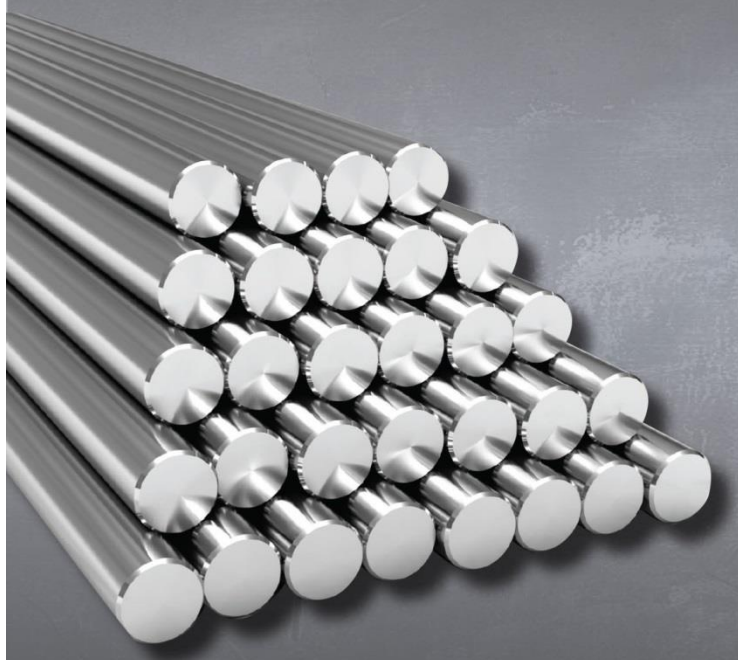
259th OMICS International Conferences

International Conference on

Smart Materials & Structures

June 15 -17, 2015 Las Vegas, USA



Exploring the Fusion of Intelligence with Materials

Operated by: Editors- Journal of Material Sciences & Engineering, Journal of Vortex Science and Technology and Journal of Nanomaterials & Molecular Nanotechnology

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A NANOFIBROUS HYDROGEL FOR BONE TISSUE ENGINEERING



Umadevi Kandalam, PhD
Assistant Professor
Department of Pediatric Dentistry
College of Dental Medicine
Nova Southeastern University
Fort Lauderdale , Florida

Various Craniofacial Defects



Cleft Defect- Hard Palate



Alveolar Cleft

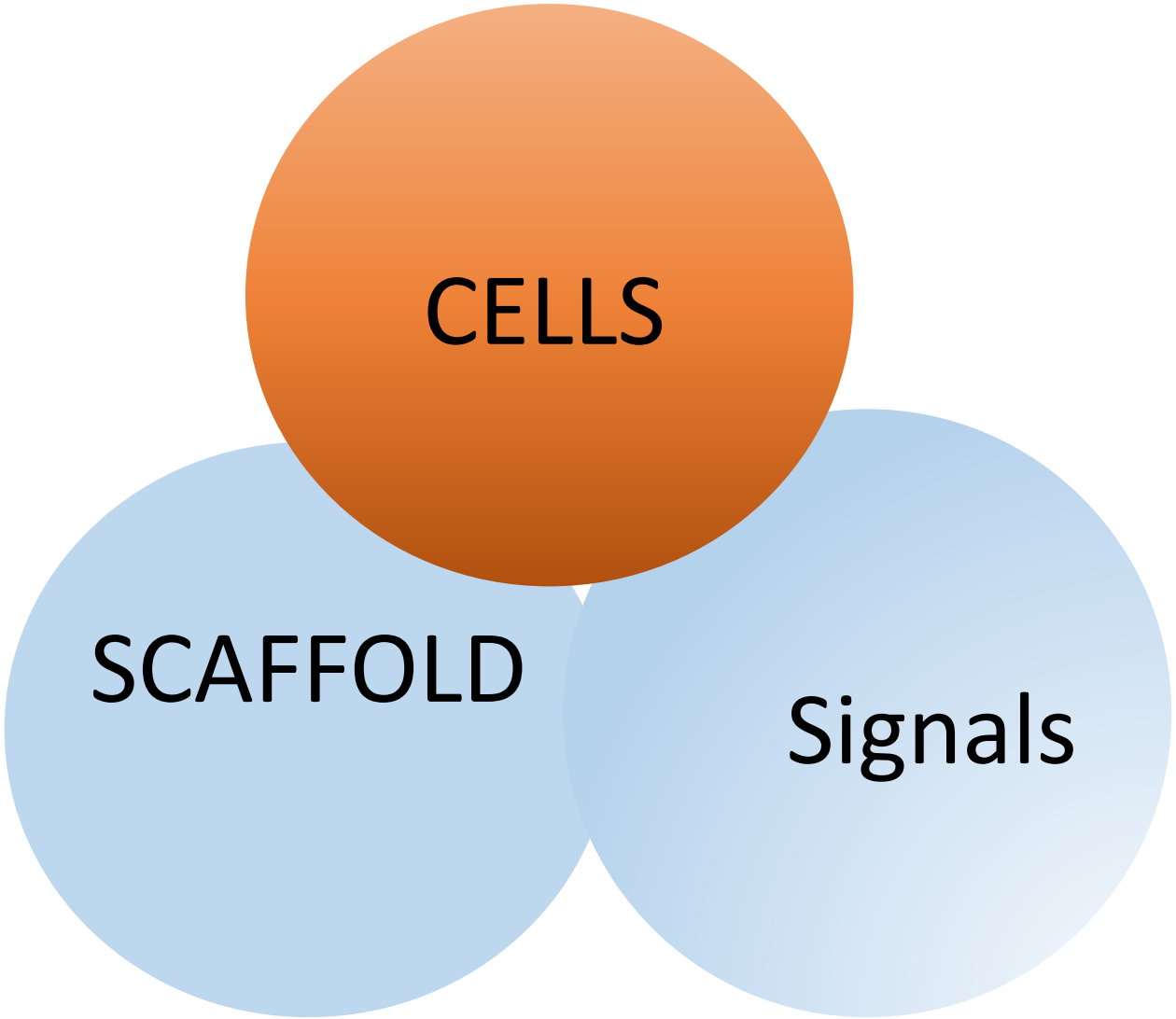


Alveolar Cleft

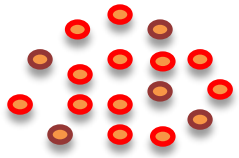


Craniosynostosis

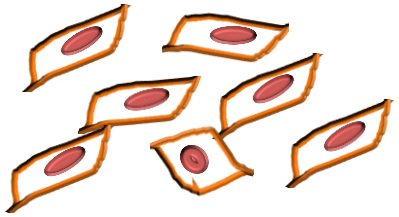
Tissue Engineering



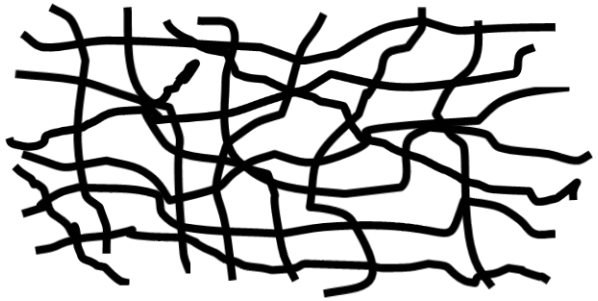
Tissue Engineering



Growth factors

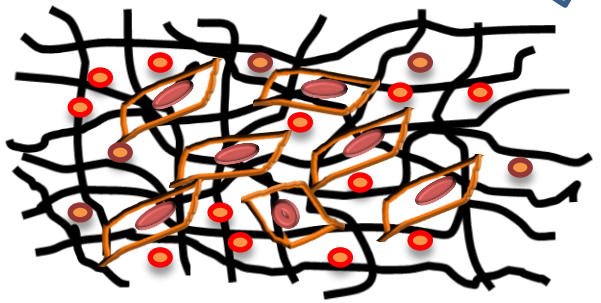


Mammalian cells



Three dimensional scaffold

- Micron/nano size and interconnected pores
- Osteoconductive
- Mechanically strong
- Biocompatible and biodegradable



In vitro culture of cells on scaffold



Bone regeneration

GOAL

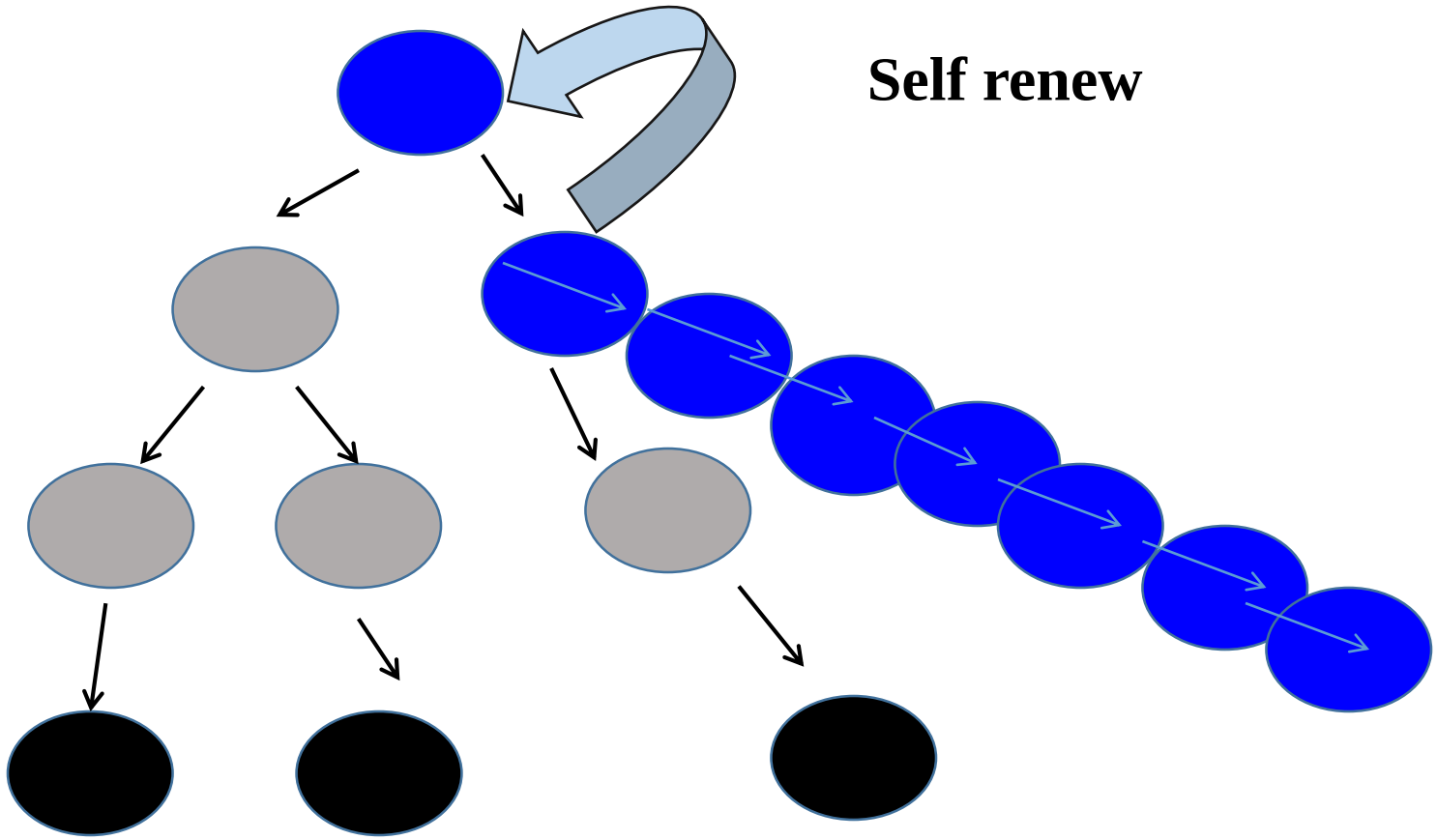
REPAIR THE BONY DEFECT IN THE CRNIOFACIAL REGION USING TISSUE ENGINEERING TECHNIQUES

