Effect of Different Pyrrole-Imidazole Polyamides as Gene Switches on the Human Mesenchymal Stem Cell Differentiation into Chondrocytes

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Overseas Research Fellowship (ORF)
2024 for Science Students
Poster No: D4
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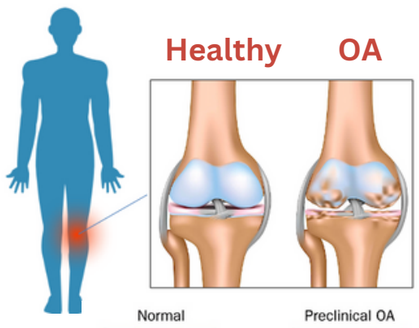
ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease necessitating innovative approaches for cartilage regeneration. This study investigates the use of pyrrole-imidazole polyamides (PIPs) as gene switches to direct human mesenchymal stem cell (hMSC) differentiation into chondrocytes. PIPs, capable of binding to DNA's minor grooves, modulate gene expression without altering the DNA sequence. We evaluated various PIPs for their efficacy in promoting chondrogenesis by assessing gene markers RUNX2, SOX9, and Osterix. Results demonstrated that specific PIPs effectively downregulated RUNX2 and Osterix while upregulating SOX9, indicating successful chondrocyte differentiation. These findings suggest that PIPs hold significant promise for cartilage repair therapies, offering a precise, reversible method to influence stem cell fate. This approach could lead to novel treatments for OA, enhancing cartilage regeneration in a clinically viable manner.

BACKGROUND & INTRODUCTION

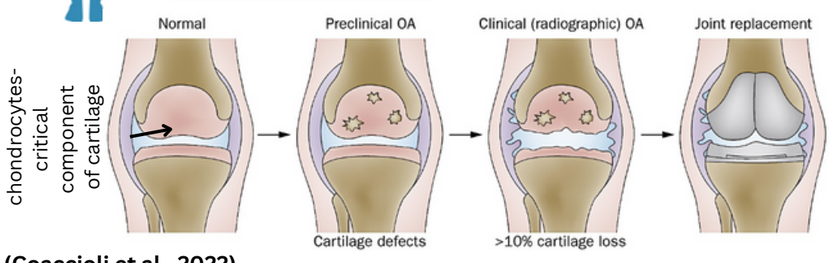
Osteoarthritis (OA)

OA - major disability affecting healthspan worldwide



No cure yet

Current modalities and interventions for symptomatic relief:
- Supportive devices
- Anti-inflammatory drugs
- Physical exercises

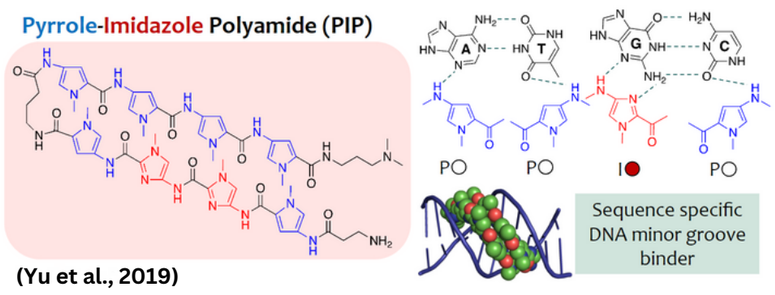


(Coaccioli et al., 2022)

Problem: Cartilage- difficult to repair
Solution: Regeneration from a suitable cell line
Issue: Precise differentiation??
Potential Approach: Transcriptional activators

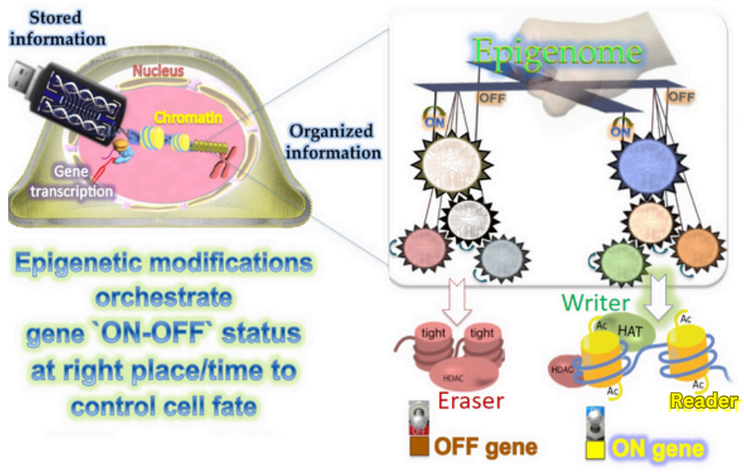
Demand: Clinical-friendly methodology to regenerate chondrocytes

