartilaginous regions of tendons ([231](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_231)).  These cell clusters have similar morphologies to the cell clusters observed in the inner AF in experimental disc degeneration in the present study and synthesise progenitor/MSC markers such as *Sox9* and *Stro-1* ([232](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_232),[233](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_233)).  In articular cartilage and meniscus it has been suggested that these cell clusters represent a reparative response by adult progenitor cell populations located adjacent to surface fibrillations ([234](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_234)).  Such OA cell clusters also express the HS-proteoglycan perlecan ([235](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_235)) which is induced by *Sox9* gene expression ([236](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_236)).  Perlecan has roles in the chondrogenic differentiation of mesenchymal progenitor cells ([31](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_31),[236](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_236)) in knee joint cartilage and IVD ([34](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_34),[81](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_81)).  Perlecan is a pericellular matrix component of disc cells, has chondrogenic properties  and also delineates progenitor stem cell niches ([31](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_31),[34](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_34),[43](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_41yz8Wnotfhx48U25xtaKBhVRx17WSKqToPeJtxh6h2JQ5uVMXEqhdUhqee8EibgQsMu3tTKAmStqTTFGqd5q1M3GL9ofzzJB7MnqEhu7aG6msQEA3tzAAYmDNTgjD9WwnjQ8QSk3357G3VNUM7ZnqaiVtcDNYiCNb9TT4JtLAxLpV8h6YobTiznjZQkFteeJKEK4UCiTw96z272qJmMXbmmNRm8EAkzHayfFrjZVPWia2Eyg#_ENREF_43)). The widespread occurrence of these cell clusters in tensional and weight bearing connective tissues is consistent with the reported occurrence of stem cell niches in various locations throughout the human body.  Their presence however only becomes apparent when these tissues are damaged and the resident stem cell populations become activated and form clusters of cells for repair purposes.

**Conclusions**

Small reactive cell clusters in the AF like those described in this review are indicative of stem cell activity. These only occur in degenerate IVDs thus they should also be considered as markers of IVD degeneration but should be distinguished from the large chondroid cell nests observed in the NP. The occurrence of these small cell clusters associated with annular defects indicates an attempted intrinsic repair response.  The large chondroid cell nests also described in which occur in normal and degenerate IVDs may represent an adult stem cell population which may serve as a cell reserve which can supply cellular replacements following apoptosis or autophagy during the normal turnover of the disc but may also be mobilized in pathological remodelling events during disc degeneration. Further experimentation is required to demonstrate if cells in these chondroid nests can be recruited in response to a disc injury and if so whether they can home to tissue defect sites to undertake repair.  A greater understanding of the biology of this chondroid cell population may identify them as a therapeutic resource which could potentially be harnessed for intrinsic repair of the IVD and is certainly worthy of further investigation.  Not only would this obviate the morbidity of stem cell harvest and delivery of an exogenous cell type into the IVD, but the repair process would utilise a cell type adapted to the harsh conditions of the IVD which may prove advantageous. Exogenous stem cells administered to the IVD have been shown to only remain viable for 3 weeks thus the therapeutic opportunities using such interventions is relatively short.

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**Figure 1 .  Variable IVD cell morphologies in foetal human, mouse, rat, canine, fallow deer and ovine IVDs showing evidence of notochordal cells. (a)**Toluidine blue stained foetal human spinal segment (12 week gestational age) showing a notochordal remnant (N) containing a condensed group of notochordal cells in the developing nucleus pulposus of the IVD interspace and adjacent vertebral cartilaginous rudiments which will become the vertebral bodies (VB).  A central population of vertebral hypertrophic chondrocytic cells is evident (H), these will develop into the ossification centres within the VB and undergo mineralisation over 14-20 weeks gestation.  A portion of the central notochordal tract is evident running longitudinally through the superior vertebral rudiment. The margins of the developing IVD are marked with dotted lines as is the anterior longitudinal ligament (ALL) which is a more condensed tissue on the outer vertebral rudiment margins.  Mid-sagittal vertical sections of newborn and 12 week old lumbar IVDs from Chloe B6 (b,c) and C57BL/6 mice (d, e).  Immunolocalisation of perlecan produced by a discrete hypertrophic progenitor cell population identified adjacent the peripheral VGP and its juncture with the outer AF are also shown, diaminobenzidene brown chromogen (f, g). The vertebral growth plates are prominent features of murine IVDs.  The central NP also contains a flattened notochordal remnant with densely interconnected cells but little GAG staining. The surrounding NP has moderate GAG staining.   Small columns of hypertrophic chondrocytes are evident in the VGPs surrounded in toluidine blue stained GAG, these columns of chondrocytes are more regularly spaced in the C57 BL/6 IVDs than Chloe B6 which have a more condensed appearance and slightly greater GAG deposition. The notochordal cells are shown in more detail in h and i. Notochordal cell populations of E19 rat (j, k, l) and non-chondrodytrophoid (m) and chondrodystrophoid canine NP (n). Immunolocalisation of the CS sulphation motifs 3-B-3(-) (j) , 4-C-3 (k) and 7-D-4 (l) expressed by stem/progenitor cell populations. Small groups of cells in NP of newborn fallow deer (o) and ovine (p) have notochordal features.  Figure modified from ([43](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_2PSKmLmM6R38VCvr8sv9vp2bRQ1qzMbfY38WHo2jS8jB3X8xPuJfMUPsjhSDWN7mijT673Zk1JAphZTaPsKNtvC49ik25gy7qr9PJeCUFC73vDYTPSpBoV1S5HFXHsnAYwHKNYba4899t1gJX3T5yderwfGJHiY3hqo7o8xUYPKE6mnnnggR5FMtYpAGmsJJP6wbVxzQQBJHSRESDpNbVWUTDQJSikriNMFc63Rz3j7KwaB6Y#_ENREF_43)) and ([9](https://mc.manuscriptcentral.com/LongRequest/scd?DOWNLOAD=TRUE&PARAMS=xik_2PSKmLmM6R38VCvr8sv9vp2bRQ1qzMbfY38WHo2jS8jB3X8xPuJfMUPsjhSDWN7mijT673Zk1JAphZTaPsKNtvC49ik25gy7qr9PJeCUFC73vDYTPSpBoV1S5HFXHsnAYwHKNYba4899t1gJX3T5yderwfGJHiY3hqo7o8xUYPKE6mnnnggR5FMtYpAGmsJJP6wbVxzQQBJHSRESDpNbVWUTDQJSikriNMFc63Rz3j7KwaB6Y#_ENREF_9)).

