



Hematology Journal Club

**Progenitor Cells Activated by Platelet Lysate in Human
Articular Cartilage as a Tool for Future Cartilage
Engineering and Reparative Strategies**

**Advised by:
Dr.Farsinejad**

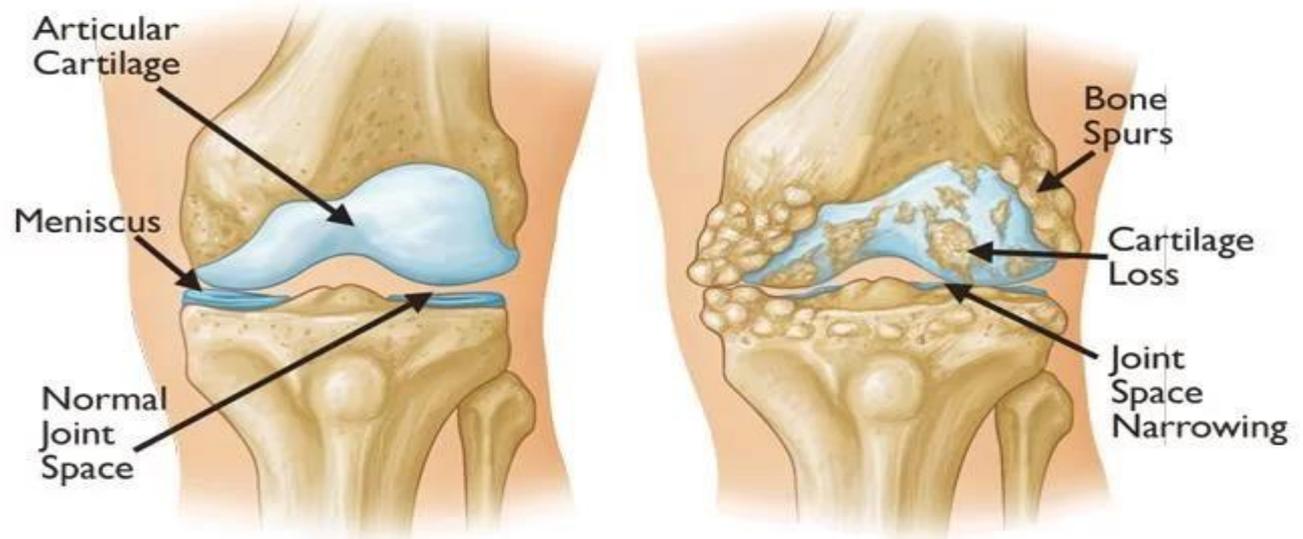
**Presented by:
S.Salek**

CELLS

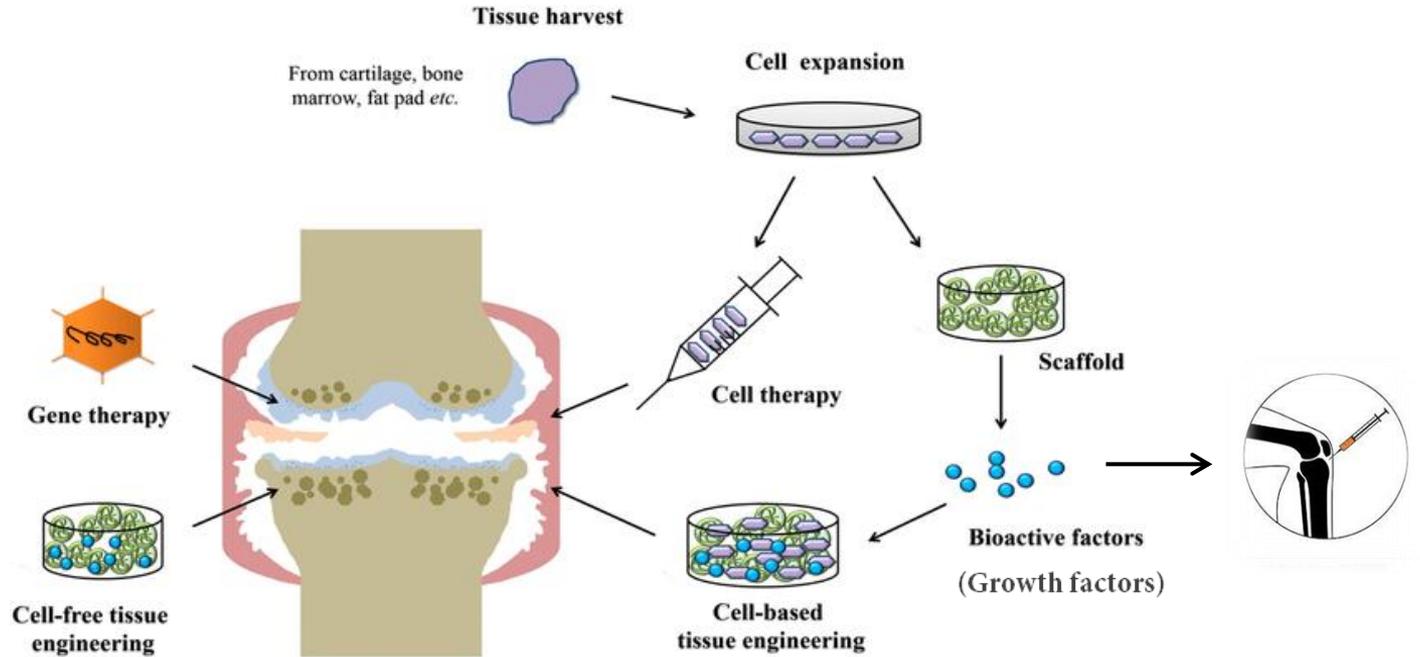
The screenshot displays the MDPI Cells journal website. At the top left is the MDPI logo. The navigation menu includes links for Journals, Topics, Information, Author Services, Initiatives, and About. On the right side of the navigation bar are buttons for Sign In / Sign Up and Submit. Below the navigation bar is a search section with the text "Search for Articles:" and three input fields: "Title / Keyword", "Author / Affiliation", and "Cells". To the right of these fields are dropdown menus for "All Article Types" and "Search", along with an "Advanced" link. A yellow circular badge indicates an "IMPACT FACTOR 7.666", and a blue circular badge indicates "Indexed in: PubMed". Below the search section is a breadcrumb trail: "Journals / Cells / Aims & Scope". On the left side, there is a "cells" logo and two buttons: "Submit to Cells" and "Review for Cells". Below this is a "Journal Menu" with a list of links: "Cells Home" and "Aims & Scope". The main content area features the heading "About Cells" and a section titled "Aims" with the following text: "Cells (ISSN 2073-4409) is an international, peer-reviewed open access journal which provides an advanced forum for studies related to cell biology, molecular biology and biophysics. It publishes reviews, research articles, communications and technical notes. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. Full experimental and/or methodical details must be provided. There are, in addition, unique features of this journal:"

Introduction:

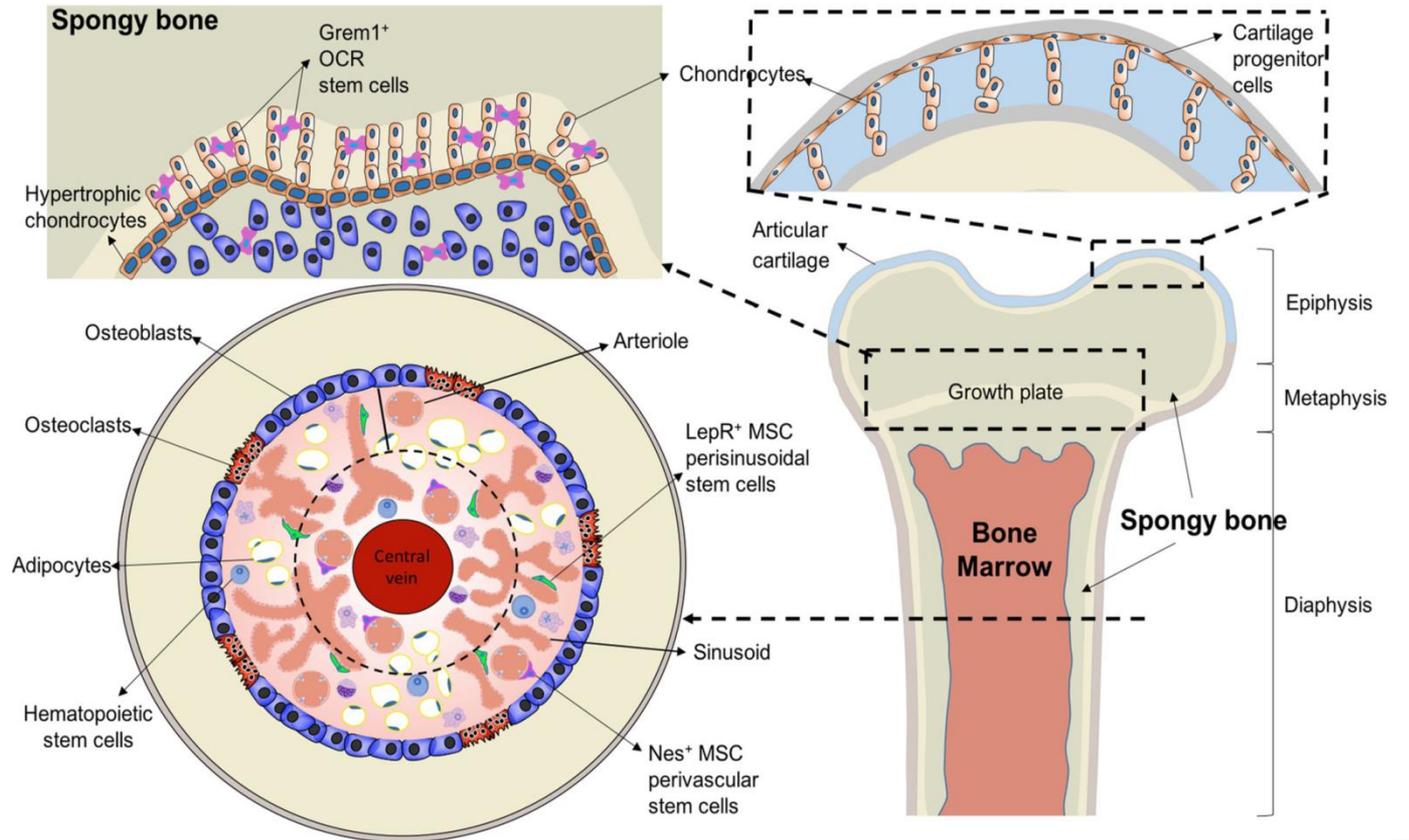
osteoarthritis (OA)



Introduction:

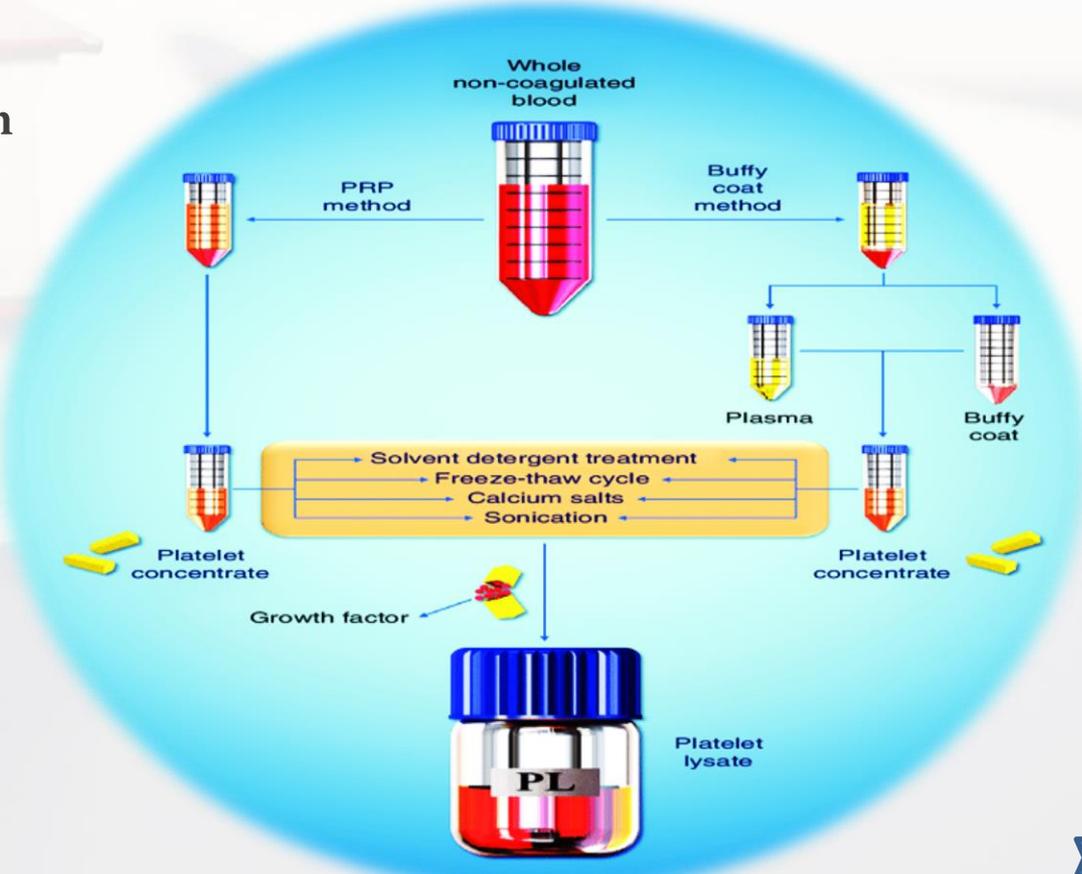


Introduction:



Materials and Methods:

1. Platelet Lysate (PL) Preparation

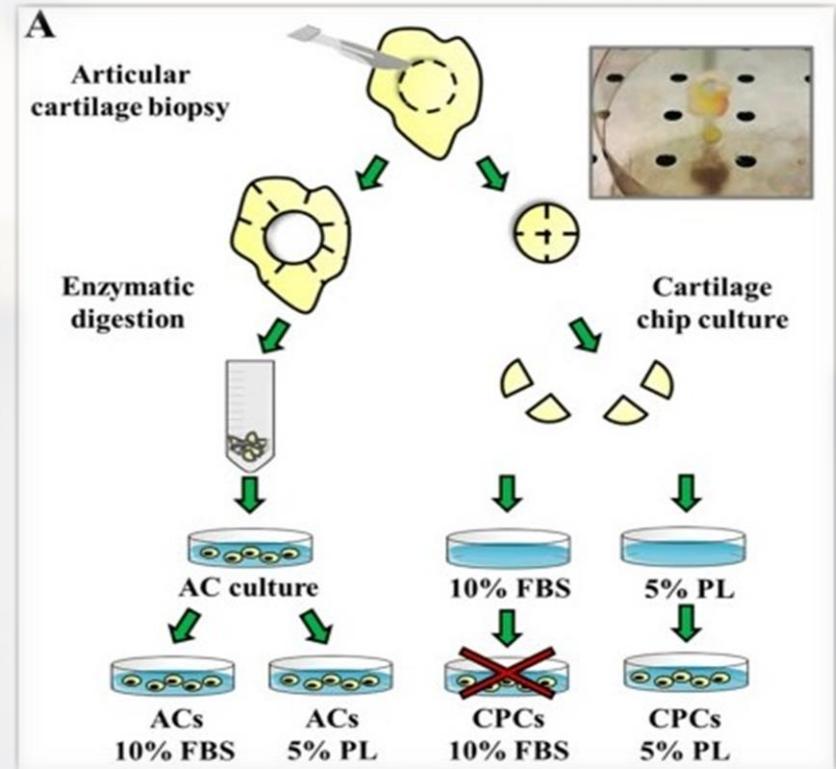


Materials and Methods:

2. Cell Primary Cultures

- Chondro-Progenitor Cells (CPCs)
- Primary Articular Chondrocytes (ACs)