Pyrrole-Imidazole Polyamides: An Epigenetic Control



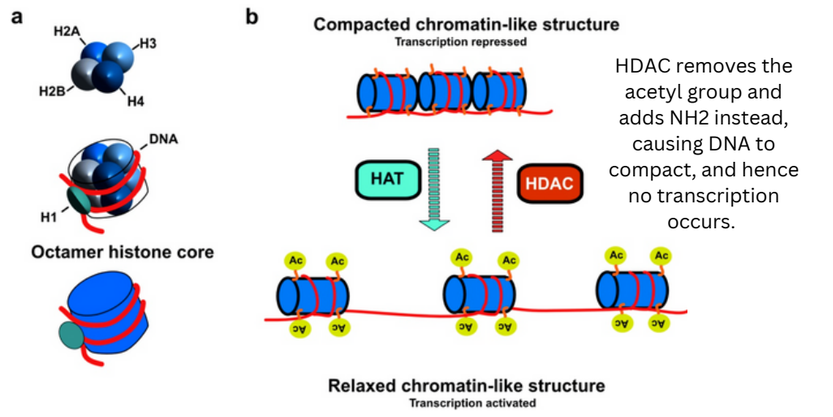
(Yu et al., 2019)

PIPs can recognize DNA base pairs with sequence specificity. An antiparallel imidazole and pyrrole (I-P) pairing recognizes a G:C base pair. A pyrrole-pyrrole pairing (P-P) recognizes A:T or T:A.



PIP's two main domains:
1. **Sequence-specific DNA binding domain**
2. **Epigenetic modulators with high selectivity:**
   - **An epigenetic eraser:** a histone deacetylase [HDAC] inhibitor
   - **An epigenetic writer:** a histone acetyltransferase [HAT] activator

The unique properties of PIP molecules that make them good candidates for therapeutic usage:
- **Reversible results**
- **Sequence specificity**
- **Smooth permeability to the cell/nuclear membrane**
- **Binding affinity is similar to natural Transcription Factors**



HDAC removes the acetyl group and adds NH2 instead, causing DNA to compact, and hence no transcription occurs.

(Elmallah & Micheau, 2019)

METHODS & RESULTS

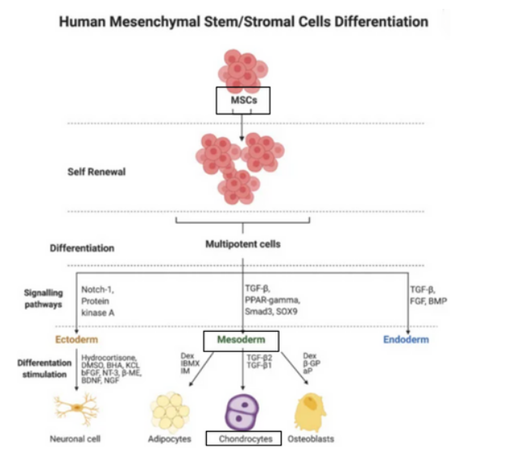
hMSCs: Human Mesenchymal (Stromal) Stem Cells

Cell Line
- Human Mesenchymal Stem Cells
- Initial cell count per well: 0.5 x 10*6 cells/ml

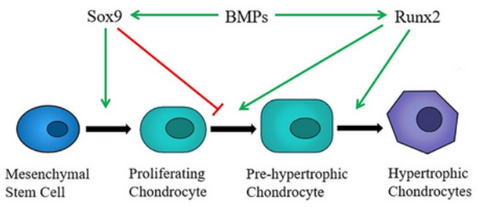
hMSCs:
- non-hematopoietic stem cells present in the bone marrow stroma
- self-renewal & multi-lineage differentiation into mesoderm-type cells (e.g., chondrocytes)

Gene Markers

1. **RUNX2**
   a. Downregulation: From mesenchymal stem cells to proliferating chondrocytes
   b. Upregulation: From proliferating chondrocytes to hypertrophic chondrocytes
2. **SOX9**
   a. Upregulation: From mesenchymal stem cells to proliferating chondrocytes
3. **Osterix**
   a. Downregulation: Marker for osteoblast differentiation



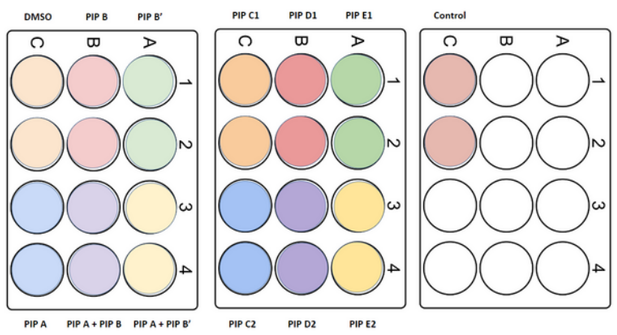
(Kulus et al., 2021)



(Chen et al., 2021)

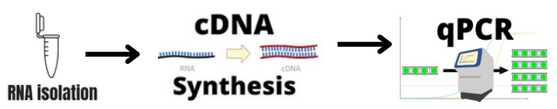
Experimental Conditions

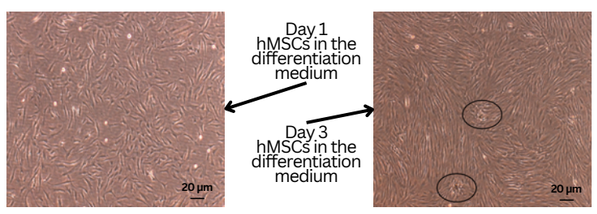
1. **Seed cells 1-3 days before treatment.**
2. **Add PIP/DMSO solution into culture.**