STEM CELL

Stem Cell

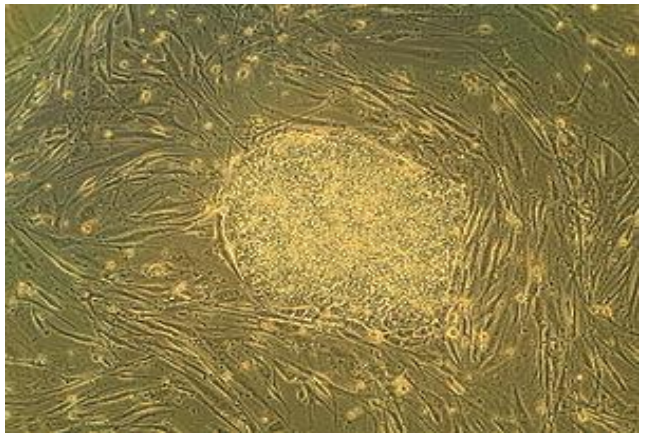
Progenitor Cell

Terminally differentiated Cell

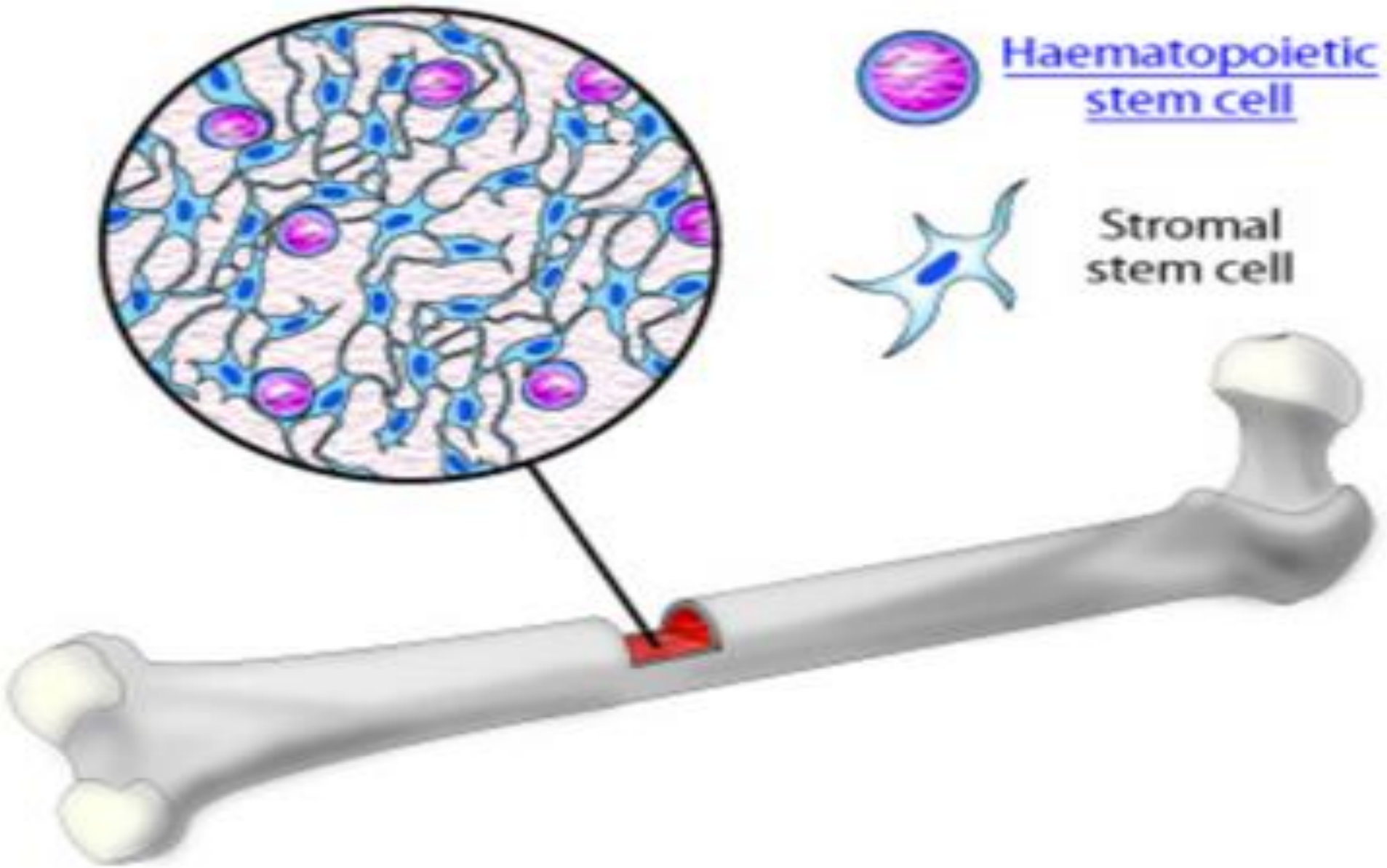


Self renew

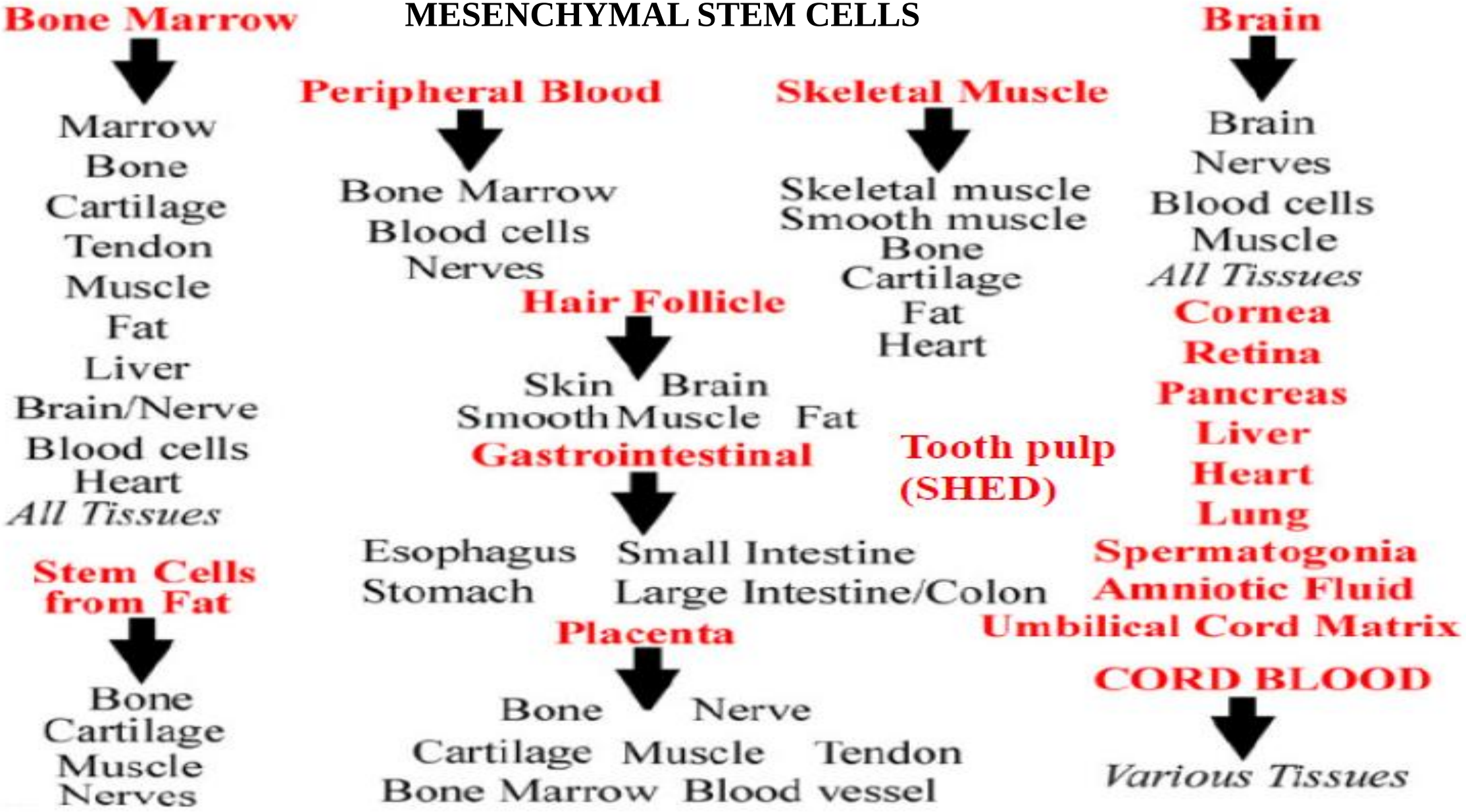
EMBRYONIC STEM CELLS



ADULT STEM CELLS

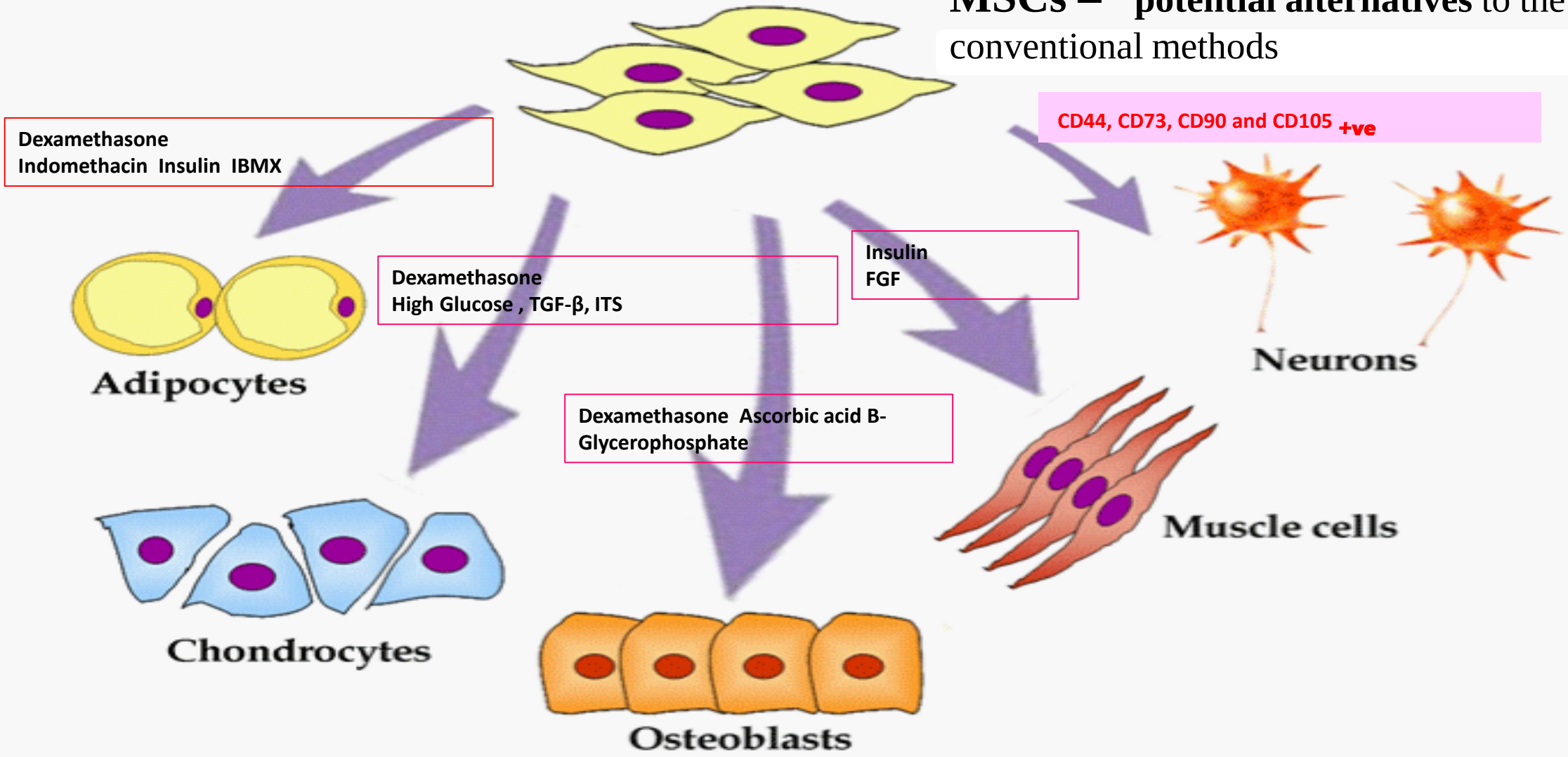


MESENCHYMAL STEM CELLS

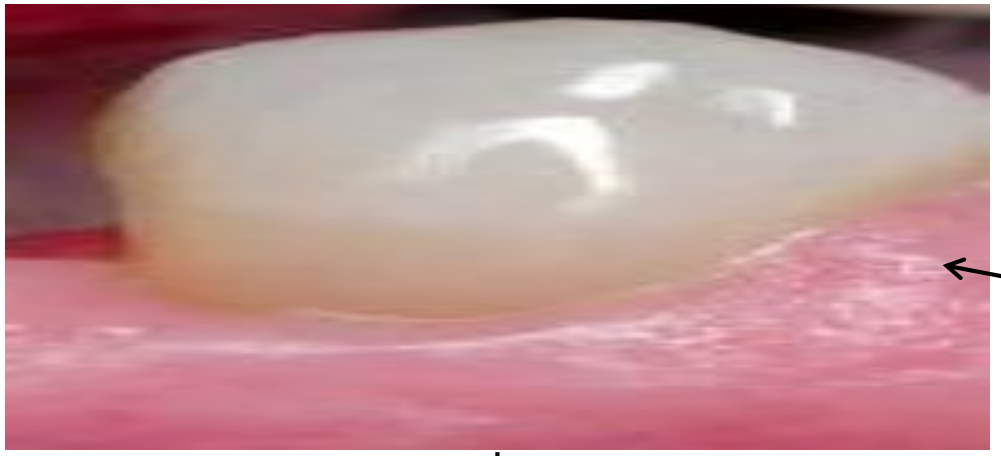


MESENCHYMAL STEM CELLS

MSCs – potential alternatives to the conventional methods

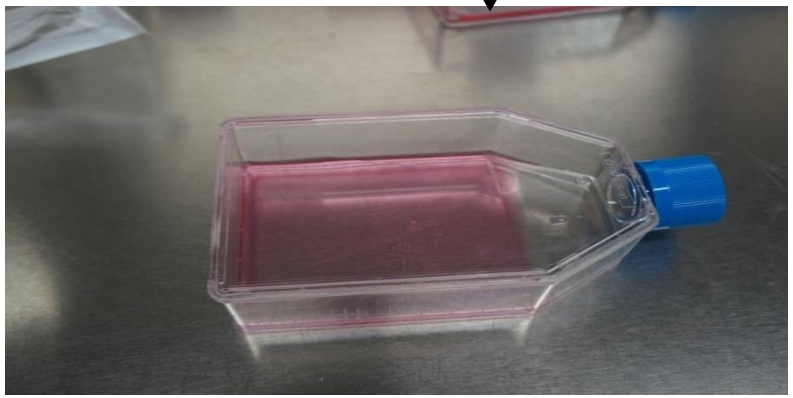


MESENCHYMAL STEM CELLS FROM OROFACIAL REGION



Human Gingiva

Enzymatic Digestion-Isolation of cells

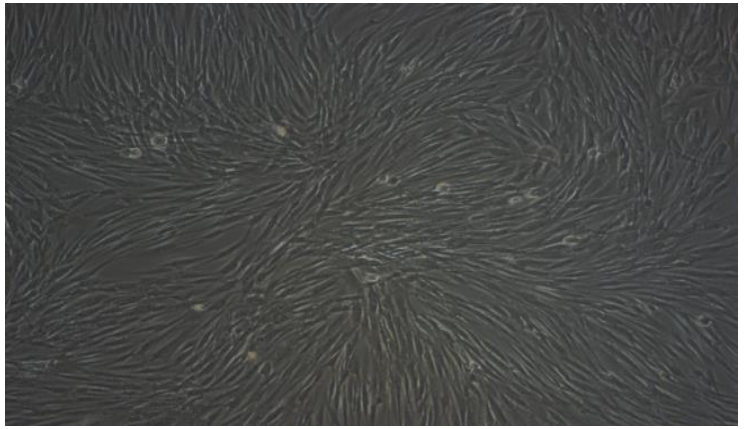


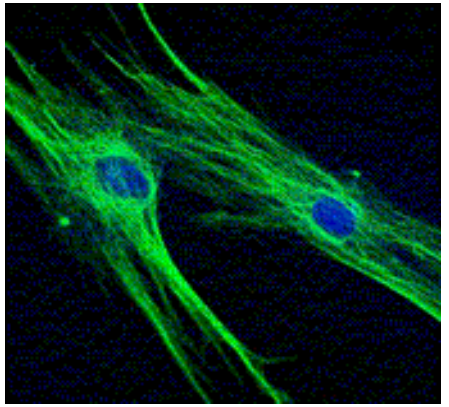
Bone marrow is **traditional** stem cell source for bone tissue engineering

Recently, **human gingival mesenchymal stem cells** (HGMSCs) have been shown to be **superior**

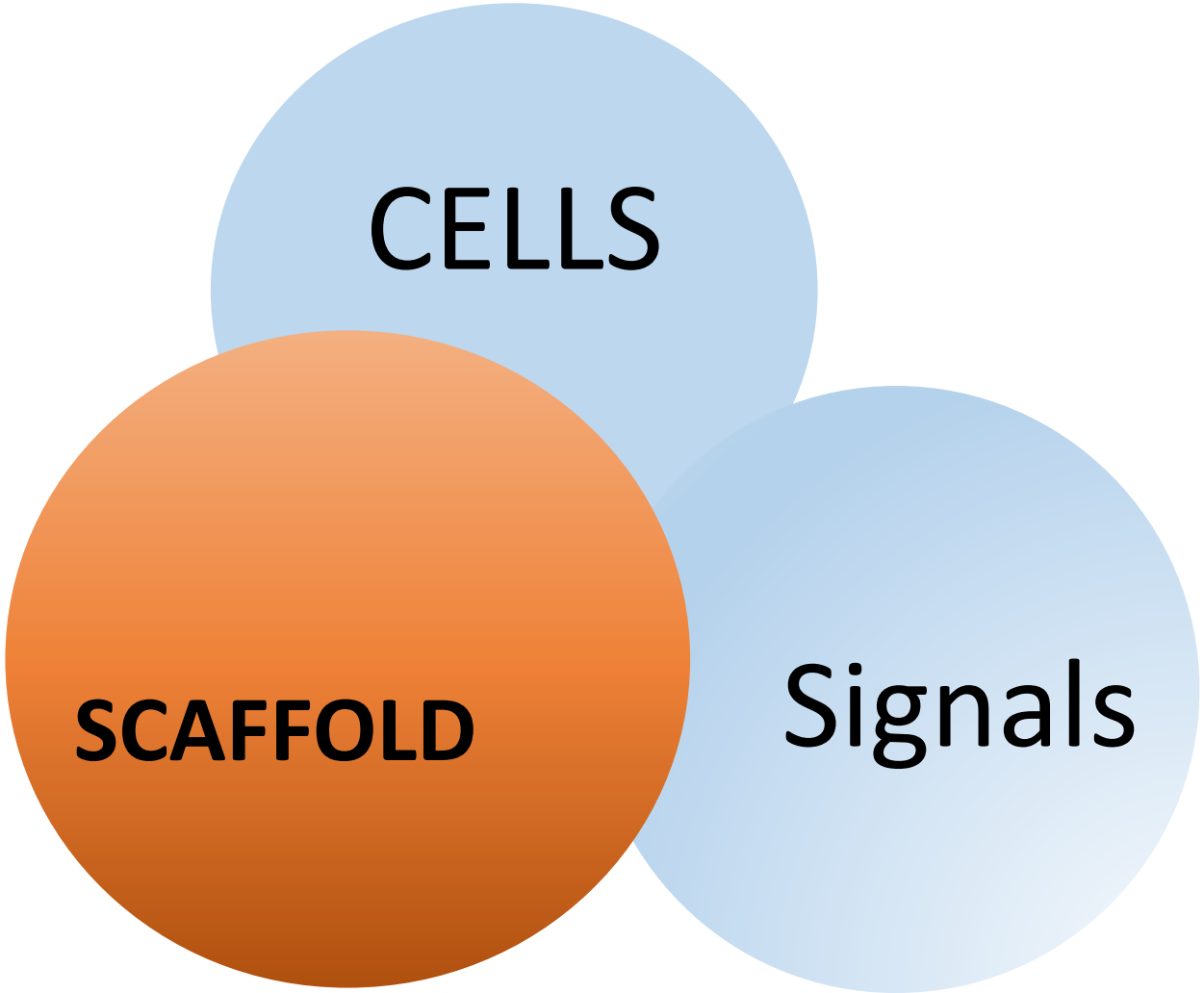
- Neural crest derived
- High Proliferation
- Osteogenic differentiation
- Immunomodulation

