Characterisation of Vertebral Mesenchymal Stem Cells from Adolescent Idiopathic Scoliosis Patients and their Osteogenic Response on Collagen–Nanohydroxyapatite Scaffolds

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Introduction

- **Adolescent idiopathic scoliosis (AIS)**
  - Lateral curvature of the spine
  - Affects 3% of adolescents[1]
- **Treatment**
  - Extensive Surgery to straighten and fuse spine using rods and screws (Figure 1)
  - A large amount of bone marrow is aspirated and discarded from the vertebrae during surgery
- **Iliac crest (IC) Autograft**
  - Used to pack the spine to promote fusion (Figure 2)
  - Creates an additional painful surgical site
- **Vertebral bone marrow** has been hypothesised as a mesenchymal stem cell (MSC) reservoir, and thus a potential alternative source of allograft[3]
- **Collagen-nanohydroxyapatite (coll-nHA) scaffolds** have been shown to promote and enhance bone formation[4]

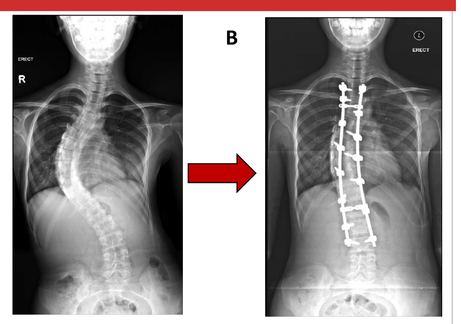


Figure 1. X-ray of scoliotic spine (A) pre and (B) post-surgery.

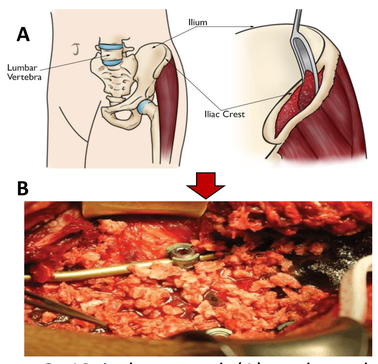


Figure 2. IC is harvested (A) and used to pack the spine (B) to promote spinal fusion.

Overall Objective

Determine whether there is an osteogenic vertebral cell population in AIS patients and if it can replace iliac crest harvesting to enhance spinal fusion

Specific Aims

- Characterise and compare the mesenchymal stem cell (MSC) population in vertebral bone marrow and IC of AIS patients
- Assess the impact of harvest site on osteogenic potential of isolated MSCs
- Investigate the full osteogenic potential of MSC through coll-nHA scaffolds

Methods

- Vertebral bone marrow from 18 AIS patients was aspirated and collected through the pedicle screw hole (Figure 3). IC bone marrow was also collected. Surgeries took place at CHI Crumlin
- Mononuclear cells were isolated through gradient centrifugation and expanded *in vitro*
- Expression of MSC markers CD73, CD90 and CD105 was assessed through flow cytometry
- Osteogenic potential was evaluated through the expression of osteogenic genes and calcium deposition
- Porous freeze-dried coll-nHA scaffolds were seeded with isolated MSCs and osteogenesis was assessed up to 28 days[4]

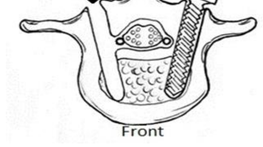


Figure 3. Vertebral pedicle perforation for screw insertion. The resulting hole is used for bone marrow aspiration.