3. Growth Kinetics



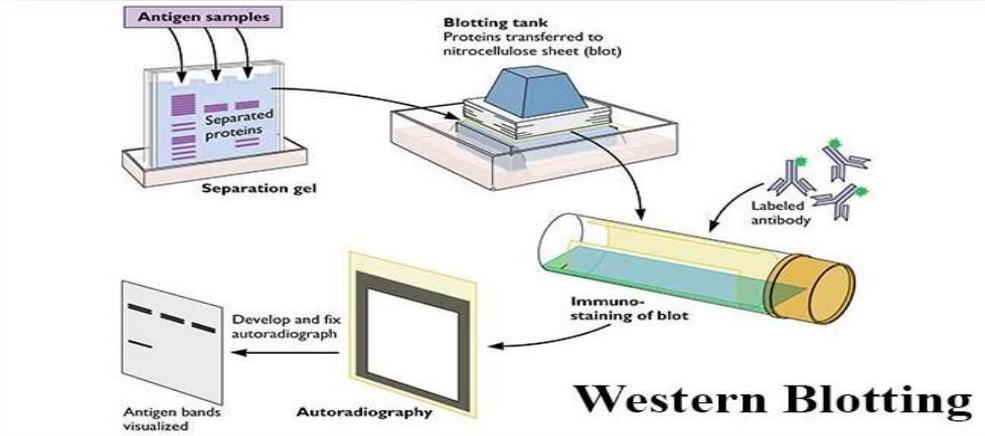
Materials and Methods:

4. Western Blot Analysis



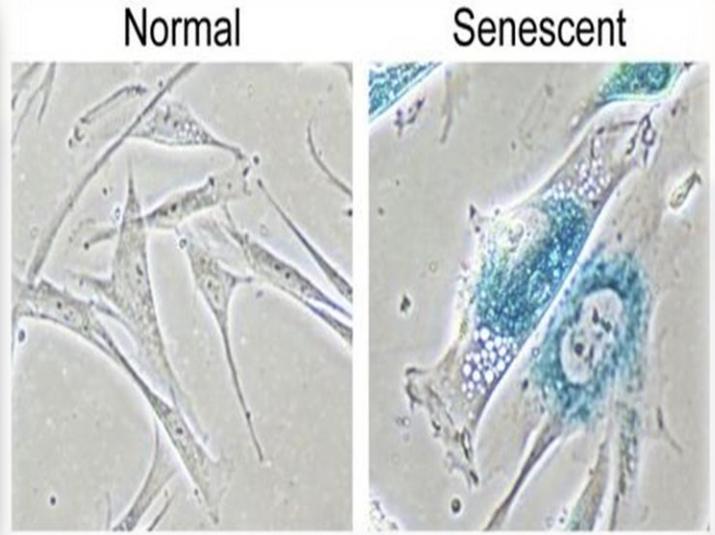
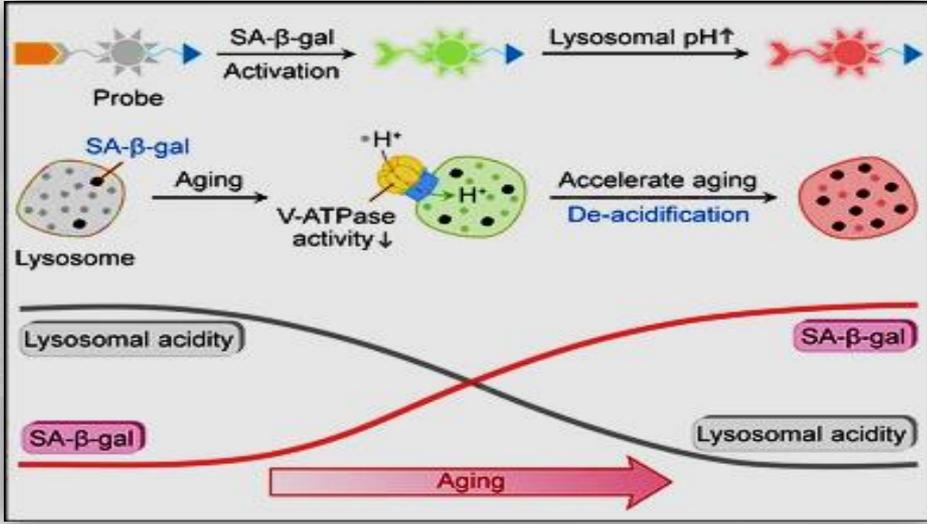
Bradford assay

Western blot



Materials and Methods:

5. Evaluation of Cell Senescence \longrightarrow senescence-associated β -galactosidase (SA- β gal)



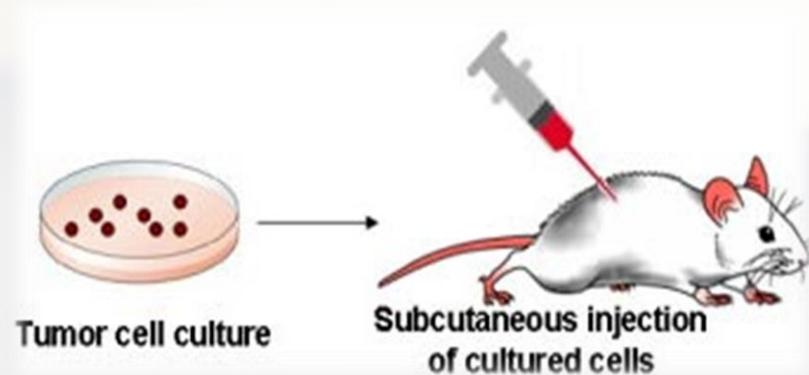
Materials and Methods:

6. Assay for In Vitro and In Vivo Neoplastic Transformation of CPCs

In vitro

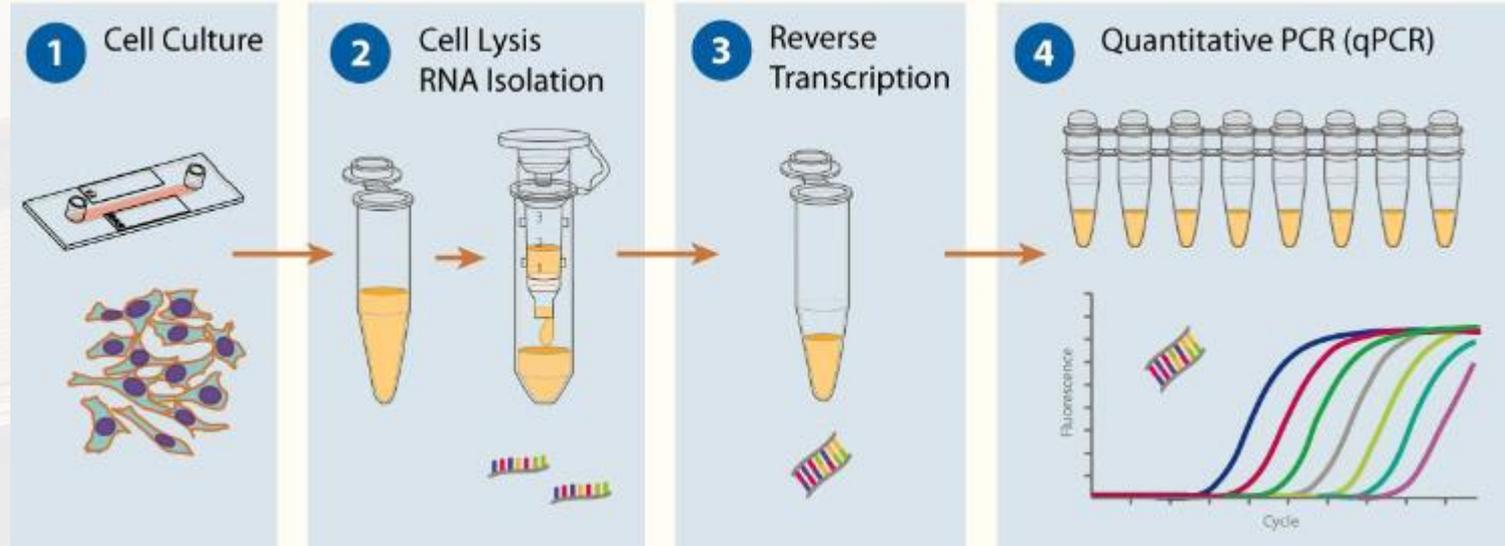


In vivo



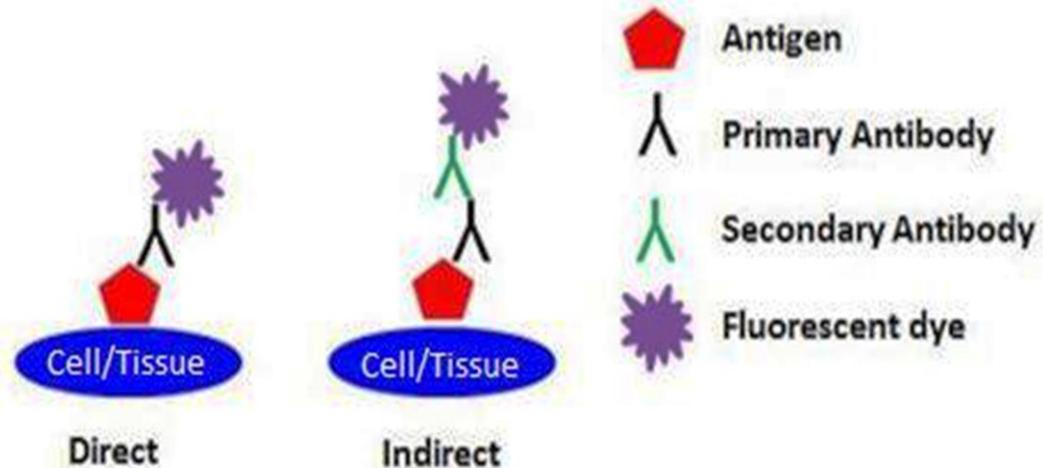
Materials and Methods:

7. RNA extraction, reverse transcription, (qPCR) → COL2A1, COL1A1, SOX9, Nestin, GAPDH



Materials and Methods:

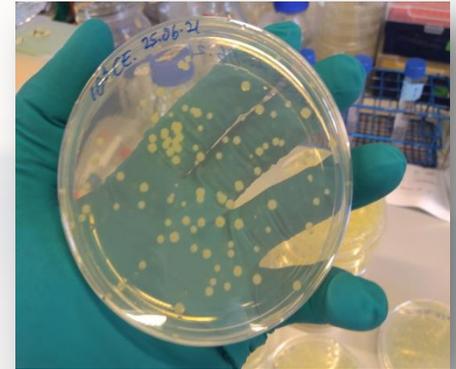
8. Immunophenotypic Characterization by Flow Cytometry → CD44-FITC, CD166-PE, HLA-ABC-PE, HLA-DR-FITC ,CD90-PE, CD105-PE, CD73-FITC, CD146-FITC, CD106-PE, CD45-FITC, CD34-PE, CD29-PE



Materials and Methods:

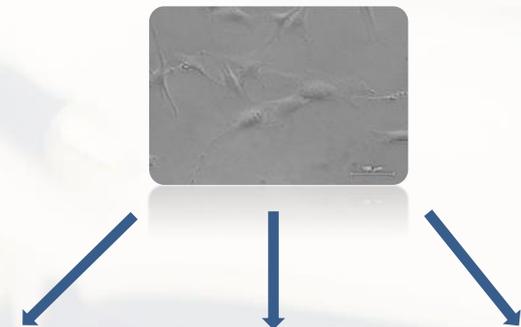
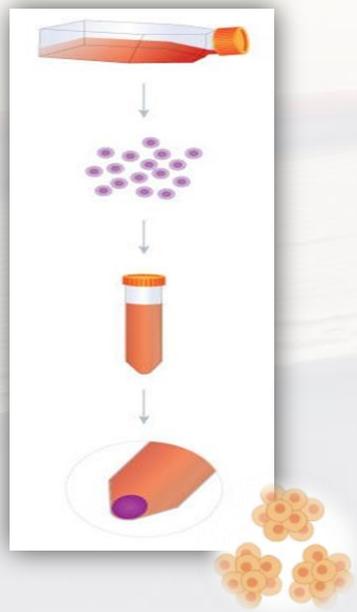
9. Colony-Forming Unit Fibroblast (CFU-F) Assay

number of colonies formed/number of plated
cells \times 100



Materials and Methods:

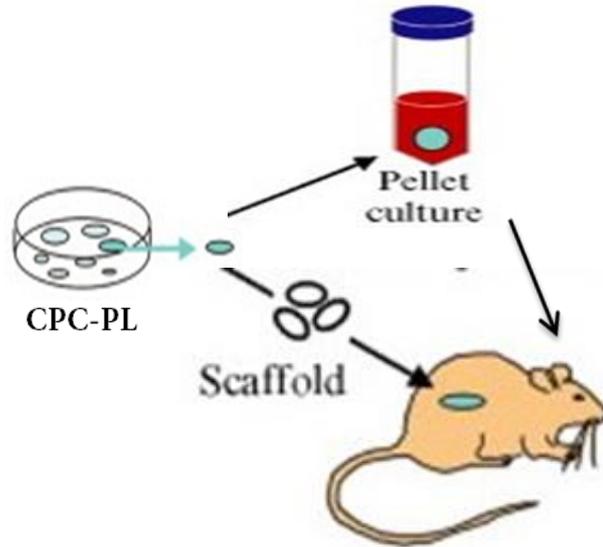
10. In Vitro Multilineage Differentiation Potential



Adipogenesis Oil red O staining	Osteogenesis Alizarin staining	Chondrogenesis Toluidine blue staining

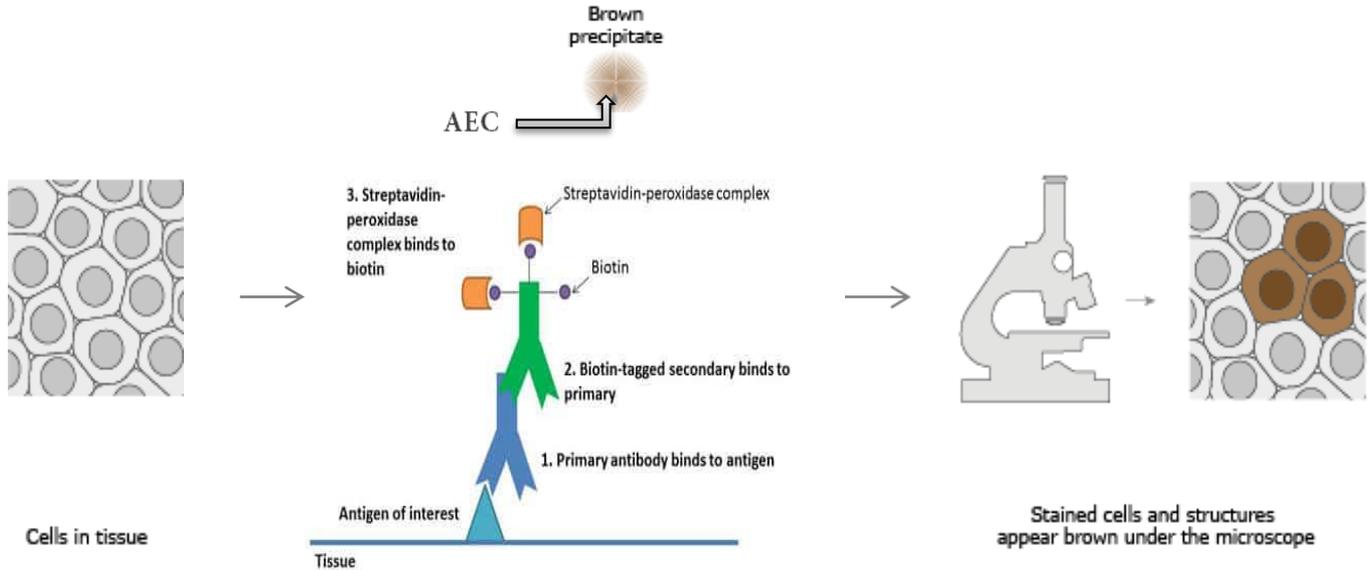
Materials and Methods:

11. In Vivo Cartilage and Bone Formation



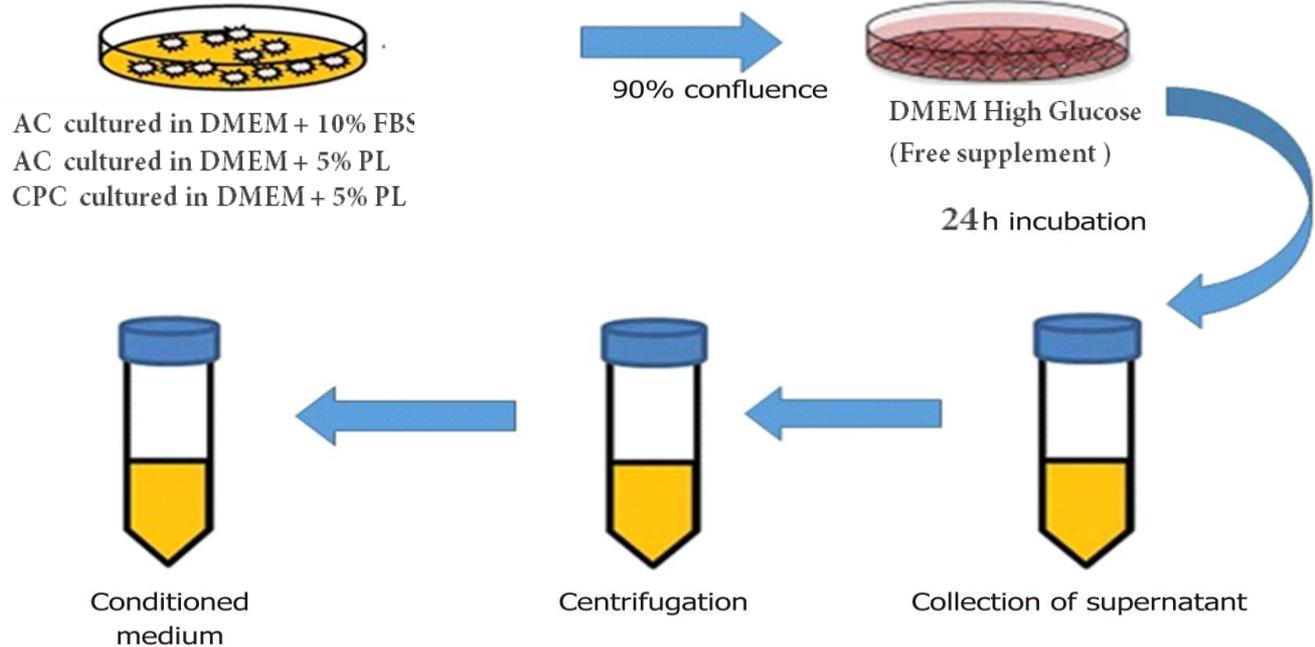
Materials and Methods:

12. Histology and Immunohistochemistry



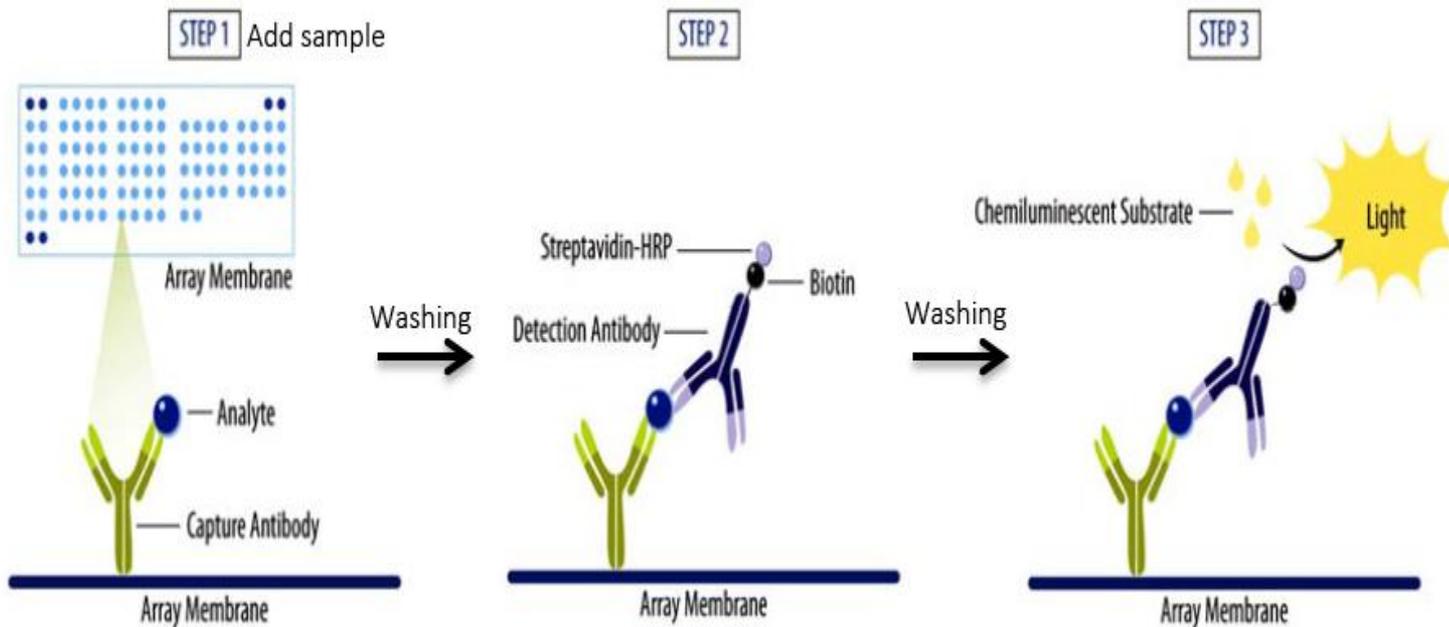
Materials and Methods:

13. Production of Cell-Conditioned Media



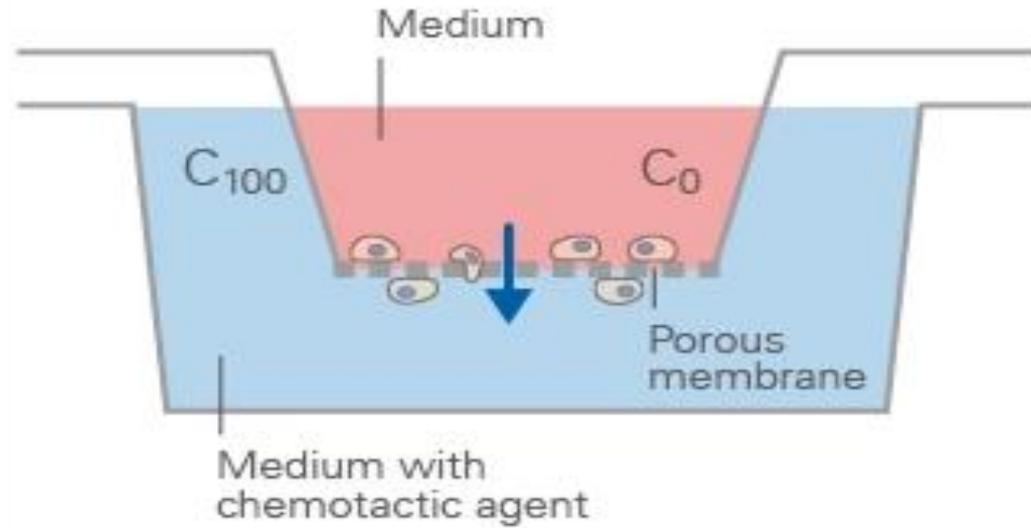
Materials and Methods:

14. Cytokine Identification in Cell Secretome



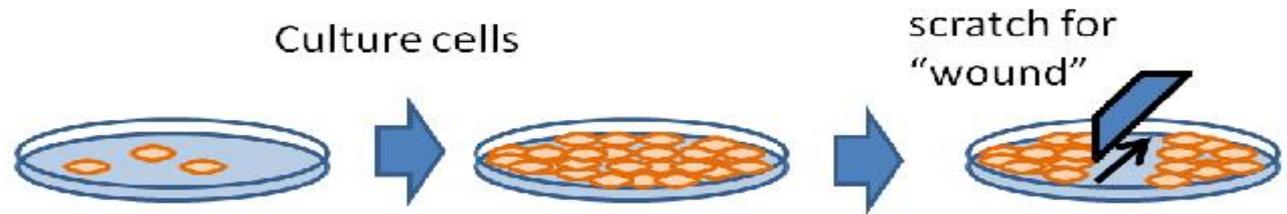
Materials and Methods:

15. In Vitro Chemotaxis of CPCs

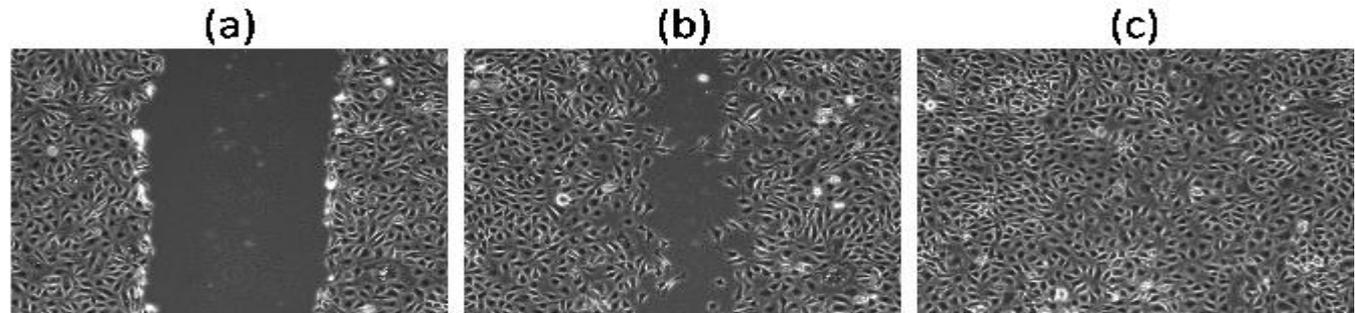


Materials and Methods:

16. In Vitro Scratch Assay

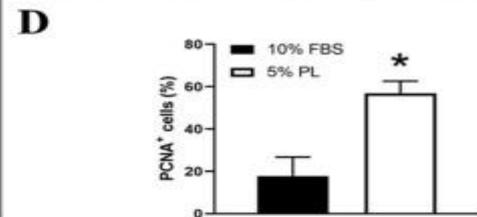
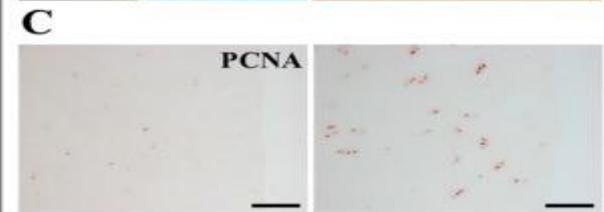
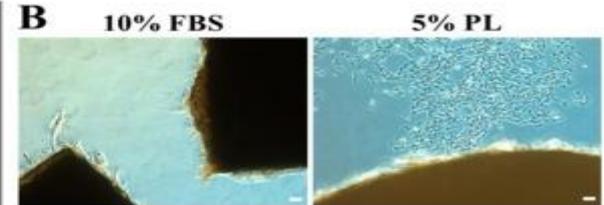
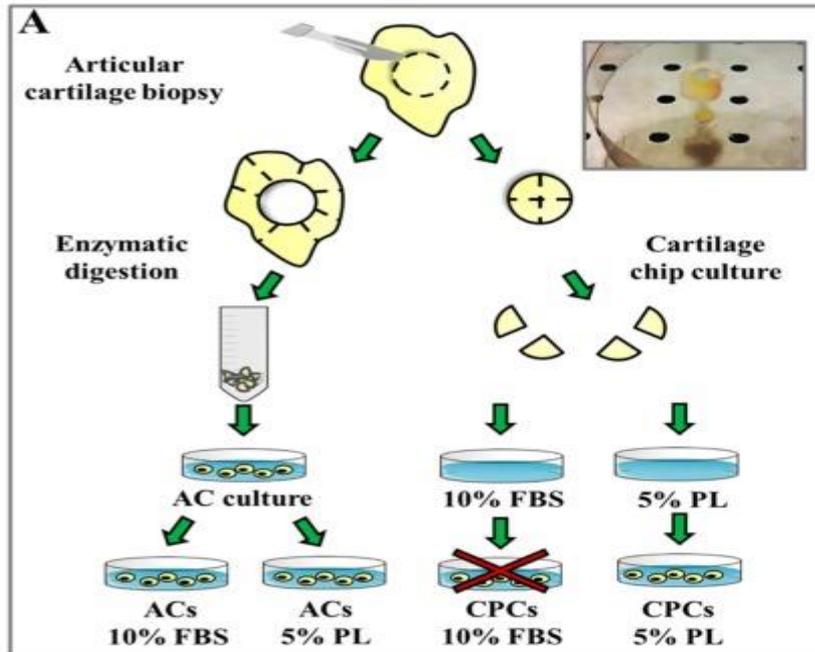


The process of making wound



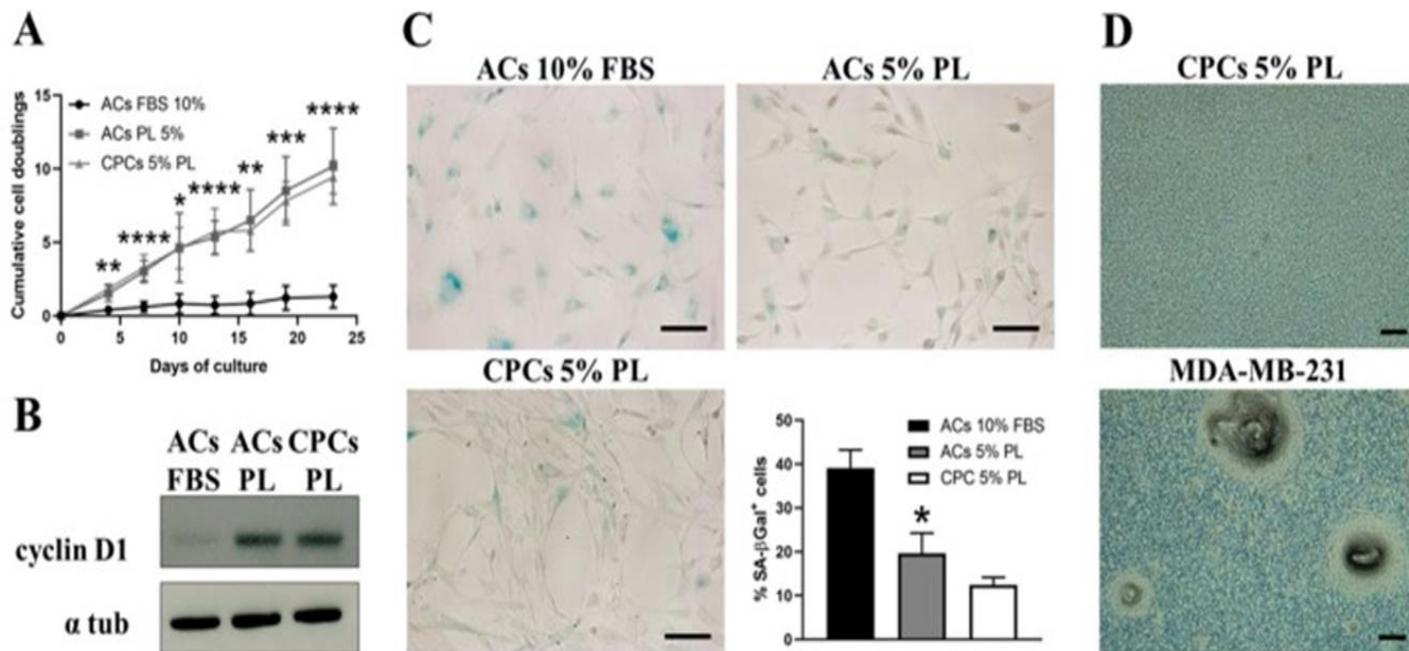
Result:

1. PL Induced Release of Cells with Fibroblastic-Like Phenotype from Ex Vivo Cultured Cartilage Chips and Promoted Their Proliferation