HGMSCs





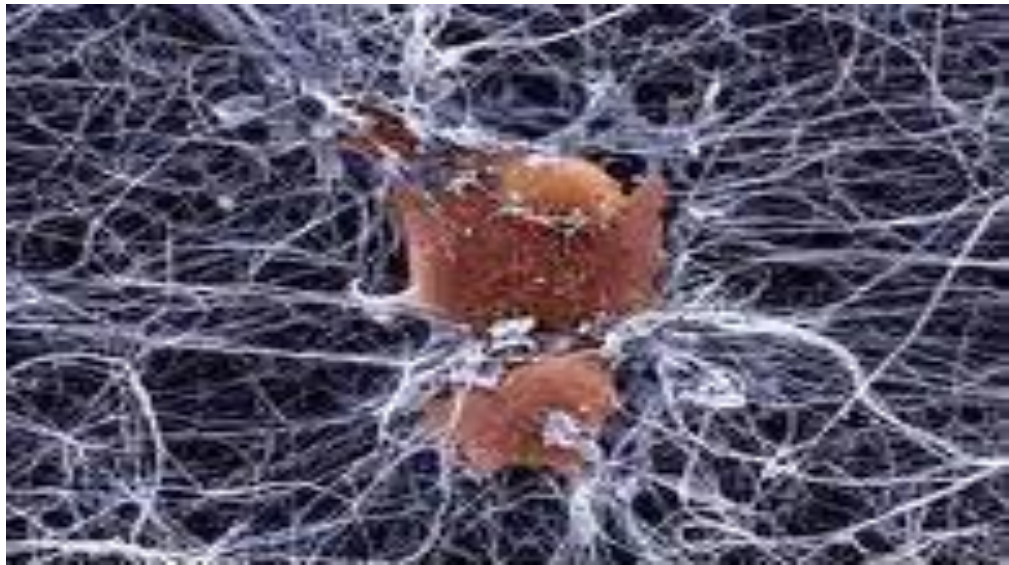
Tissue Engineering



SCAFFOLD

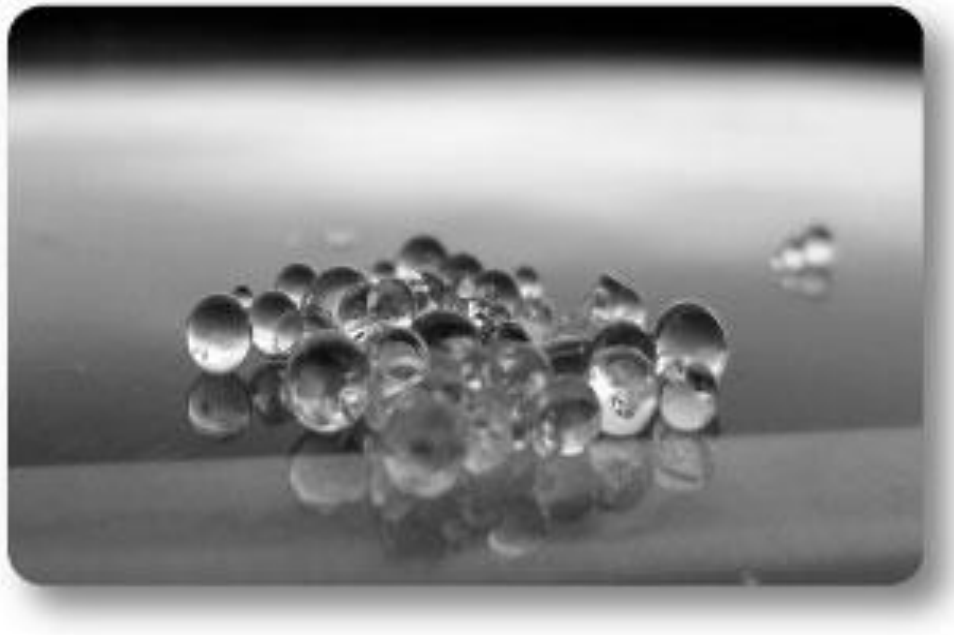
❖ The Biomaterial that is used for tissue-engineering applications often serve as **scaffold** for a specific cell type

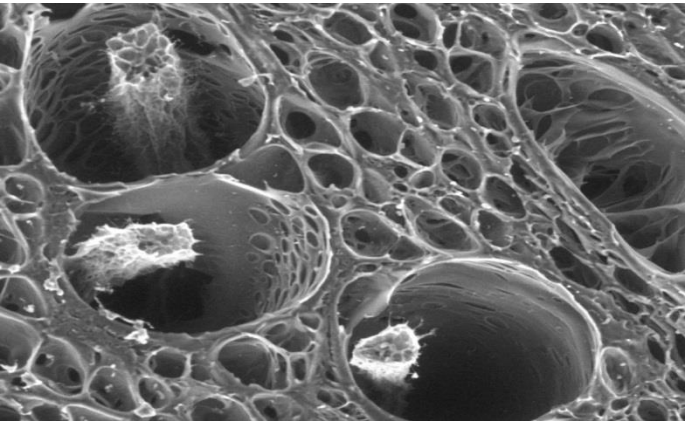
- Natural or synthetic
- Provide framework, mechanical strength
- Promotes natural healing
- Supports cell adhesion and proliferation
- **Carry living cells, drugs, and growth factors**
- Biocompatible, biodegradable



Hydrogels

- Non invasive manner
- **Injectable delivery method**
- Easy to apply
- No scar formation
- Osseoconductive
- Can adapt to **irregular defects** such as bony cranial defects