Results

Vertebral and IC MSC isolation, expansion and surface markers expression

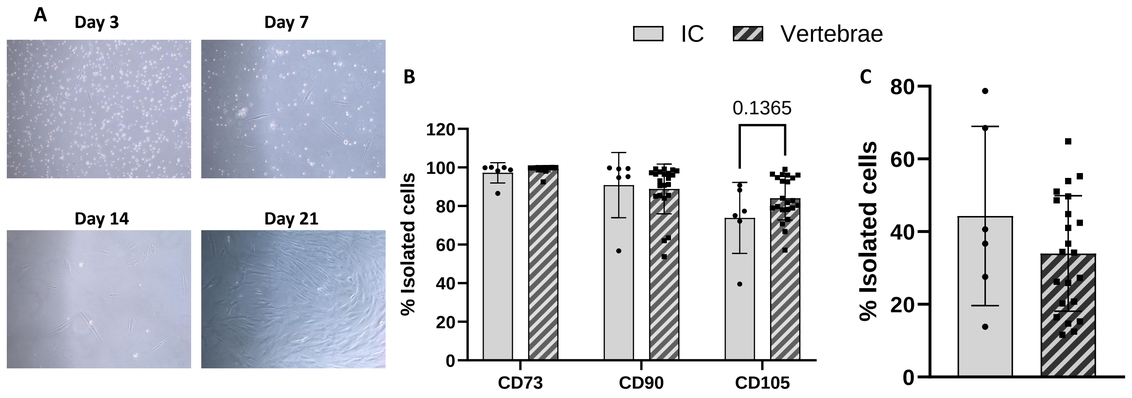


Figure 4. (A) Isolated cells start adhering by day 14, displaying a fibroblast-like morphology. (B) Vertebral MSC CD105 expression was increased compared to IC MSC. (C) IC MSC expressed increased levels of negative markers compared to vertebral derived MSC.

Harvesting site impact on osteogenic genes overexpression and calcium deposition

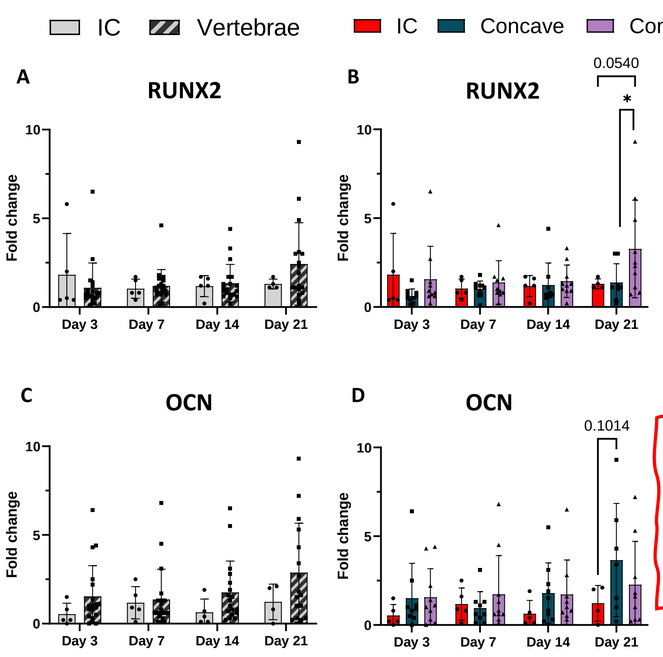


Figure 5. Vertebral MSC displayed enhanced osteogenic potential compared to IC MSC. (A,B) Convex MSC displayed RUNX2 overexpression by Day 21. (C,D) Vertebral – and concave – MSC displayed OCN overexpression at Day 14.

Lack of significant differences in osteogenic gene expression between vertebral and IC MSC

Results

Impact of harvesting site on osteogenic genes overexpression and calcium deposition

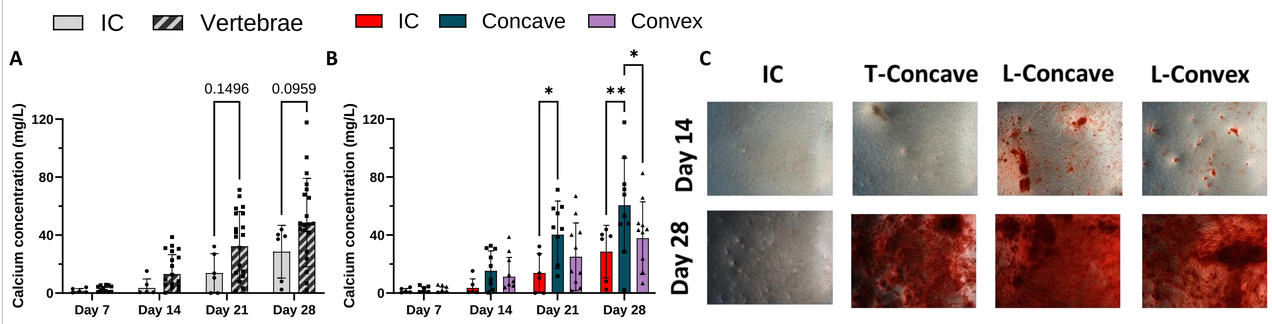


Figure 6. (A,B) Vertebral, both concave and convex, MSC displayed increase calcium deposition compared to IC MSC. (C) Calcium staining of a representative patient confirmed differences observed in quantification assays between vertebral and IC MSC.