Serum-Free Chondrogenic Differentiation Medium:
- MEM + Dexamethasone + Ascorbic acid
- Duration: 4 days total
- Medium refreshed with fresh PIPs every 48 hours

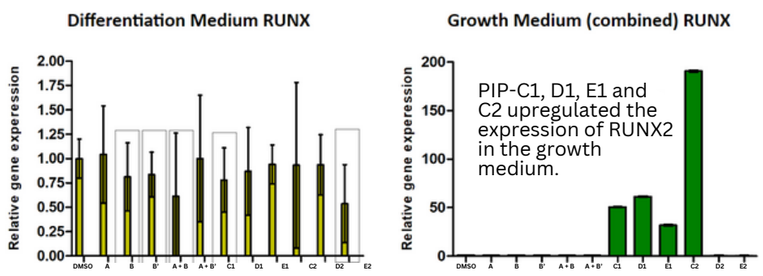
Growth Medium (two repeats):
- DMEM + L-Glutamine
- Duration: 2 days total
- No medium change



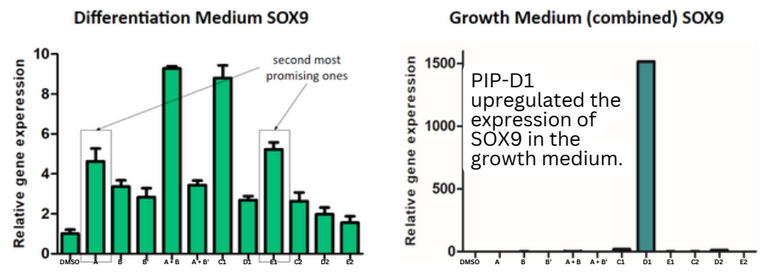


Future work:
COL1 and COL2 gene markers
Focus: Growth medium ✓
Promising PIPs: D1 and C2
Longer treatment (complete differentiation: 21 days)

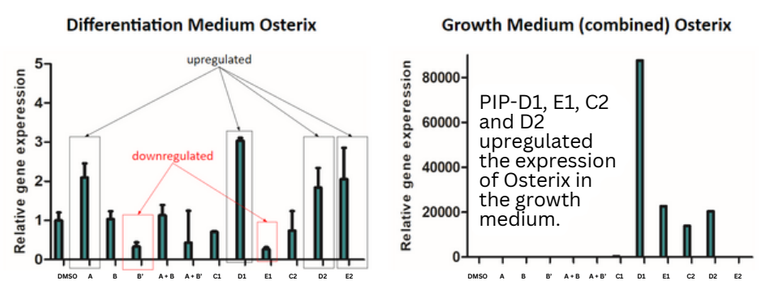
qPCR Results



PIP-B, B', the co-treatment of A and B, C1 and E2 downregulated the expression of RUNX2 in the differentiation medium.



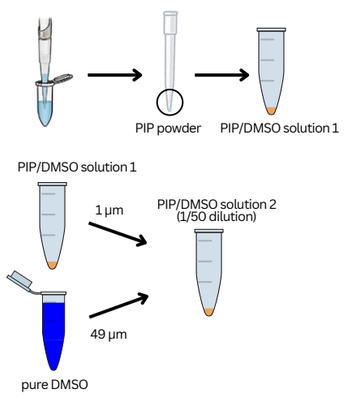
All PIPs upregulated the expression of SOX9 in the differentiation medium. Among all PIPS; the co-treatment of PIP-A and B and C1 are the most promising ones.



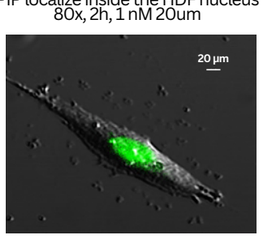
PIP-A, D1, D2 and E2 upregulated; while B' and E1 downregulated the expression of Osterix in the differentiation medium.

Conclusion: The possibility of directing the hMSC differentiation into the chondrocyte pathway by using some promising PIPs in growth medium to generate cartilage in a clinical-friendly matter

PIP Treatment



PIP localize inside the HDF nucleus
80x, 2h, 1 nM 20um



$c = \frac{Abs}{\varepsilon a d}$ (Abs. should be 0.2-1.0 in 300-310 nm)
- c (M): concentration
- ε, extinction coefficient = 9900
- a = total number of pyrrole and imidazole rings
- d = 0.1 cm (NanoDrop ND-1000)

Final conc.: 5 µM with 0.1% DMSO

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Acknowledgement: I would like to express my deepest gratitude to my supervisor, Professor Ganesh Pandian Namasivayam, for his patient guidance and valuable advice. My heartfelt appreciation also goes to Ms. Mahima Kumar and Ms. Arooma Jannat and all the members of the laboratory for their assistance and support. Finally, I would like to thank the Faculty of Science at HKU for providing financial support.