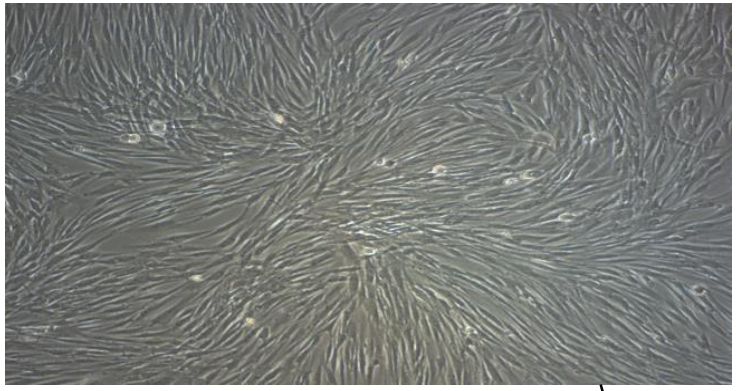




Examples

Alginate
Collagen
Gelatin
Fibrin
Agarose
Polyvinyl alcohol

Self Assembling Injectable hydrogel Scaffolds



Cells



Self assembling peptide hydrogel

Cell gel Mix



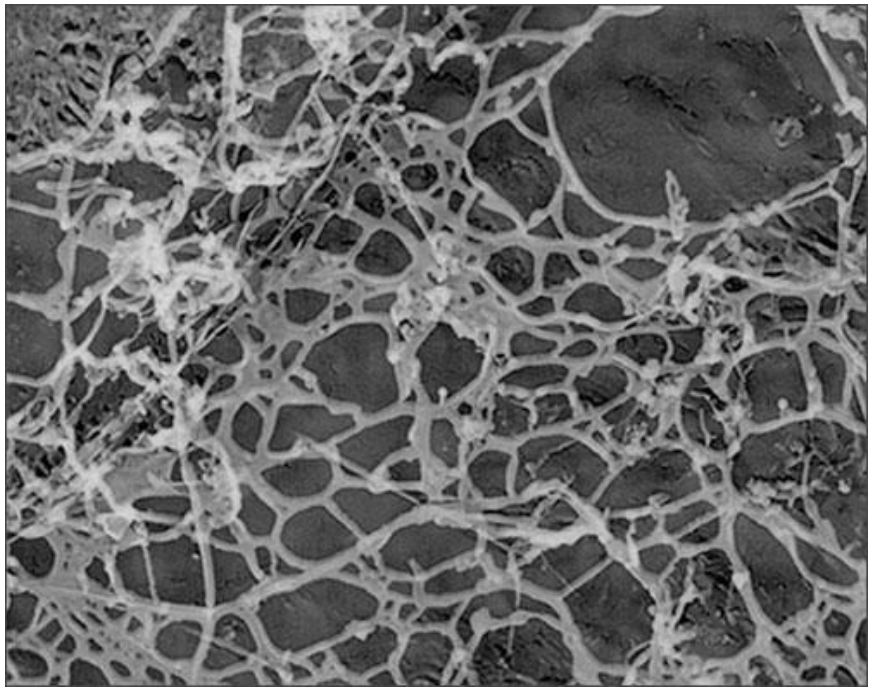
Injection

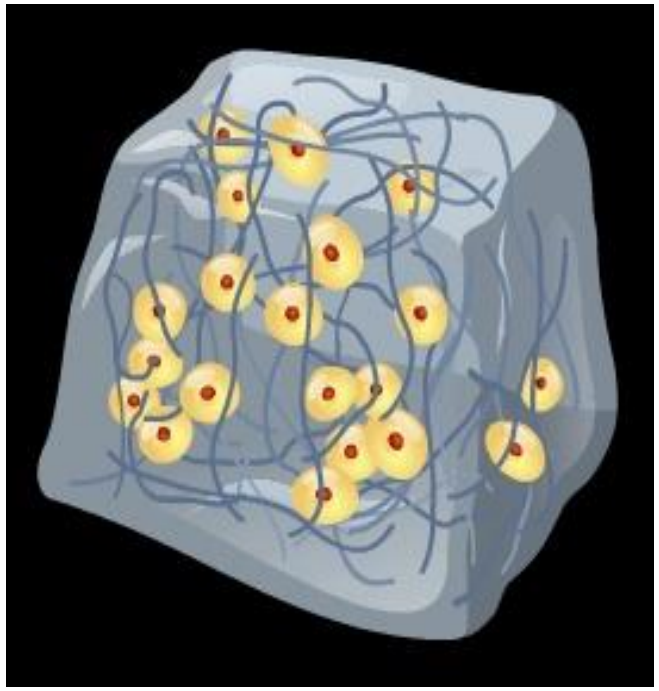
***In Situ* Gelation**

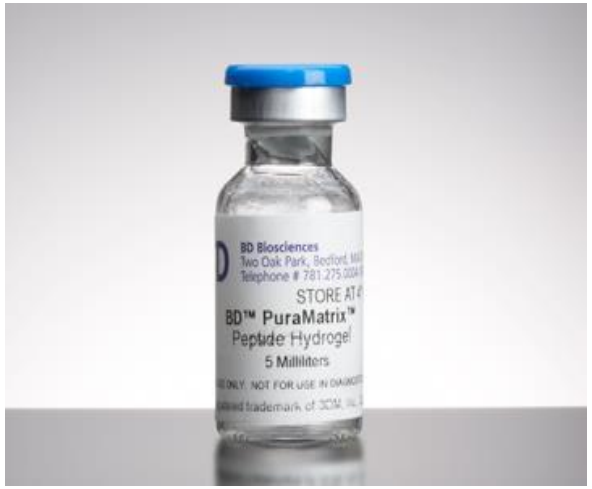


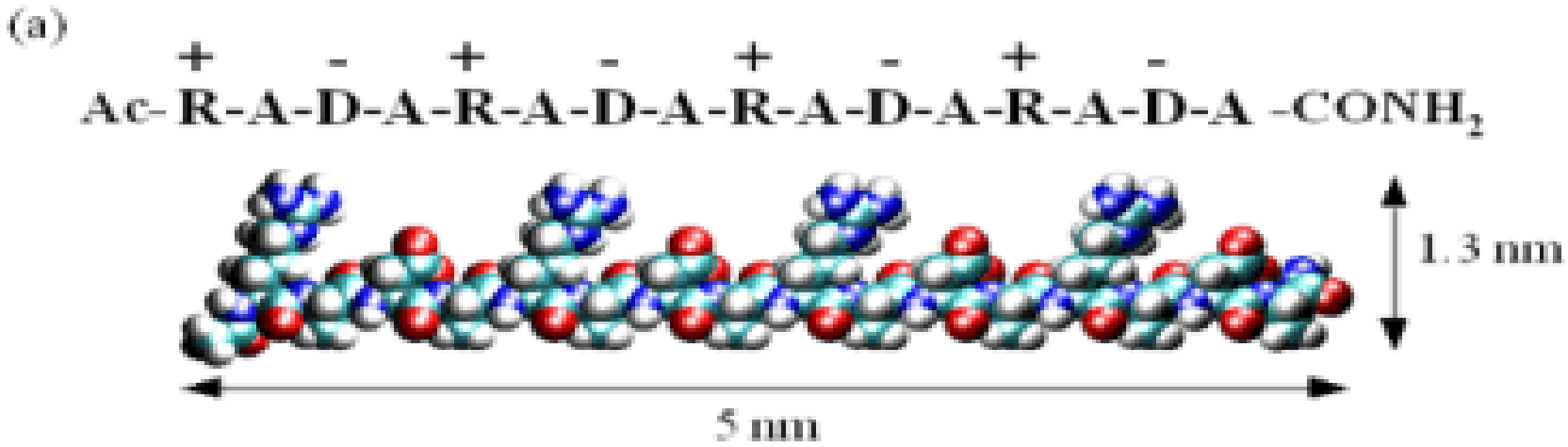
INJECTABLE HYDROGEL: PURAMATIX™



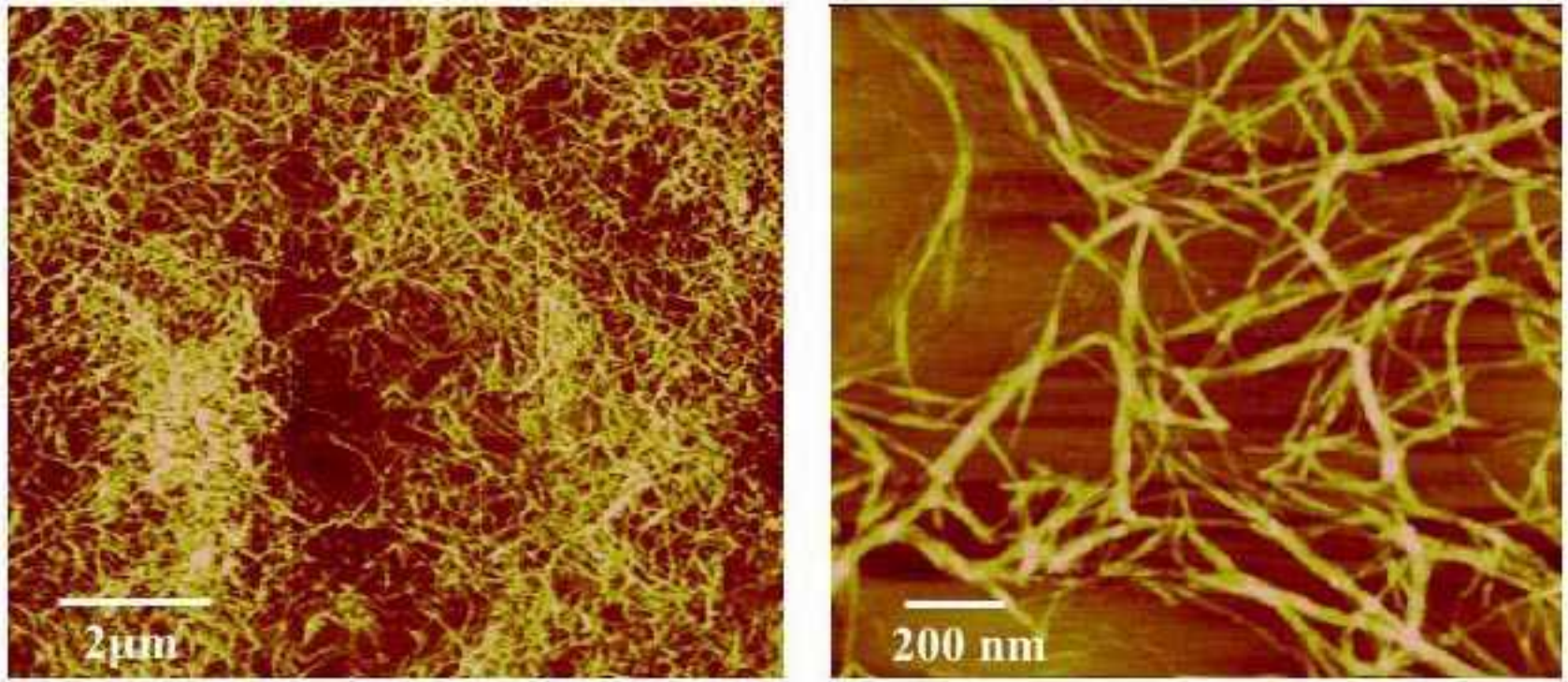








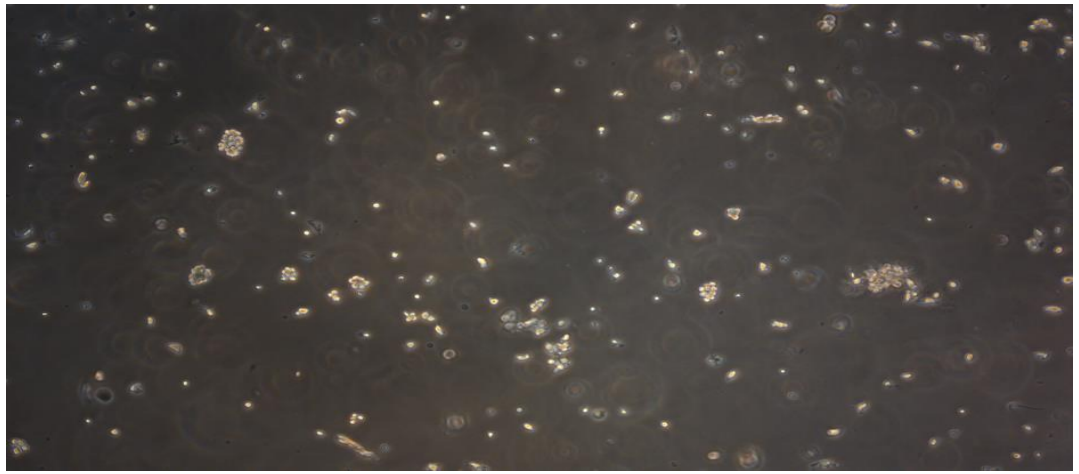
PuraMatrix



AFM image of PuraMatrix 3mM (5mg/ml) in water

CELL- SCAFFOLD SYSTEM

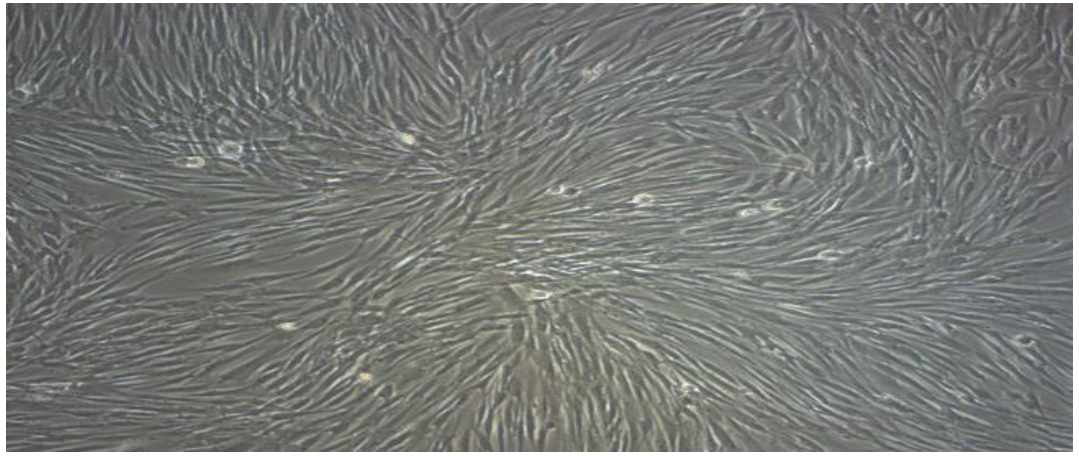
Culture of Human Gingiva Derived Mesenchymal Stem Cells