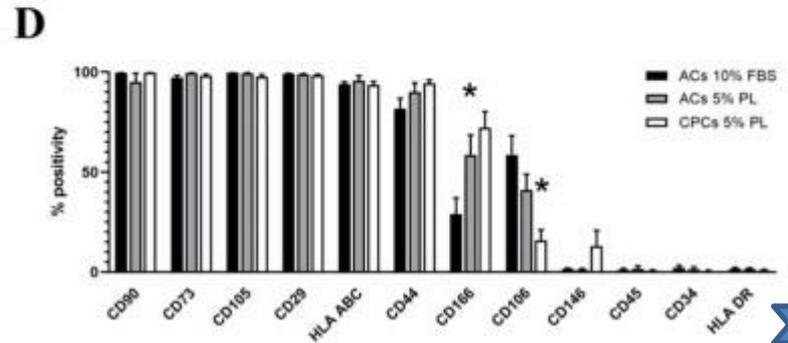
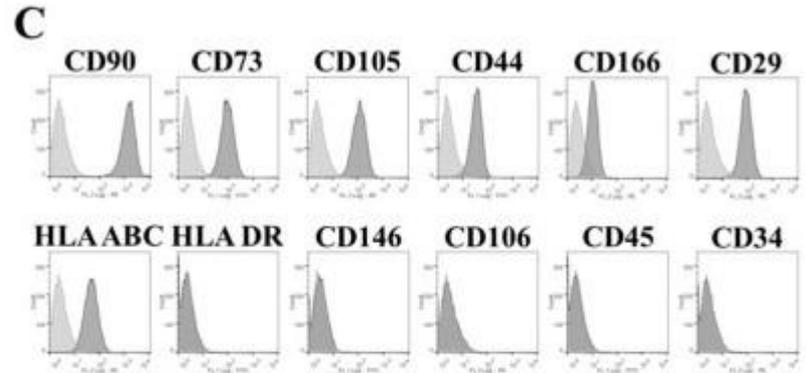
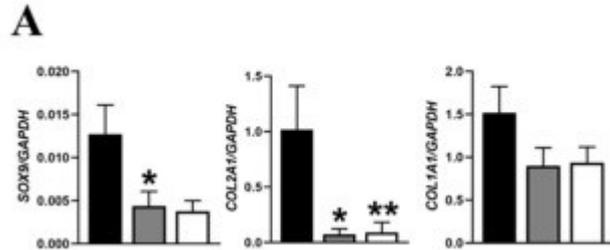
Result:

2. PL Increased the Proliferation of ACs and Reduced Their Senescence



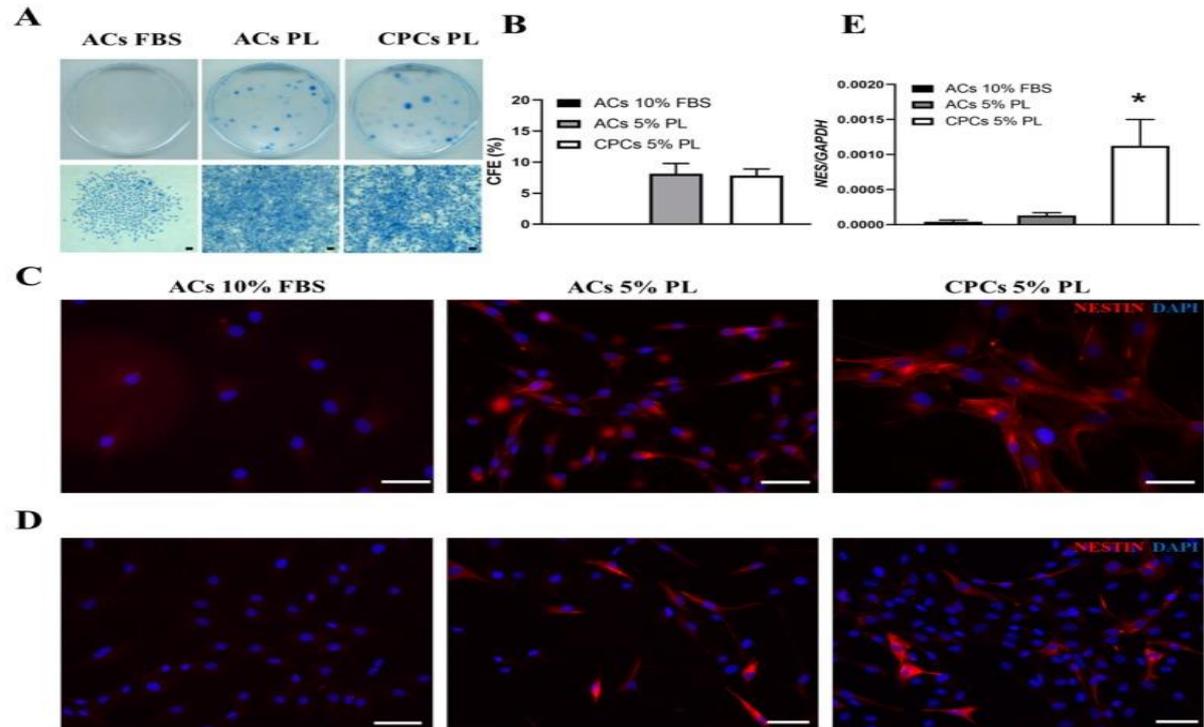
Result:

3. Effect of PL on Gene Expression and Phenotype in Cartilage-Derived Cells



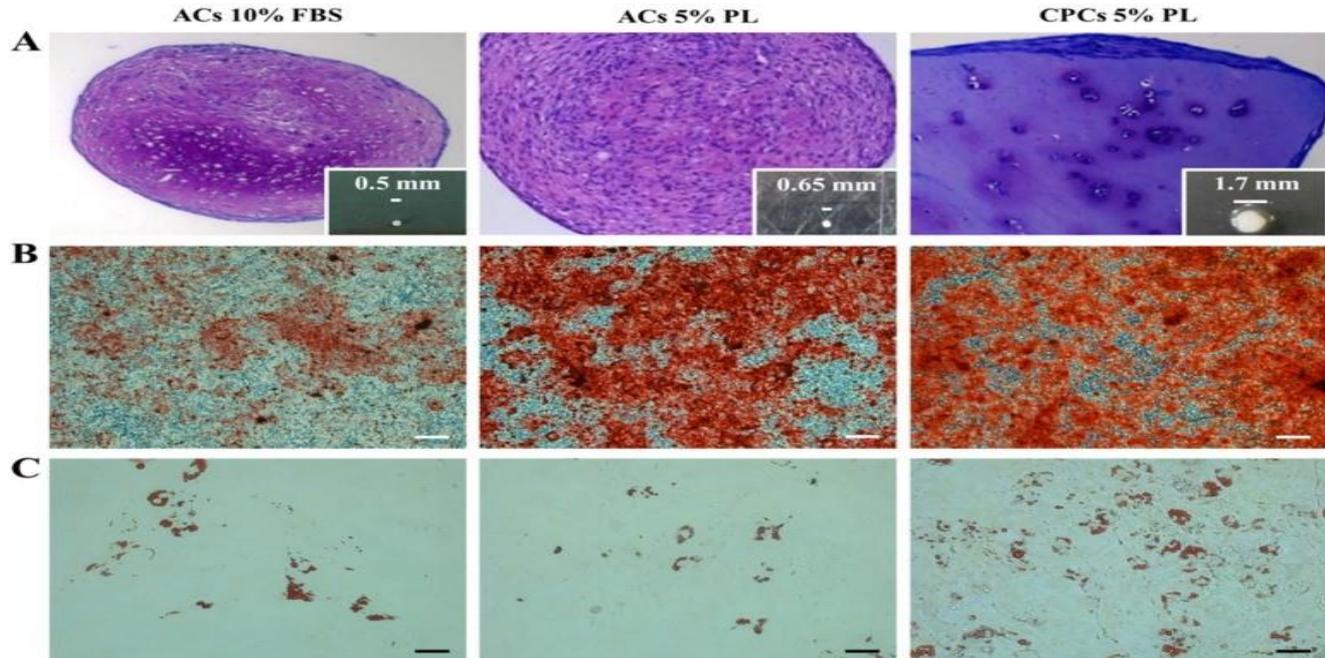
Result:

4. PL Modulated the Clonogenic Potential and Expression of Nestin Stem Marker in Cartilage-Derived Cells



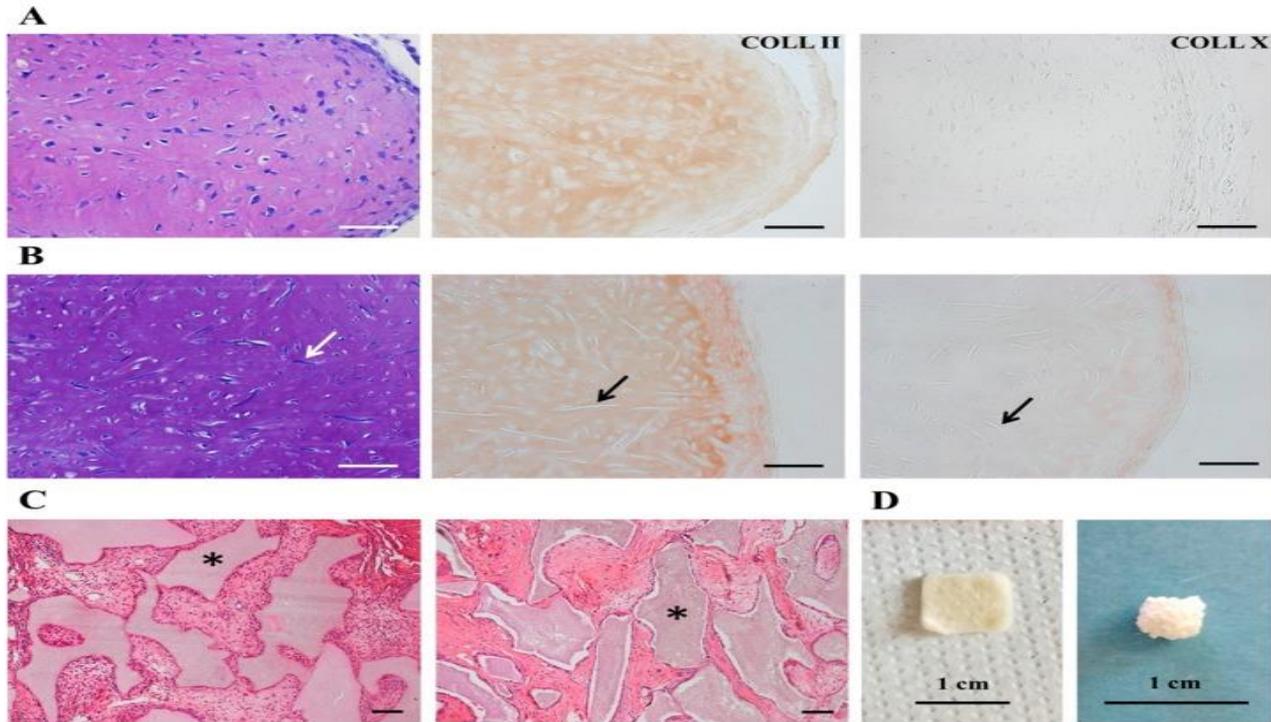
Result:

5. Comparison of In Vitro Multilineage Differentiation Potential Between CPCs and ACs



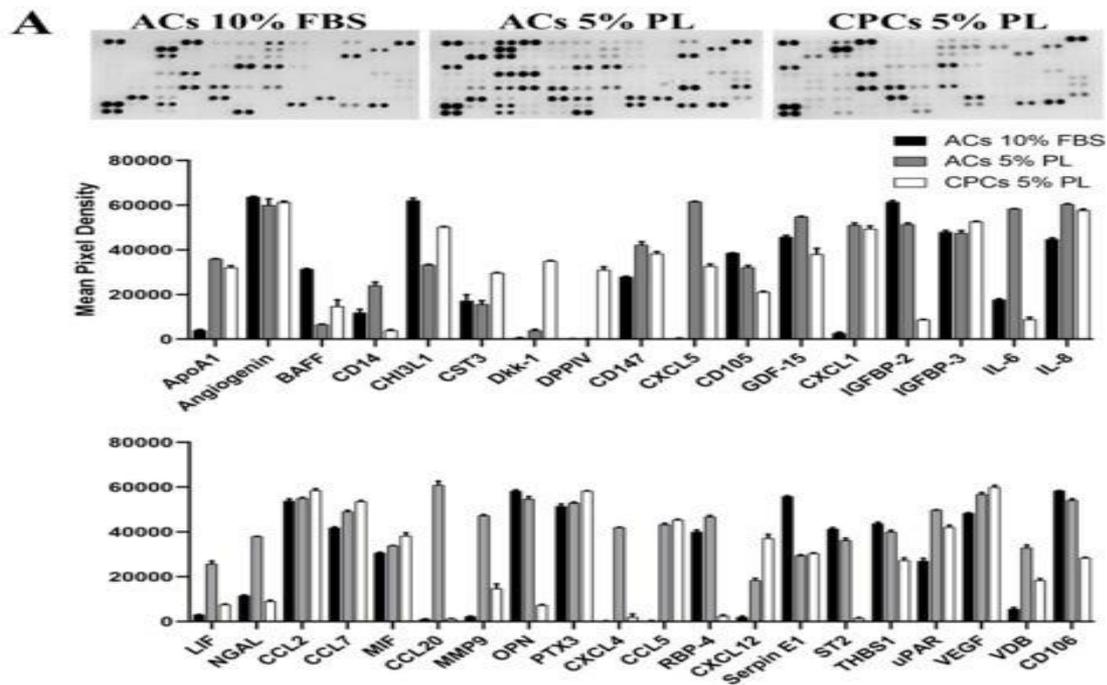
Result:

6. CPCs-PL Produced Hyaline-Like Cartilage In Vivo Suitable for Tissue Engineering Strategies



Result:

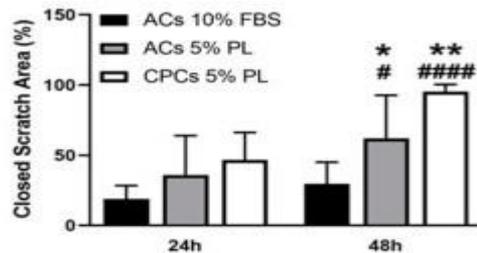
7. Secretory Profile of CPCs and ACs Revealed an Intricate Scenario Including Tissue Turnover, Hypertrophy Counteraction, PL-Induced Pro-Inflammatory Effects and Chemoattractive Capability



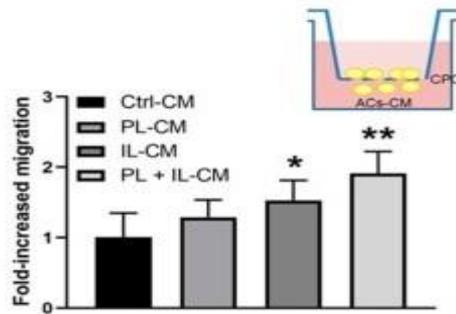
Result:

8. PL-Recruited CPCs Showed Enhanced Motility Under Inflammatory Conditions

B



C



Discussion:

- this study sustains that articular cartilage from joints of patients with ongoing OA contains chondro-progenitor cell population that can be mobilized and subsequently ex vivo recruited from intact tissue by treatment with PL.
- PL is suitable for expansion of chondrocytes intended for therapeutic applications, especially since senescent cartilage cells are involved in the development of OA in joints and their clearance may attenuate its progression promoting a pro-regenerative milieu.
- Nestin-expressing cells compose a quiescent reserve in adults that, if properly reactivated, is able to proliferate, differentiate and migrate. These events are triggered after injury, implying the involvement of these cells in processes of tissue regeneration.
- PL-induced activation of such CPC population able to counteract cell hypertrophy within articular cartilage could be considered beneficial in therapeutic treatments for OA, Furthermore they are able not only to release into the surrounding environment chemoattractant factors but also to actively migrate in response to signals coming from inflamed chondrocytes.
- Finally, PL exerted a general strong chemotactic action on the entire cartilage cell population, including mature chondrocytes (ACs) that usually are considered not provided with a mobile phenotype.



Conclusion:

PL exerts stimulant effects on articular cartilage, such as the promotion of chondrocyte proliferation, cell mobilization and activation of nestin-expressing progenitors. In particular, PL-recruited progenitor cells (CPCs-PL) are able to migrate in response to inflammatory stimuli, show paracrine activity in attracting other cells (ideally toward injured sites) and display high chondrogenic potential and resistance to hypertrophy. Thus, they might replace damaged chondrocytes in a compromised cartilage environment, thus representing a promising target for future therapeutic approach for cartilage disorders.