Increased matrix mineralisation observed in vertebral MSC compared to IC MSC
In some patients, mineralisation was observed in vertebral MSCs and not IC MSCs

Investigating MSC osteogenic potential in coll-nHA scaffolds

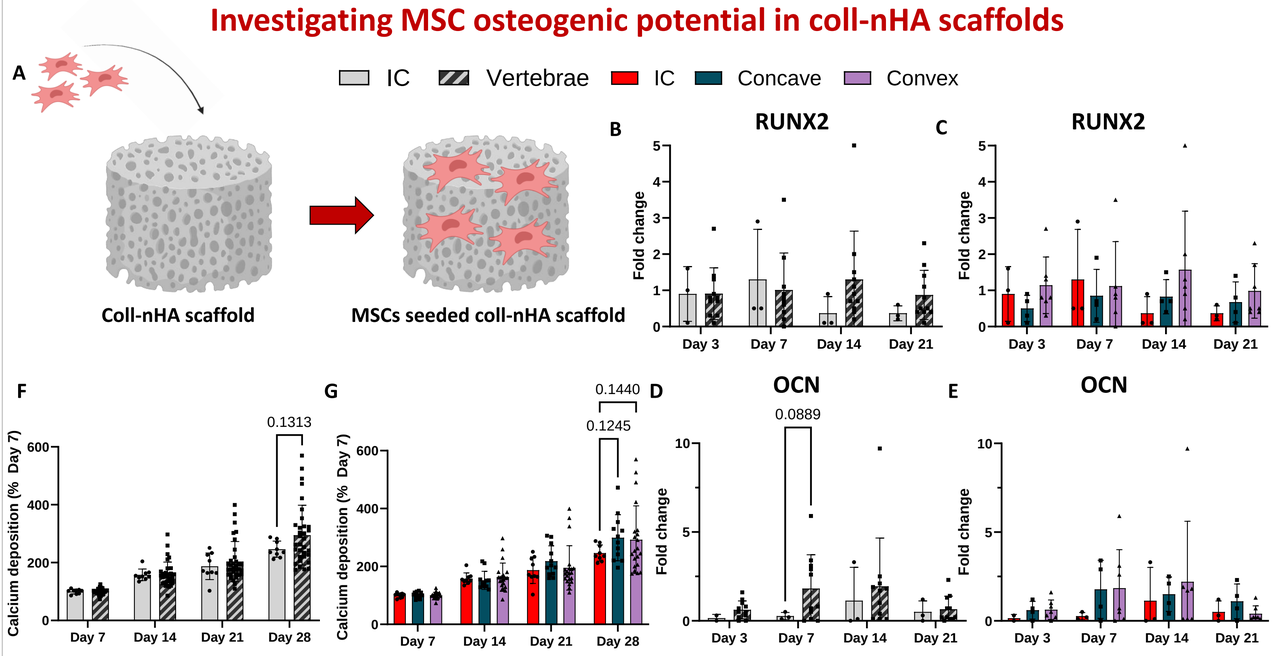


Figure 7. (A) Coll-nHA scaffolds enhance MSC osteogenic potential. (B,C) Convex MSC displayed RUNX2 overexpression by Day 14. (D, E) Vertebral – and concave – MSC displayed OCN overexpression at Day 21. (F,G) Vertebral, both concave and convex, MSC displayed increase matrix mineralisation compared to IC MSC.

Osteogenic potential of all MSC is enhanced by coll-nHA scaffolds

Discussion

- High variability between patients (n=18) lead to very high standard variation throughout study
- Vertebral MSCs present more stem-like surface markers expression than IC MSCs
- In 2D culture, vertebral and IC MSC have similar levels of osteogenic genes overexpression
- In 2D culture, matrix mineralisation by vertebral MSCs tends to be higher, mainly at late time points
- In coll-nHA scaffolds, gene expression and calcium deposition follow the trend observed in 2D culture
- Scaffolds support osteogenic differentiation of all MSCs – including IC

Conclusions

- IC MSC are not superior to vertebral MSC regarding surface markers expression, osteogenic gene overexpression or calcium deposition
- Lack of significant differences between IC and vertebral MSC point to an underlying pathology that affects the cellular population of both harvest sites
- Both IC and vertebral MSC osteogenesis was enhanced by coll-nHA scaffolds, displaying the osteogenic potential of these materials

Limited osteogenic potential of IC point to an underlying systemic pathology

Coll-nHA scaffolds can potentially be used with vertebral MSC without need for IC harvesting in AIS patients, removing the additional surgical site

References & Acknowledgements

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