HGMSCs Plated in T75 flask DAY 0



HGMSCs DAY 1

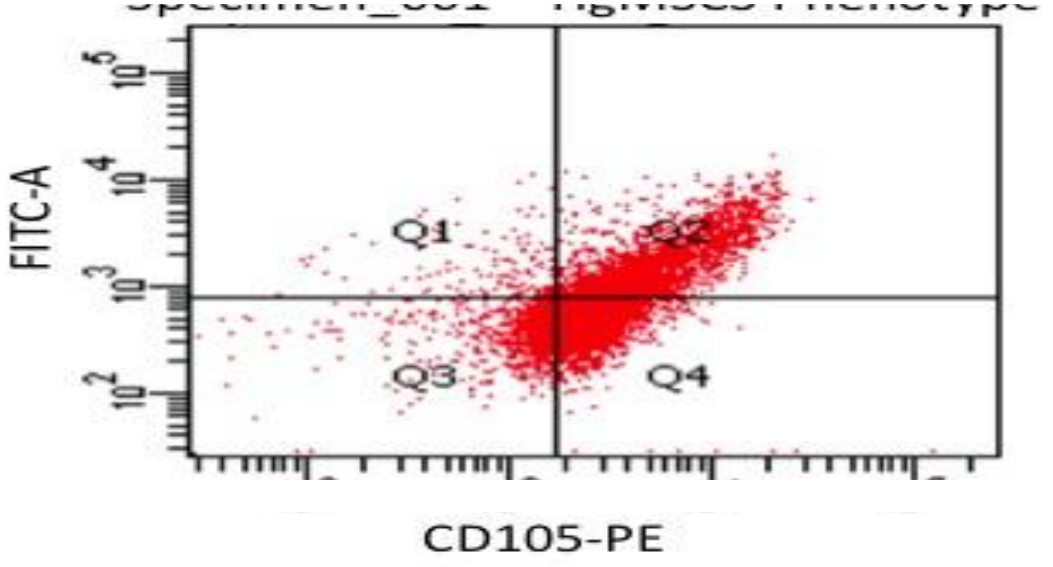


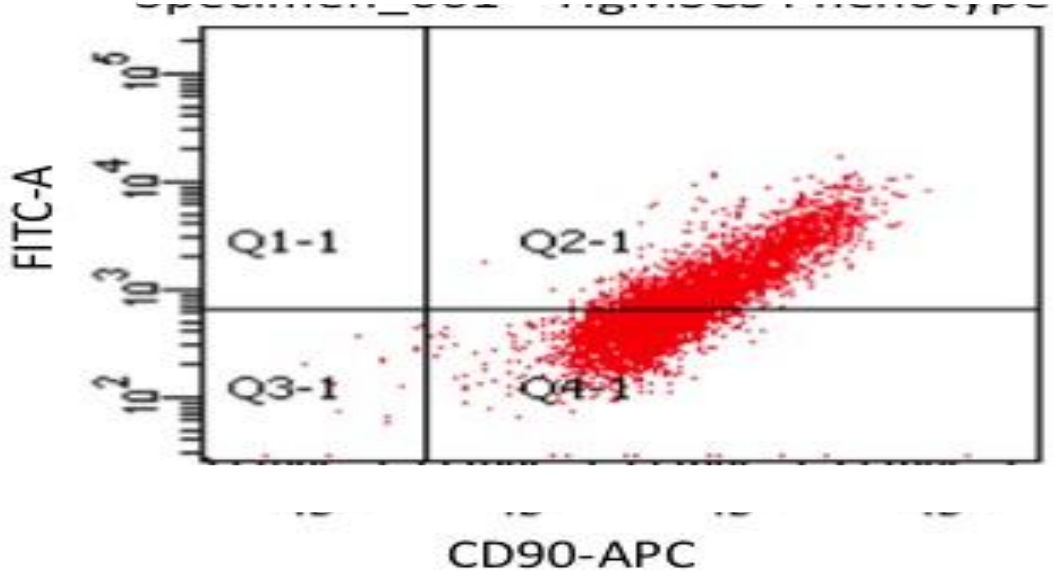
HGMSCs at sub-confluence

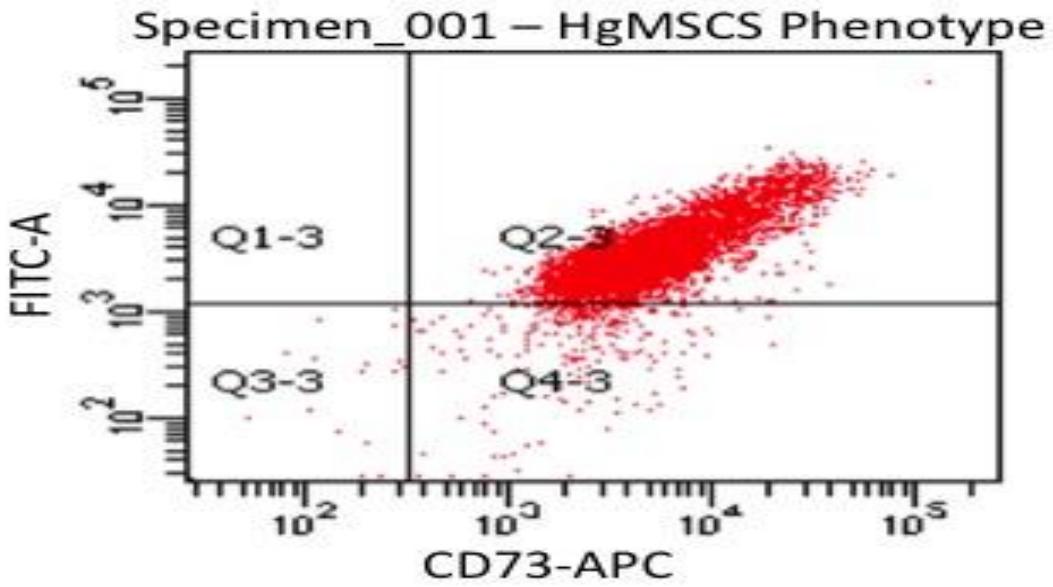


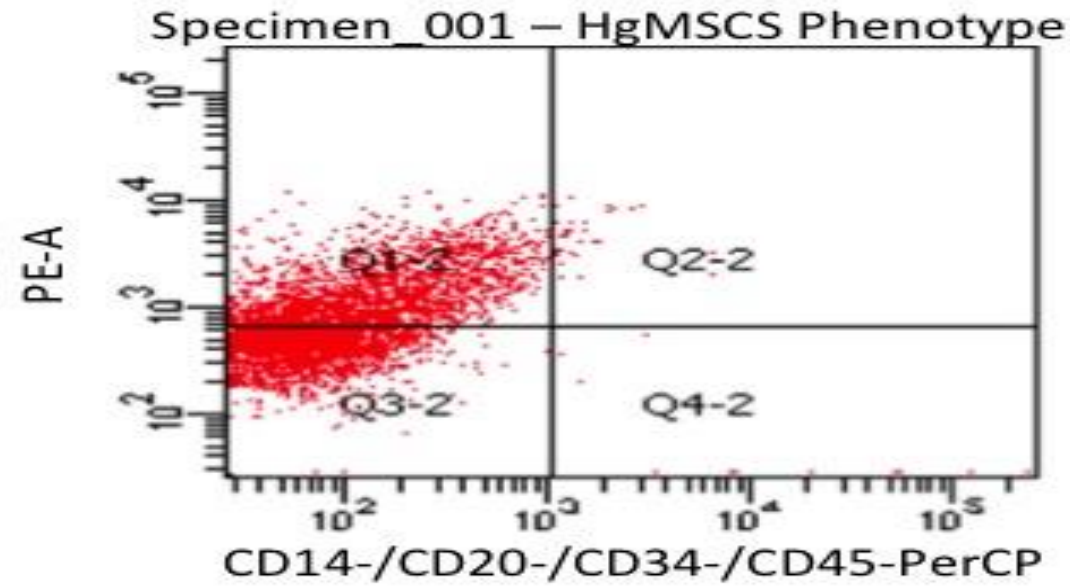
HGMSCs at confluence

Flow-cytometry



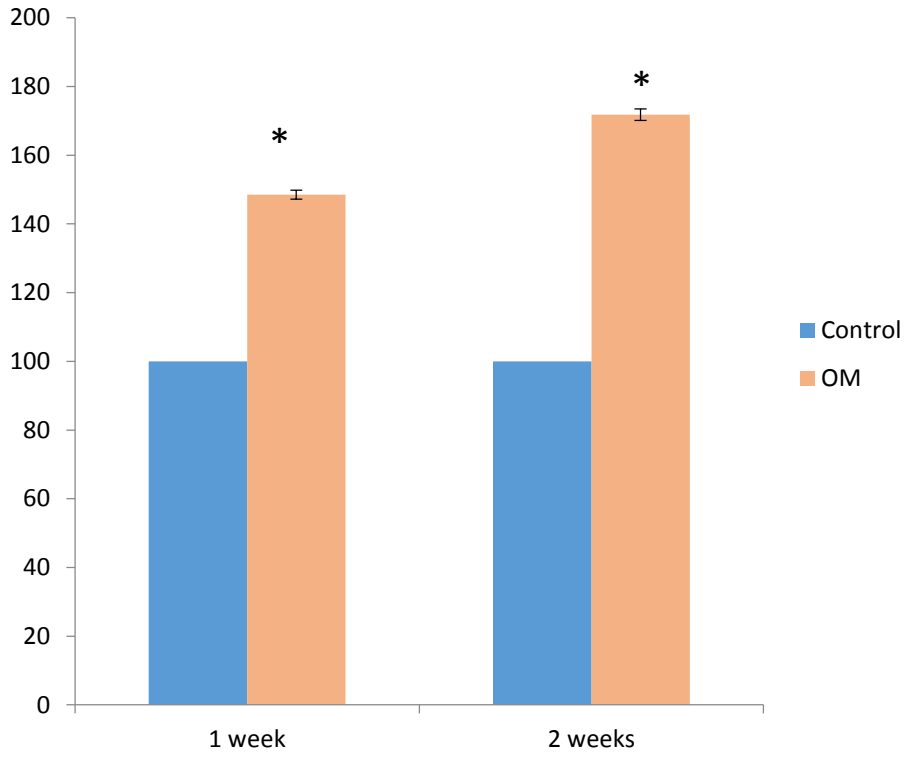




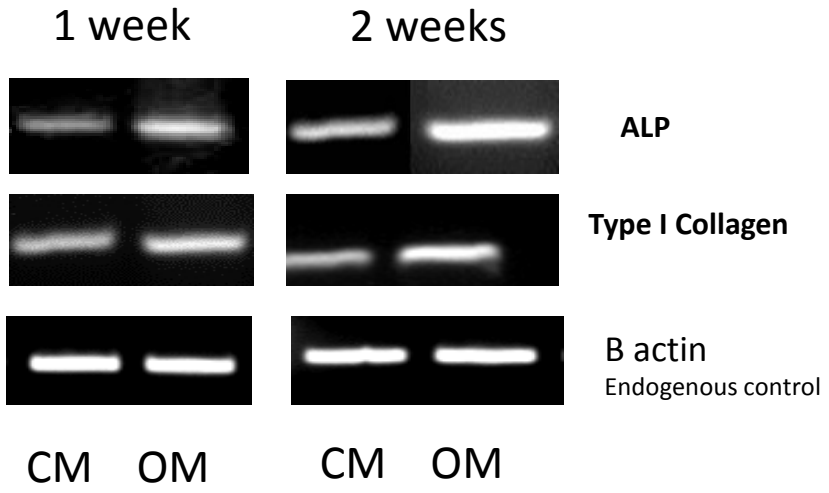


HGMSCS – Osteogenic Differentiation

ALP Enzyme activity



GENE Expression



Protein Expression

1 Week



OPN

Beta Actin

Osteopontin

2 Weeks



Osteopontin

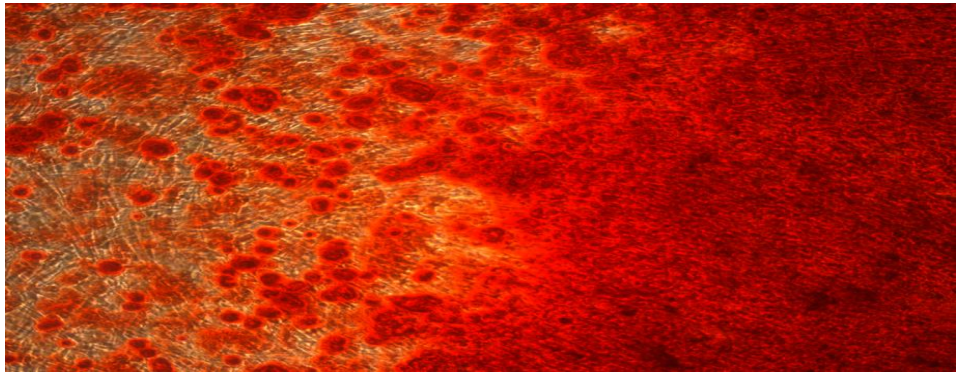
MINERAL DEPOSITION

A. ALIZARIN RED

Cells cultured in basal medium

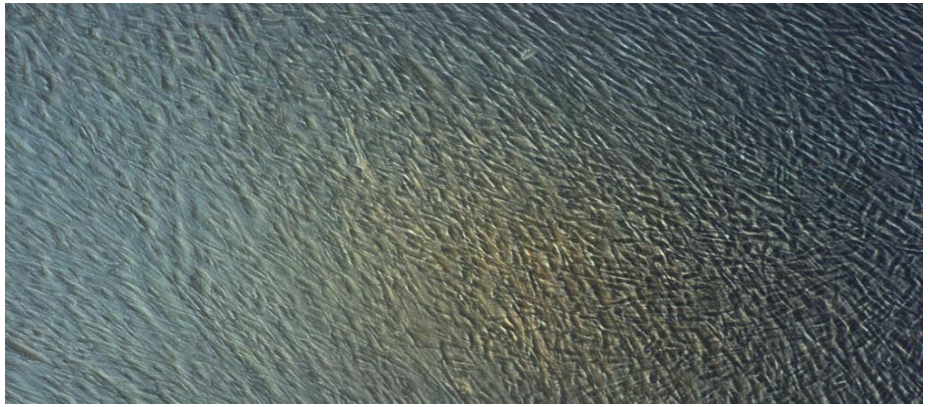


Cells treated with osteogenic medium

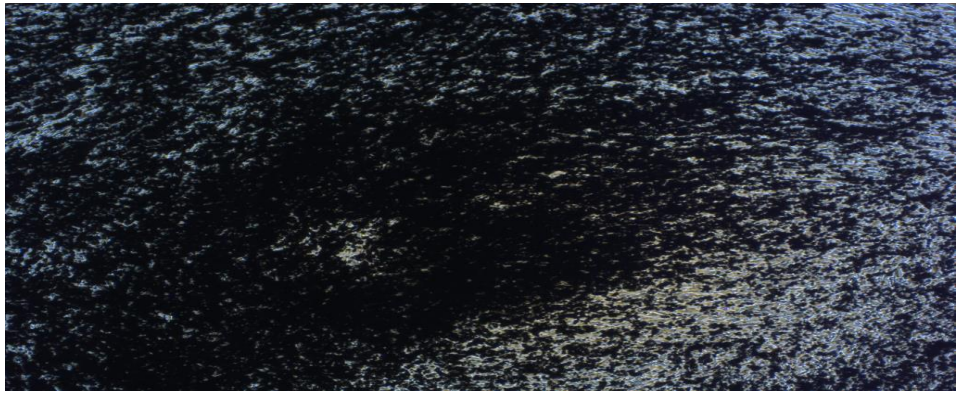


B. VON KOSSA STAINING

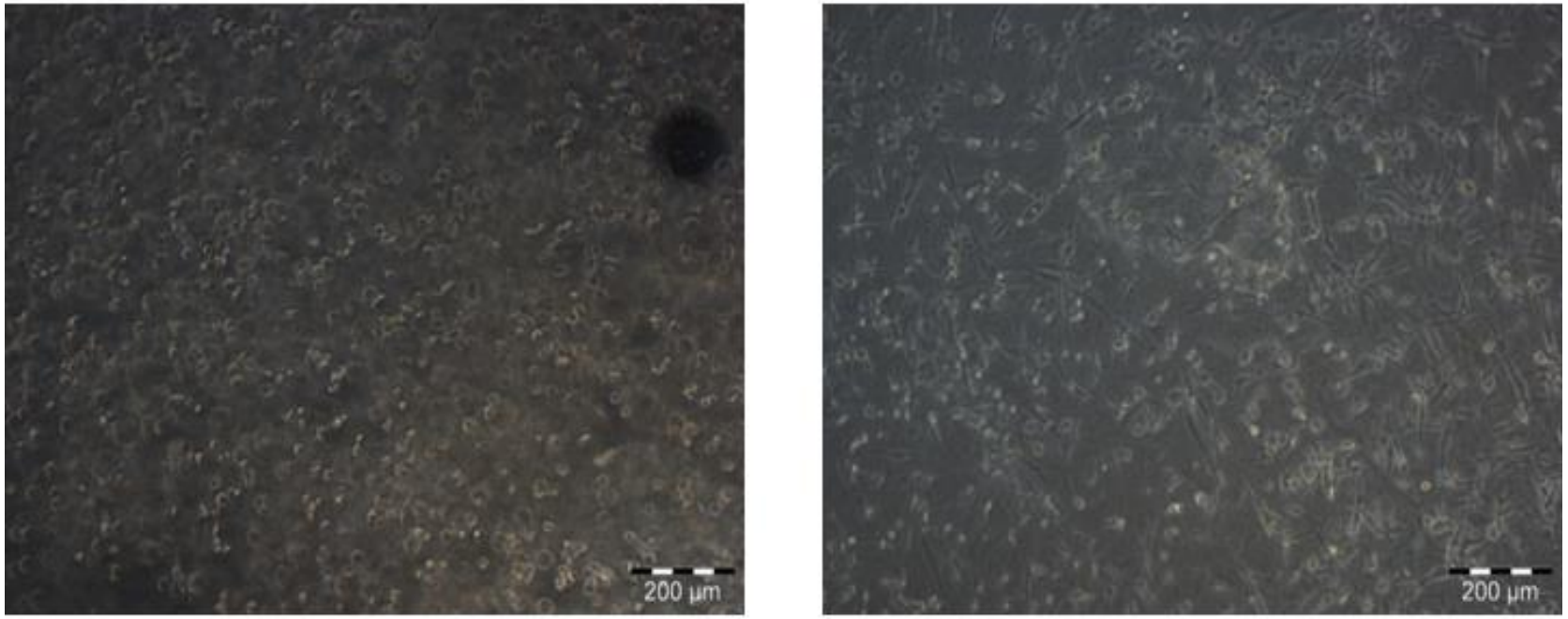
Cells cultured in basal medium



Cells treated with osteogenic medium

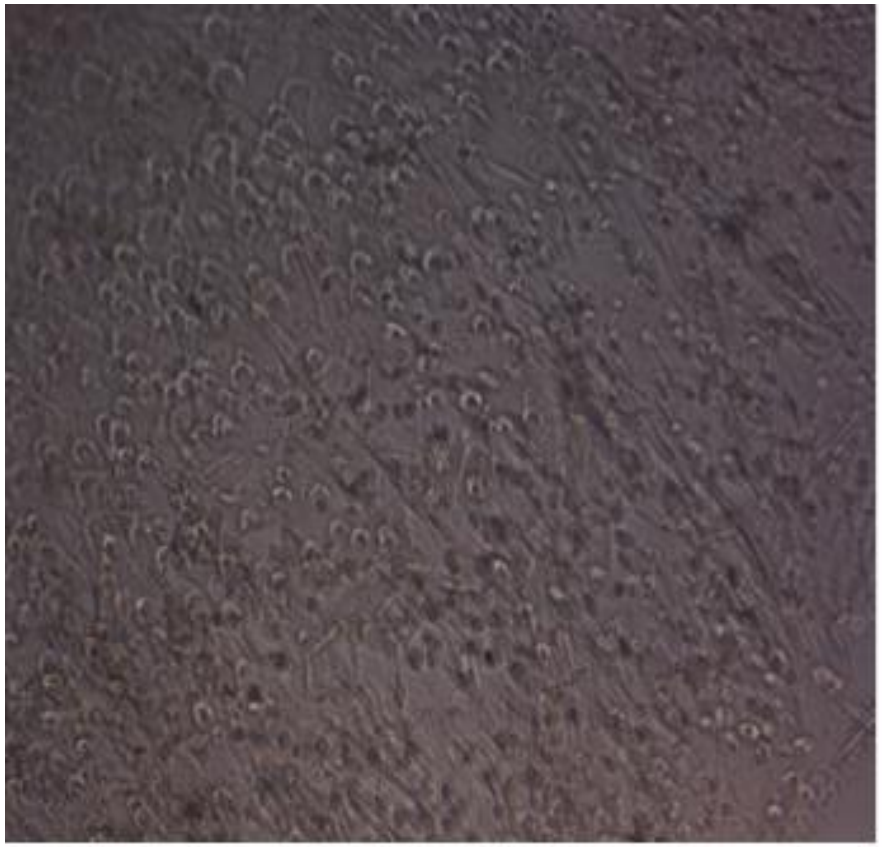


Cells Encapsulated in Puramatrix™



Cells encapsulated in PuraMatrix hydrogel; Phase contrast images showing the morphology and cell proliferation at different time points. A) Day 1- Cells show round structures. B) Day 3- Cells attained spindle shape- cell growth can be observed.