**Figure 2.** Demonstration of a peripheral putative progenitor cell population in the outer Annulus Fibrosus (AF) margins with the vertebral growth plate (VGP) in murine IVDs.  Henricksson et al have described a progenitor cell population at this location in a number of animal species.  At 6 weeks of age this progenitor cell population is located at the VGP – AF margins (a) however by 12 weeks this cell population has undergone hypertrophy and now occupies the full width of the VGP (b). Hypertrophic cells are also evident in the inner AF and NP (c), red arrows indicate possible routes of progenitor cell migration to these regions of the IVD.  Plates (a-c) are from *Hspg2* exon 3 null mutant mice which have the perlecan domain-1 deleted, thus are devoid of perlecan HS substitution. The peripheral progenitor cell population in this mouse strain display higher proliferation rates, differentiation to a hypertrophic maturational status and higher levels of GAG deposition in IVD tissues.  A VGP of a C57 BL/6 wild type mouse (12 week old) is shown for comparison (d).  In this case the growth plate chondrocytes are arranged into regular columns of cells and the progenitor cells remain as a discrete population at the periphery of the VGP with its juncture of the outer AF. CEP, cartilaginous endplate; NP, nucleus pulposus.**Abbreviations:** VGP, vertebral growth plate; CEP, cartilaginous endplate; IAF, inner annulus fibrosus; OAF, outer annulus fibrosus; NP, nucleus pulposus.

**Figure 3.**  Chondroid cell colonies in the NP of ovine IVDs. Toluidine blue stained ovine IVDs identifying discrete populations of chondroid like cell populations in the NP of a 4 year old (a-d), 2 (e) and 6 year old (f) ovine IVD.  These cell populations are not particularly obvious in macroscopic view (dotted area) (a) however can be identified in higher power views (b-d).  A chondroid-like cell population is indicated by dotted lines in (b).  These cells have a rounded morphology dissimilar from NP fibrochondrocytes which have a more elongated morphology clearly seen in (c) and are contained within cigar shaped basophilic arrangements in a matrix devoid of the fine fibrillar material evident by toluidine blue staining elsewhere in the NP (c).  Some of the chondroid cells show evidence of cell division in small cell clusters within these cigar shaped cell colonies (d).  Immunolocalisation of aggrecan (g, h), versican (i, j, k) and hyaluronan (n) in chondroid cell nests and adjacent NP in a 2 year old ovine IVD.  Negative control sections for the versican localisation (j) and hylaluronan localisation (l) are also shown.  Plate (m) is a toluidine blue stained tissue section depicting a chondroid cell colony on the right hand side and cells in the surrounding NP.  The dotted lines depict the margins of the chondroid cell nests.  Chondroid cell colonies are surrounded by aggrecan but do not contain versican which is a prominent component of the surrounding NP.  The chondroid cell colonies are surrounded by a pericellular cell coat of hyaluronan (n).  Figure modified from (9).  **Abbreviations:** VGP, vertebral growth plate; CEP, cartilaginous endplate; AF, annulus fibrosus; NP, nucleus pulposus; CSN, chondroid cell nest.

**Figure 4.**  Immunolocalisation of matrix components synthesized by single chondroid cells (a) or groups of chondroid cells (b) isolated from human IVDs grown in monolayer culture. These cells synthesise a number of pericellular and ECM proteoglycans containing the CS sulphation motifs  4-C-3 (c), 6-C-3 (d), 3-B-3[-] (e) and the stem cell markers cytokeratin-8 (CK8) (g) and cytokeratin-19 (CK19) (h).  A number of negative control sections are also shown (f, I, j).  These CS sulphation motifs have been proposed as markers of activated stem cells.  Scale bars 100 mm.  Figure modified from  (29).

**Figure 5.** Identification of cell clusters in IVD tissue sections from an ovine experimental model of disc degeneration (a-f) and in degenerate grade IV human IVDs (g, h).  Macroscopic image of a vertical section through an IVD and superior and inferior vertebral bodies showing the extent of the annular lesion (red arrow) which is used to induce disc degeneration in this model.  The two areas indicated by dotted lines are also shown at higher magnification, area 1 is shown as an H & E  (c ) and toluidine blue stained section (e ) area 2  is also shown as a toluidine blue stained section (f).  The contrast of this image has been manually adjusted to depict details of the cell clusters  which are otherwise obscured by the toluidine blue staining.  Plate (d)  is a diagrammatic depiction of the IVD and the annular lesion which propagates through the IVD to the contralateral AF in this model.   Small and large cell clusters are also evident in degenerate grade IV human IVDs as shown in toluidine blue stained sections for the AF and NP in a 36 year old female L5S1 IVD (g) and 58 year old L4L5 herniated IVD (h). The penetration of blood vessels along the lesion tract is shown in (b).  Figure modified from (97).  **Abbreviations:** VGP, vertebral growth plate; CEP, cartilaginous endplate; AF, annulus fibrosus; NP, nucleus pulposus; CSN, chondroid cell nest.