Cells Encapsulated in Puramatrix™

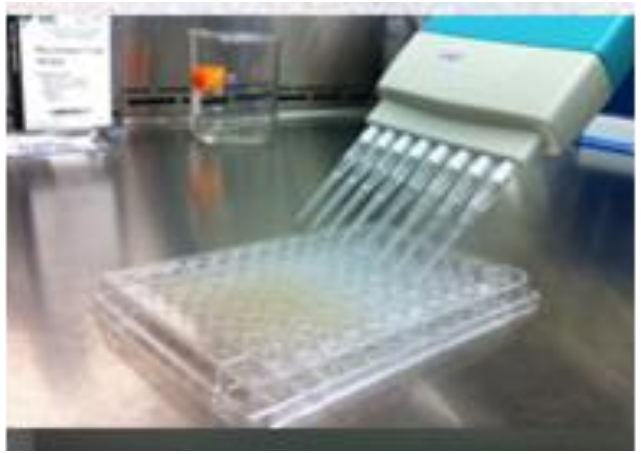


A) Cells encapsulated in PuraMatrix in terconections

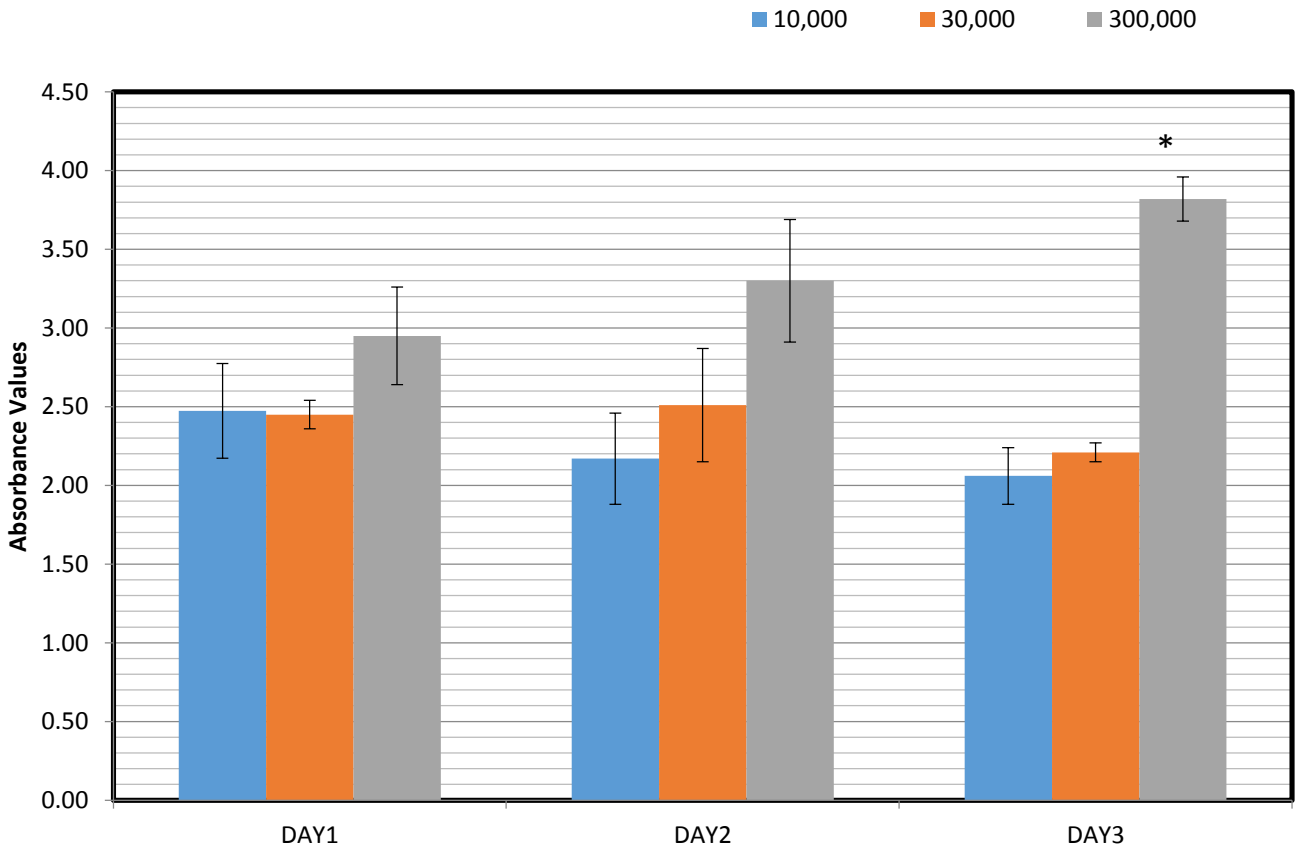


B) Day 5- Cells at peripheral region

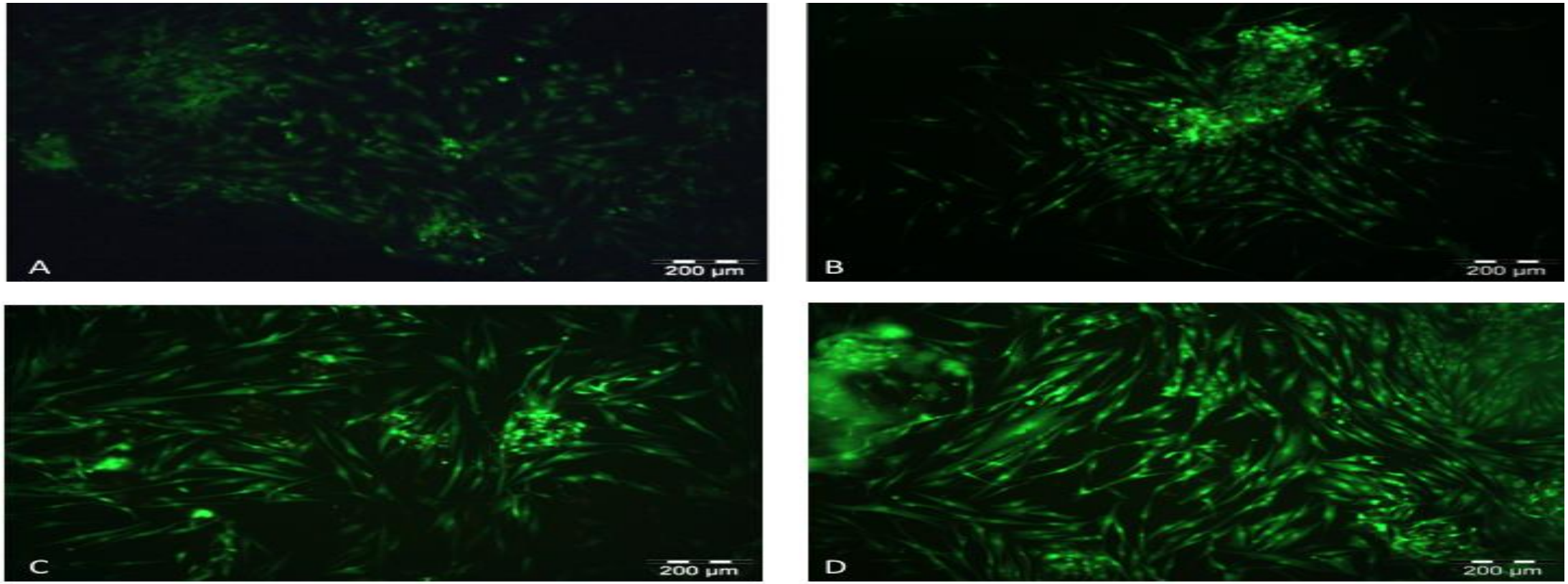
Cell Proliferation in 3 D Gel





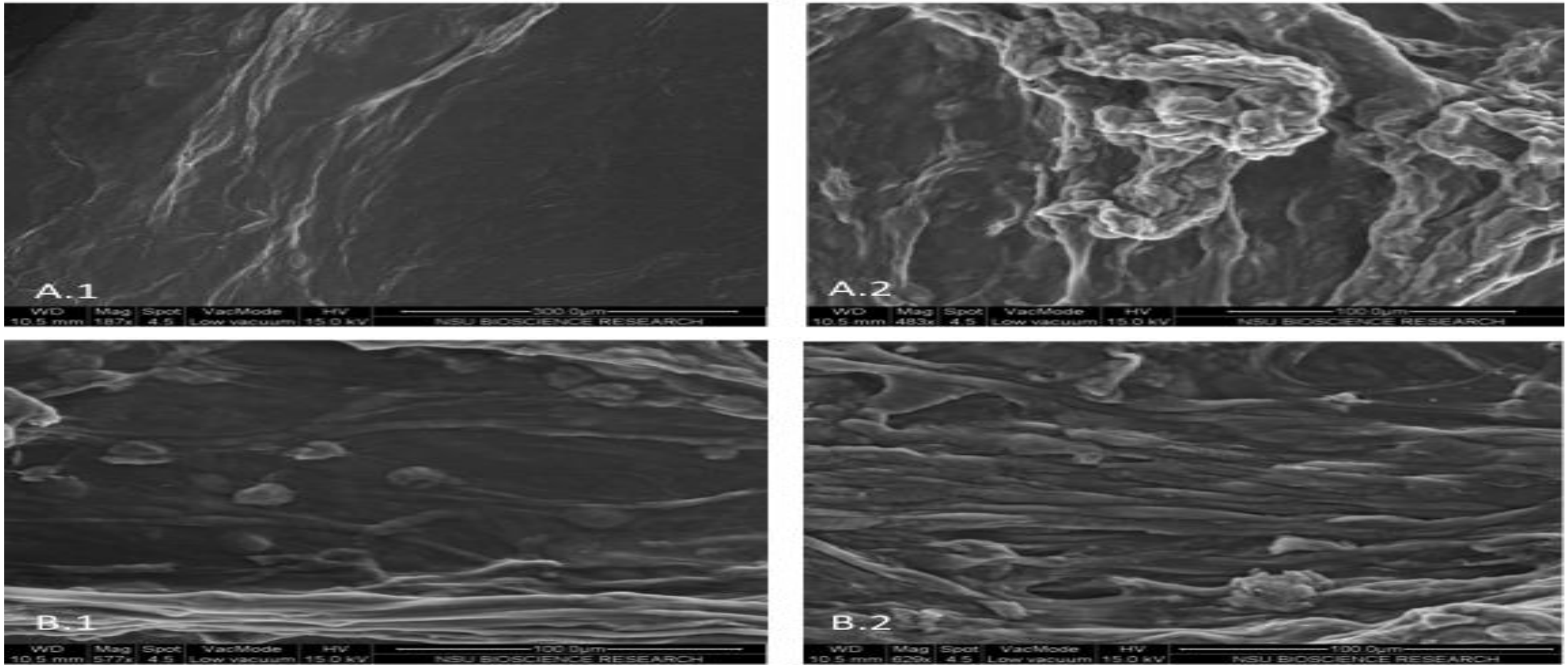


LIVE DEAD CELL ASSAY



Live/Dead Cell Assay at day 1, 3, 5, and 7. Fluorescent microscope pictures - cells in PuraMatrix™ hydrogel with Live/Dead staining. A) Day 1-Cells encapsulated in puramatrix B) Day 3-Cells with spindle shape and they are spread out evenly. C) Day 5– Cells are alive and express growth. D) Day-7 Cells have increased in proliferation and show adhesion to fibrous network.

SCANNING ELECTRON MICROSCOPY STUDIES

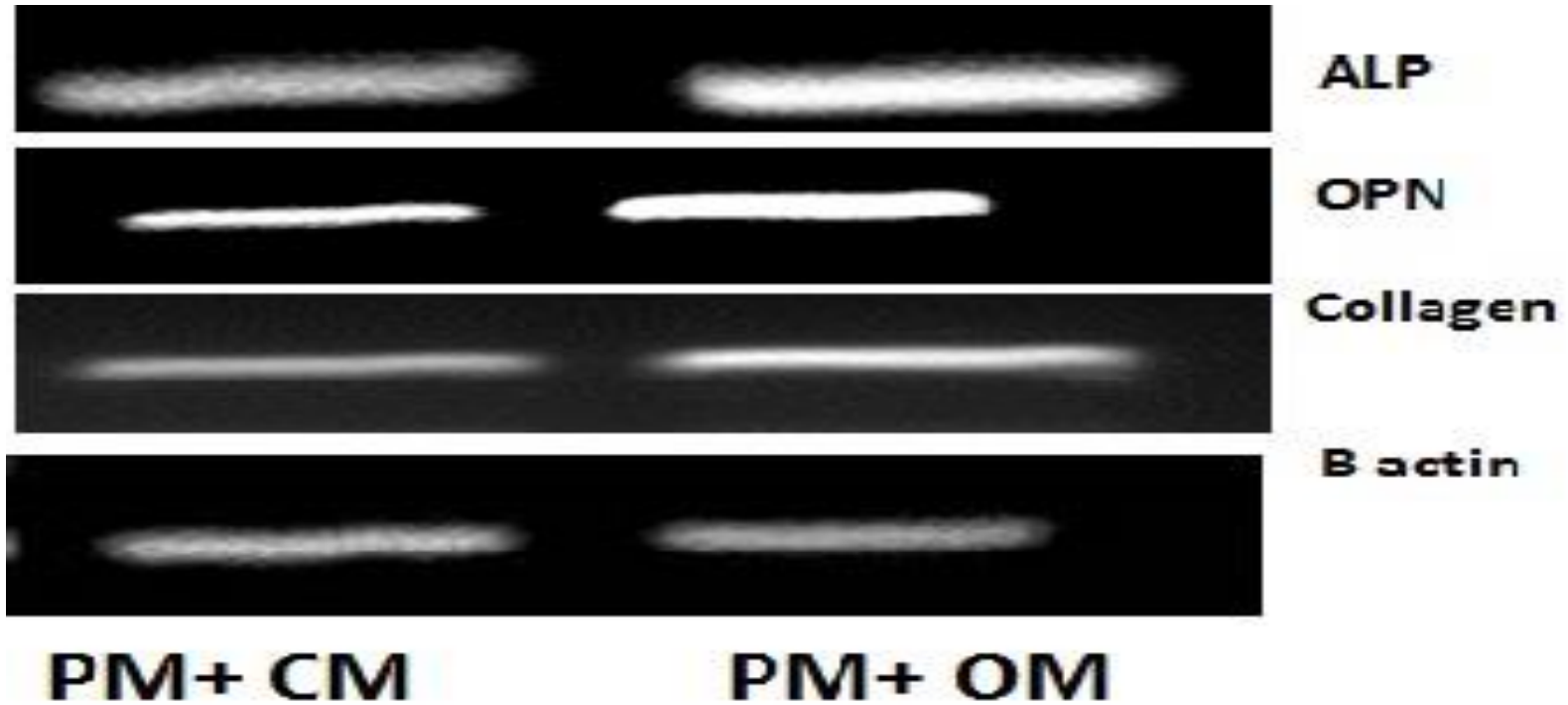


SEM of PuraMatrix™ and HGMSCs seeded PuraMatrix™. A) Scanning electron microscopy of PuraMatrix™ scaffold only. A.1 Shows surface appearing like a sheet under low magnification, 187x. A.2 Shows peptides appearance to be like interwoven nanofibers at higher magnification, 483x. B) Scanning electron microscopy of PuraMatrix™ with HGMSC cells. B.1 PuraMatrix™ with HGMSC under 577x magnification. Shows linear assembly of cells within matrix. B.2 PuraMatrix™ with HGMSC under 629x magnification. Shows linear assembly of cells within matrix

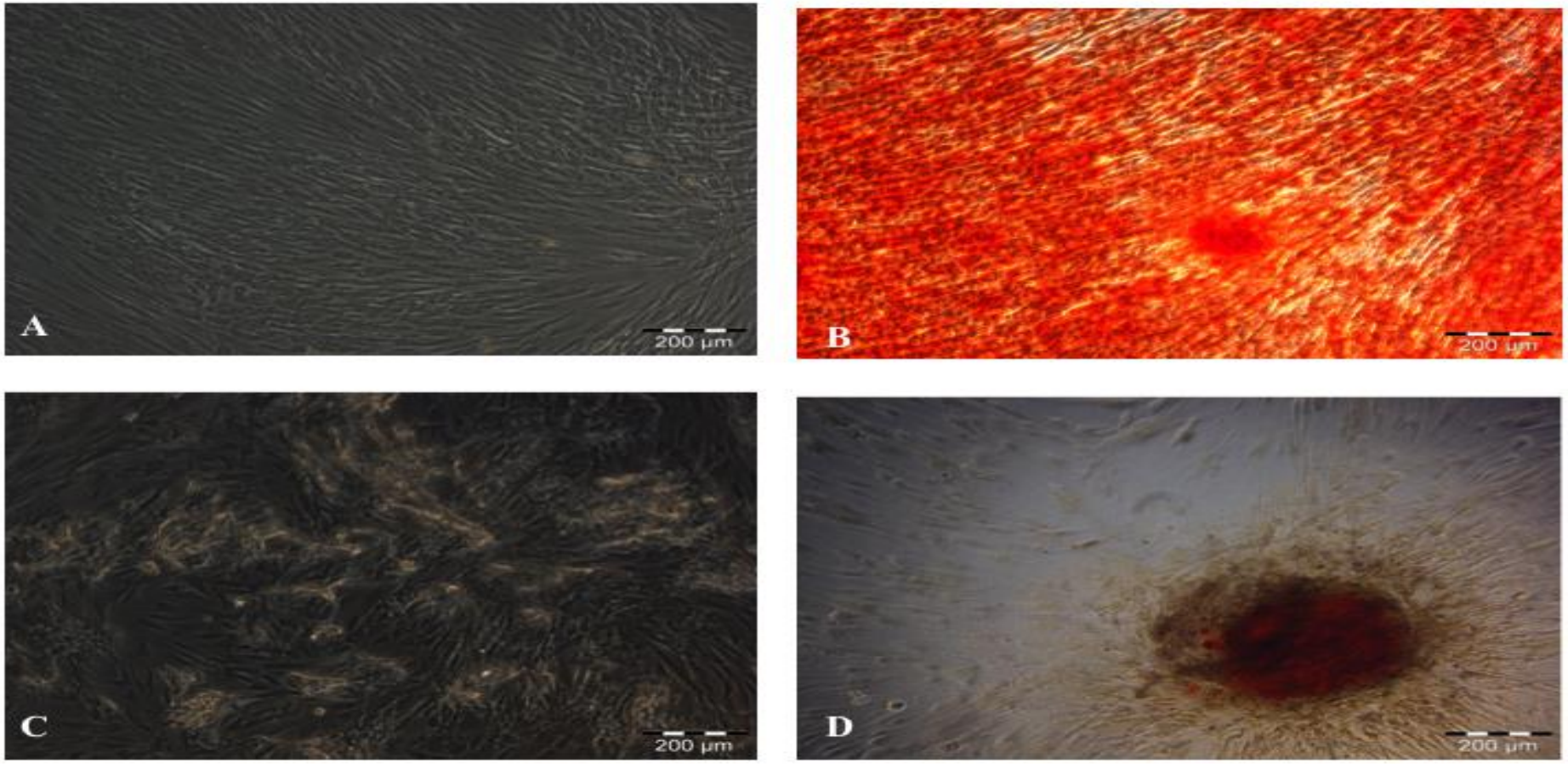
OSTEOGENIC DIFFERENTIATION

Gene expression Studies





Mineralization



HGMSCs as monolayer culture (2D) and cells encapsulated in PuraMatrix (3-D). The morphology of differentiated cells A) Cells in monolayer in osteogenic medium at week 4 B) Cells induced with OM for 4 weeks and stained with Alizarin Red – orange color indicates calcium deposition C) Cells in PuraMatrix in OM (clustering of cells can be observed) F) 4 weeks -Cells in PuraMatrix were stained with Alizarin Red to detect the mineral deposit, presence of mineral deposition is observed for cells with in PuraMatrix gel.

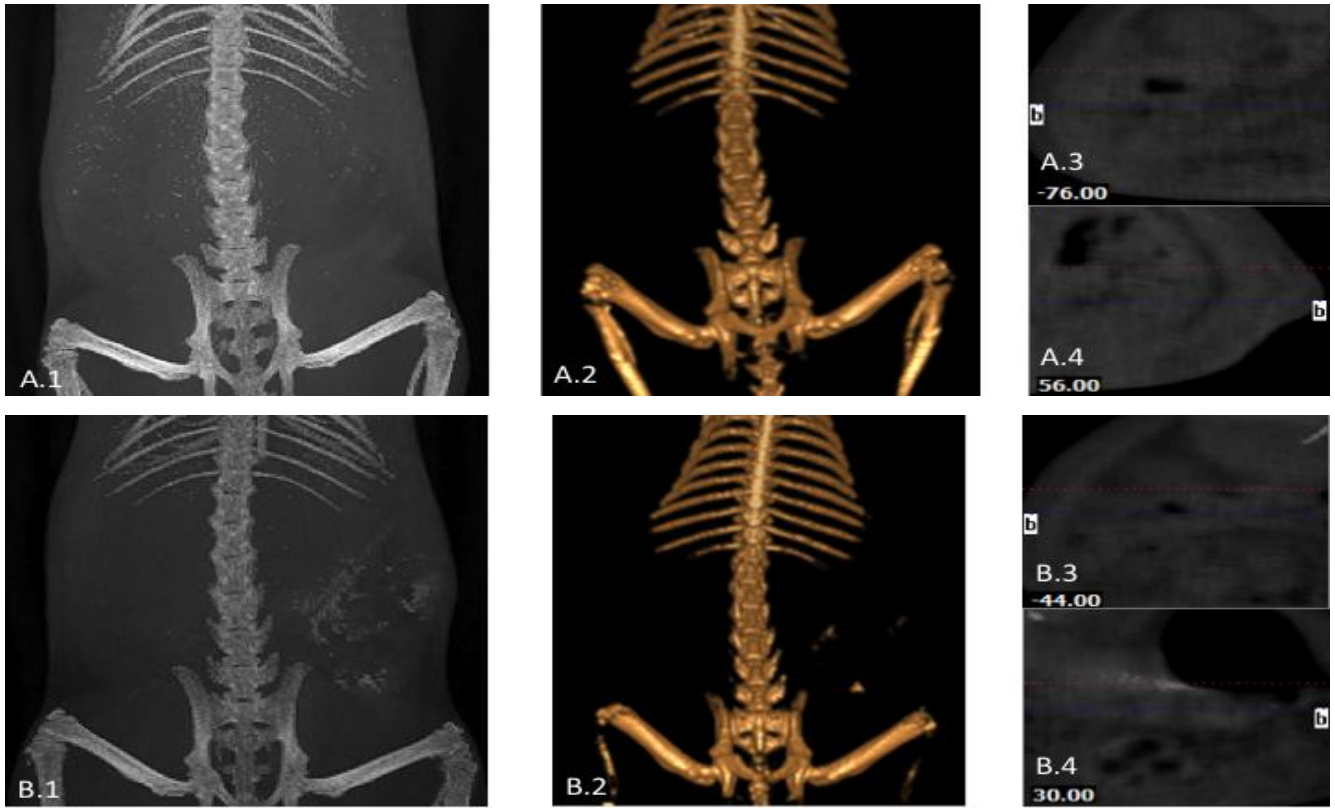
Summary of *In Vitro* Study

- HGMSCs demonstrated osteogenic differentiation
- The cells grown in **PuraMatrix** nanoscaffold showed high survival rate and cell growth was also observed in time dependent manner
- The cells attained **spindle shape within 3 days** of encapsulation and cell growth was observed from day 3
- Scanning electron microscope studies revealed that the **cell bodies embedded in PuraMatrix started to develop a dense network process**.
- The Live/Dead cell assay and WST assay revealed that PuraMatrix nanofibers are **cytocompatible**
- Cells **expressed positive mineralization** at 4 weeks

INJECTION OF CELLS AND SCAFFOLD

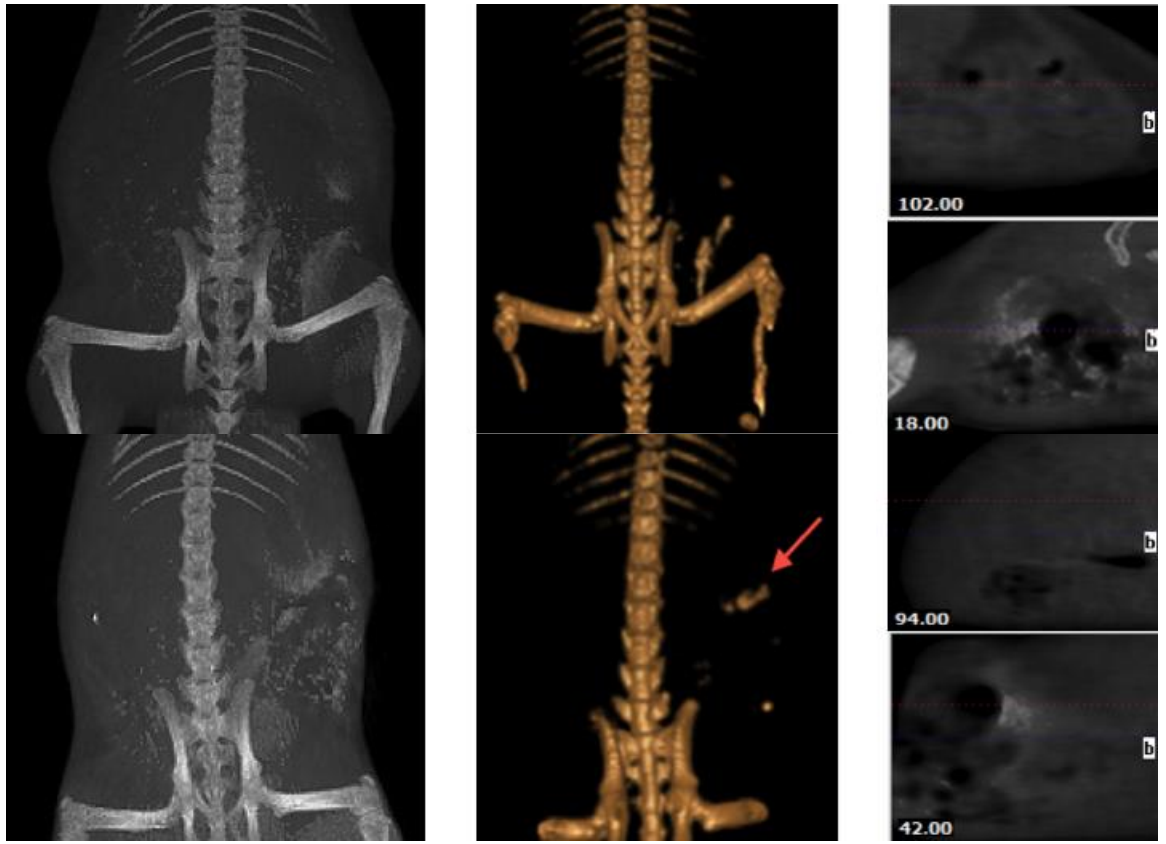


CT SCAN



CT Scans of Control and Week 2. Series of images from CT Scan of rat subject post surgery using i-CAT vision software showing 2D scans, 3D scans, and control and experiment cross sectional slices. A) CT scans of control rat shows no radiopacity at week 4. B) Shows 2 week CT scan of rats showing radiopacities starting to form.

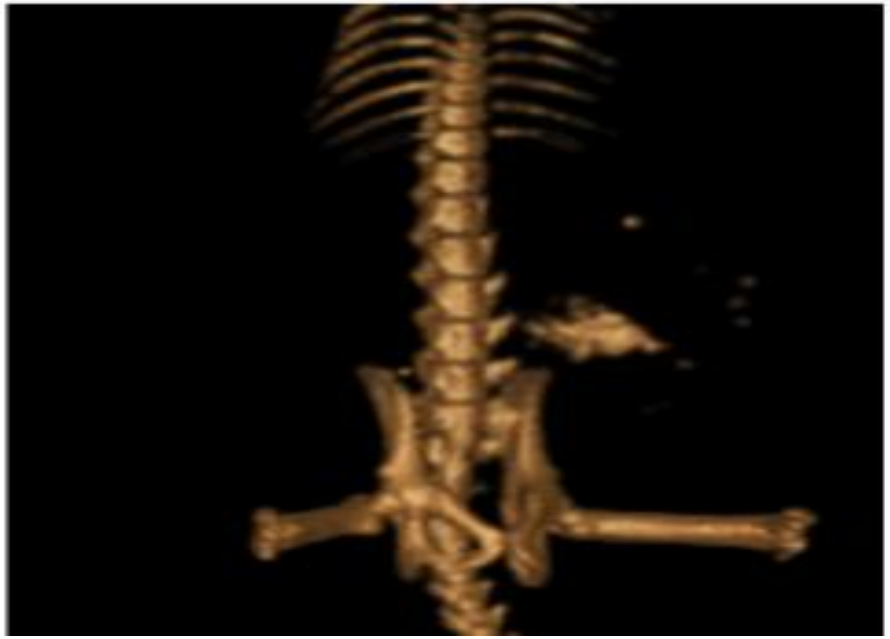
CT SCAN

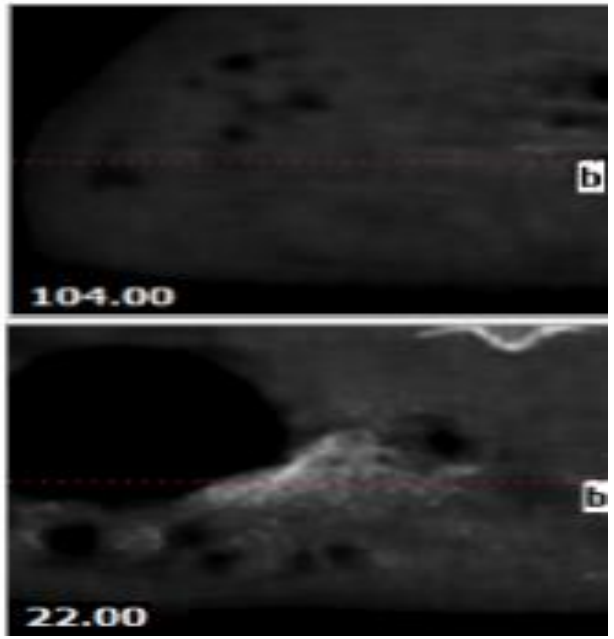


A) Clusters of radiopacities presented on injectable experimental side at 4 weeks. B) 3D representation of radiopacities at 4 weeks. C) Clusters of radiopacities presented on injectable experimental side at 4 weeks. D) 3D representation of radiopacities at 4 weeks.

Ct Scan

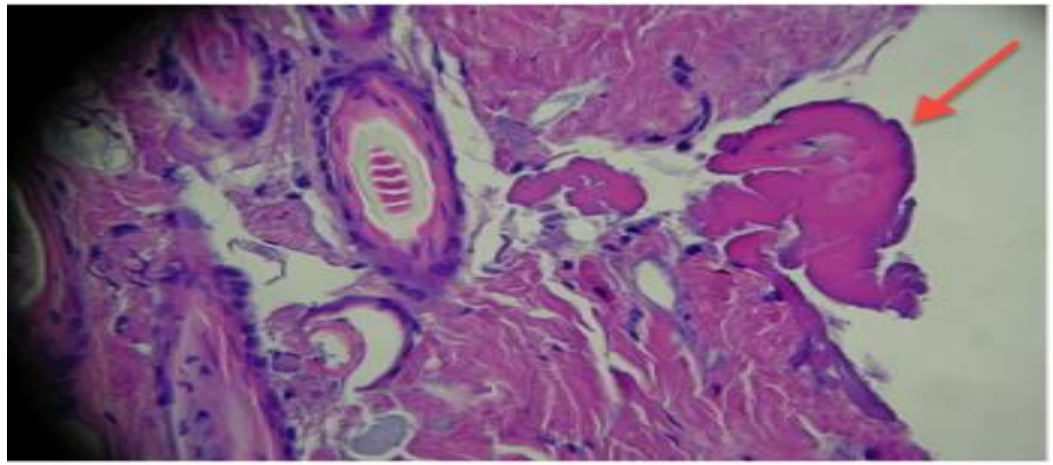
Clusters of radiopacities presented on injectable experimental side at 4 weeks.

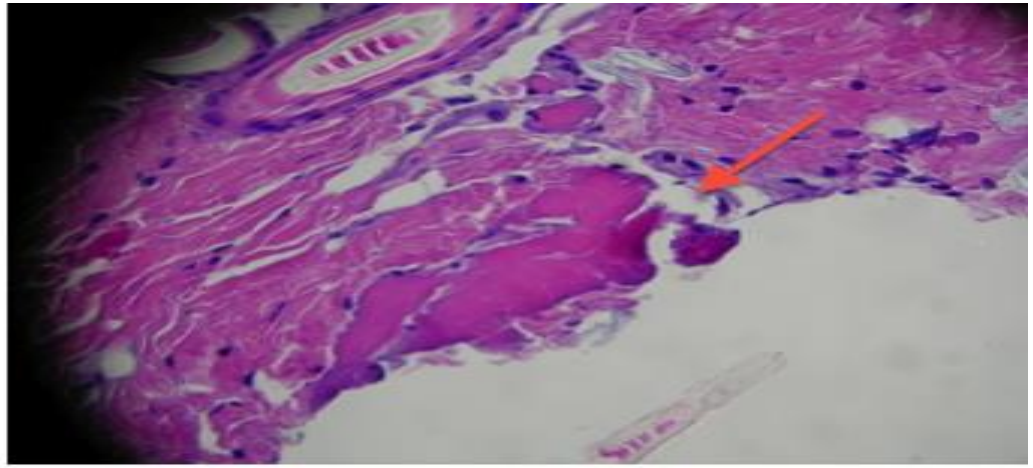


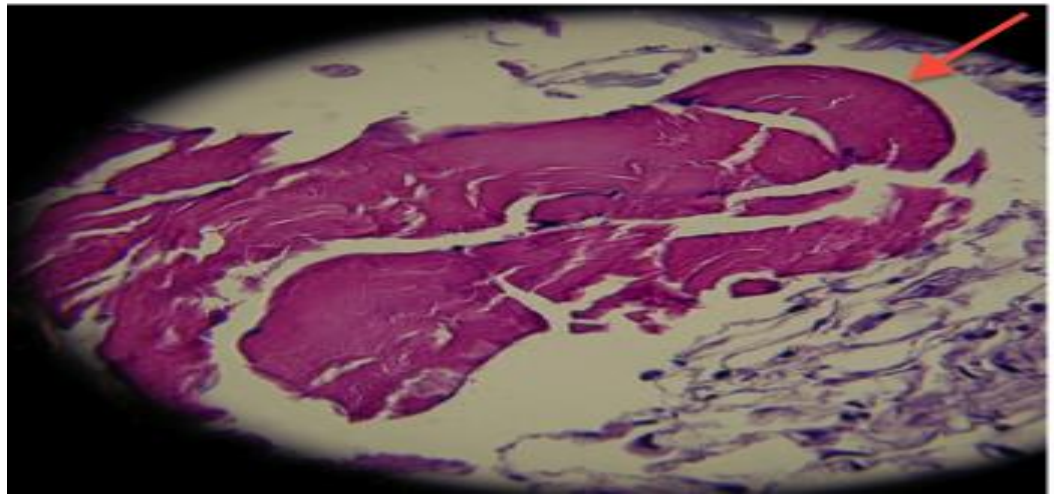


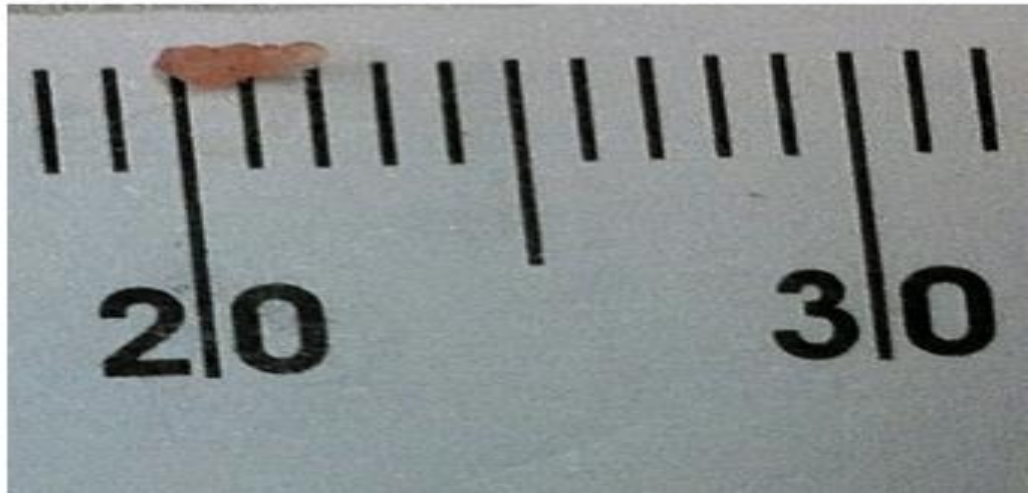


Histology



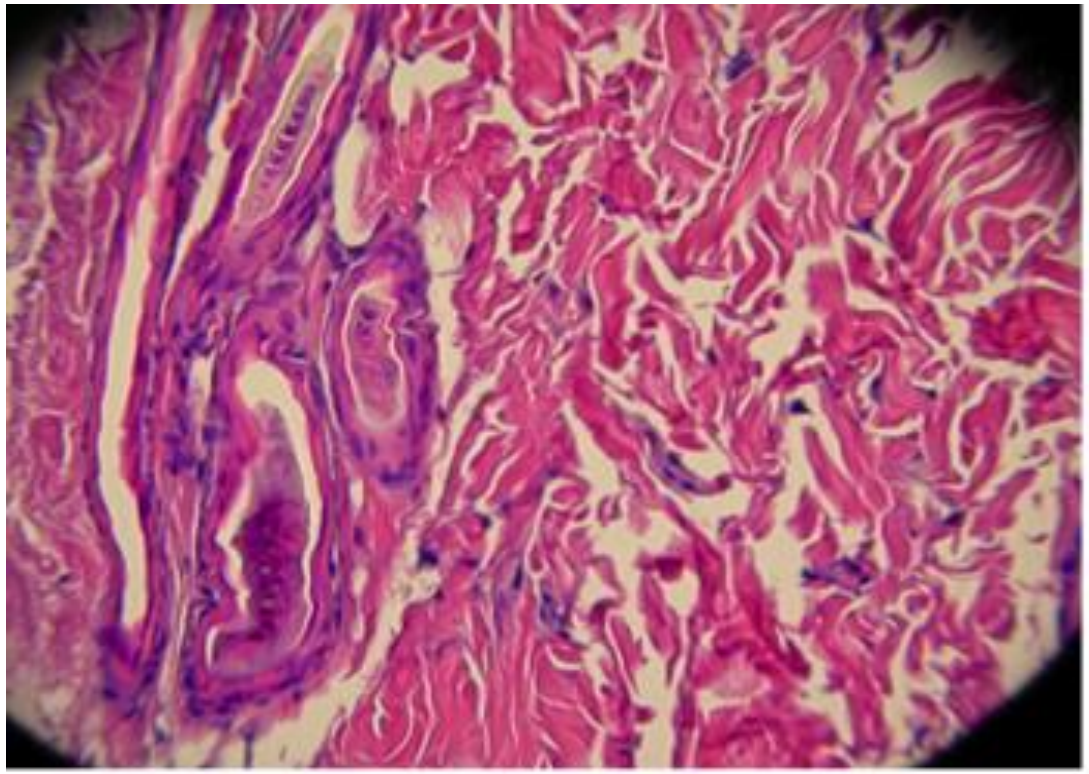


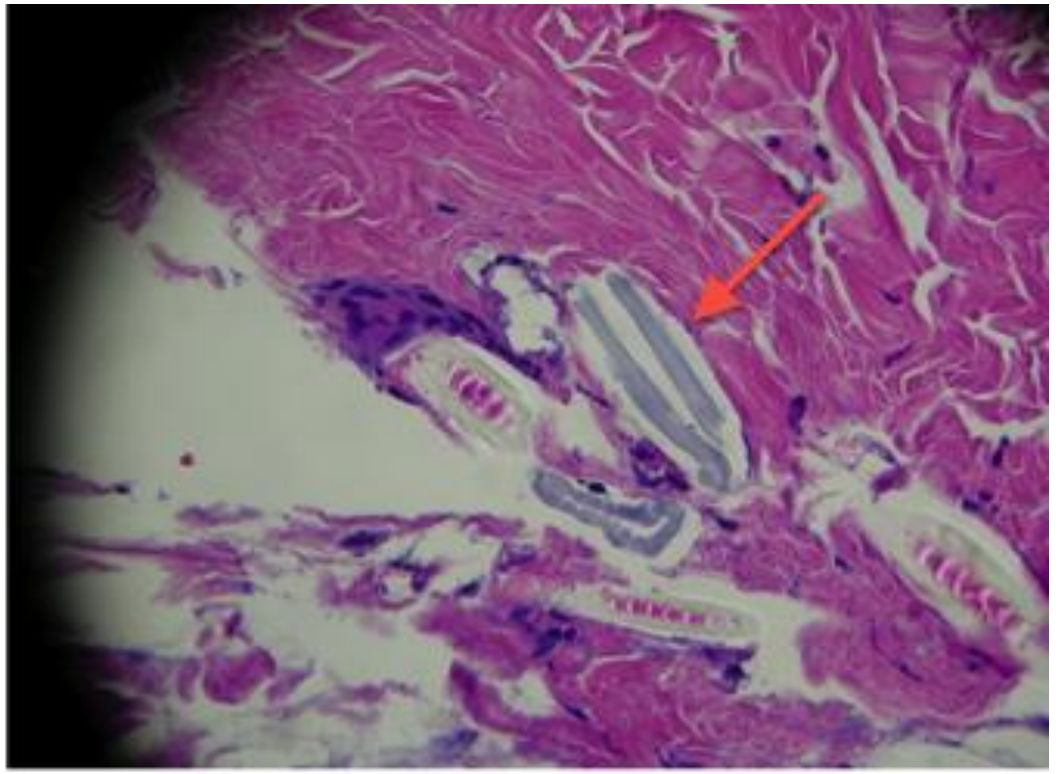




Histological slides at 400x. A) Bone tissue formation at 4 weeks. B) Bone tissue formation at 4 weeks. C) Bone tissue at 4 weeks. D) 2mm bone nodule explant excised from dorsum of rat at 4 weeks.

Histology





A) Tissue sample of control side showing connective tissue only. B) Tissue sample of control side showing presence of remanants of PuraMatrix scaffold.

ANIMAL MODEL





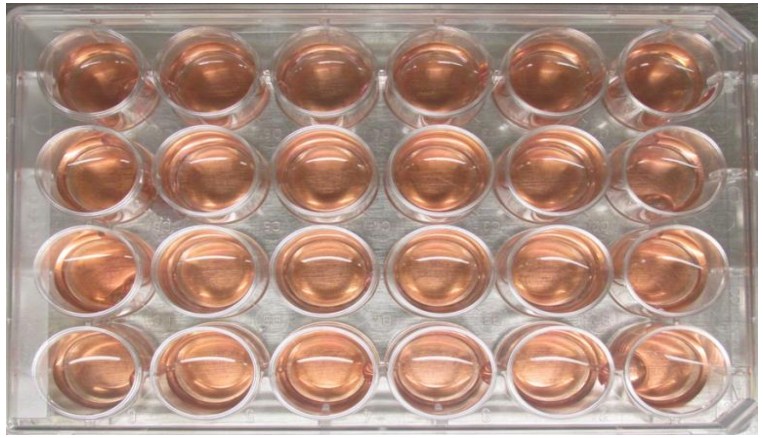
Summary: In vivo Study

- ***In Vivo*** Study
  - CT scans show **radiopacities** suggesting that PuraMatrix scaffold with HGMSC supported ectopic bone formation
  - Histological studies demonstrated that PuraMatrix in combination HGMSCs **supported the ectopic bone formation**.
  - We are establishing the animal model

CONCLUSIONS AND FUTURE STUDY

- The self-assembled, injectable PuraMatrix scaffold in combination with HGMSCs can support bone tissue formation
- Further research needed to investigate the bone growth in terms of density and volume at week 8 and later time points.
- Also, further studies will investigate the potential of HGMSCs seeded PuraMatrix combination for the repair of critical size defects in hard palate of rats.
- Using growth factors to enhance bone growth

CELL CULTURE AND BEAD GENERATION



Beads ~30mg,60mg,120mg

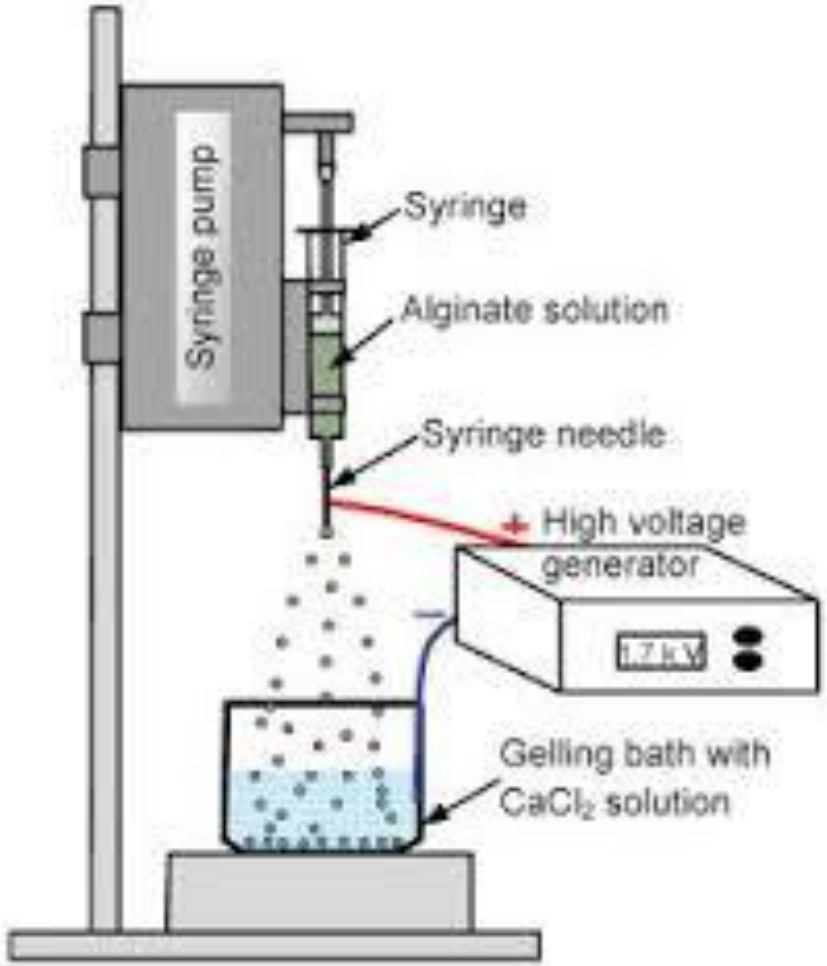


2% alginate (w/v) solution

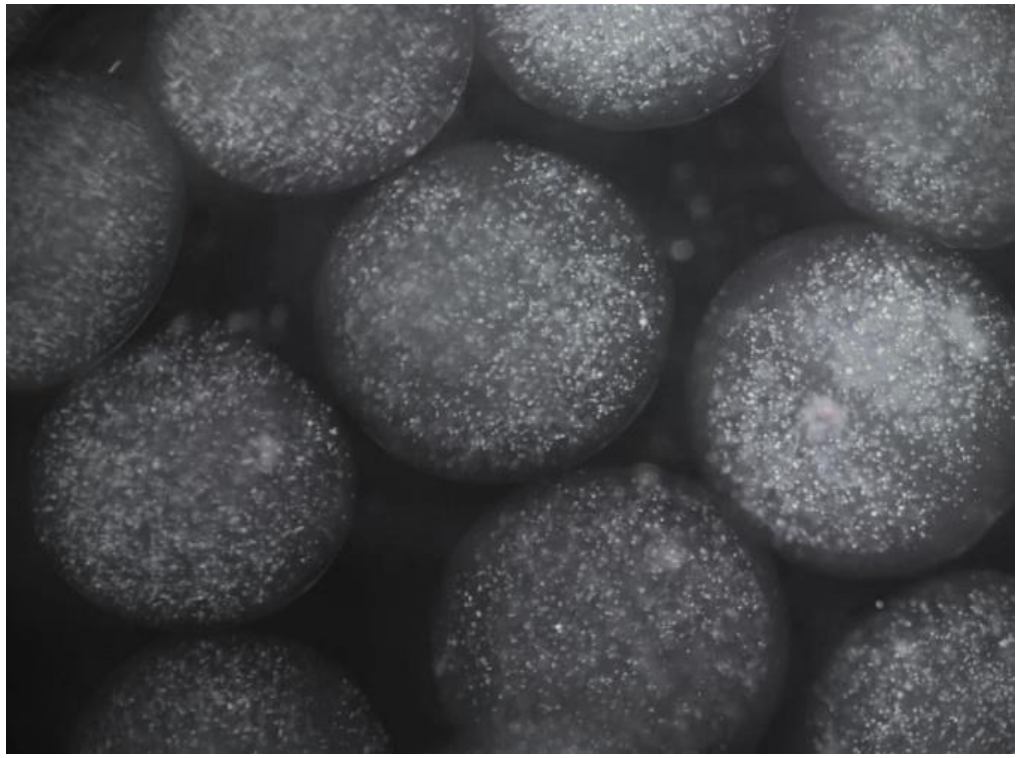


Ca++

Cell Encapsulation



ENCAPSULATED CELLS



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